Biochimica et Biophysica Acta 1864 (2017) 125–137

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Protein trafficking at the crossroads to mitochondria

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ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form 25 October 2016
Accepted 27 October 2016
Available online 31 October 2016

Keywords:
Mitochondrial protein biogenesis
Protein transport
Retrograde signaling
UPRmt
UPRam

ABSTRACT

Mitochondria are central power stations in the cell, which additionally serve as metabolic hubs for a plethora of anabolic and catabolic processes. The sustained function of mitochondria requires the precisely controlled biogenesis and expression coordination of proteins that originate from the nuclear and mitochondrial genomes. Accuracy of targeting, transport and assembly of mitochondrial proteins is also needed to avoid deleterious effects on protein homeostasis in the cell. Checkpoints of mitochondrial protein transport can serve as signals that provide information about the functional status of the organelles. In this review, we summarize recent advances in our understanding of mitochondrial protein transport and discuss examples that involve communication with the nucleus and cytosol.

1. Introduction

Mitochondria are involved in a plethora of vital processes in the cell. Apart from the generation of adenosine triphosphate (ATP), they serve as a metabolic hub that interconnects various anabolic and catabolic processes, including oxidative phosphorylation, the tricarboxylic acid (TCA) cycle, the β-oxidation of fatty acids, and the steroidogenesis or heme synthesis. The important metabolic role of mitochondria is reflected by their involvement in multiple signaling pathways in the cell, including calcium signaling and apoptosis. To fulfill these multiple functions, mitochondria encompass a large proteome of roughly 1000 proteins [1,2]. Although mitochondria contain an autonomous genome, the great majority of mitochondrial proteins are encoded in nuclear DNA and translated in the cytoplasm. These precursor proteins are imported into mitochondria by specialized machineries that decode structural signals in the amino acid sequence of precursors to properly distribute them into four mitochondrial compartments: outer membrane (OM), intermembrane space (IMS), inner membrane (IM), and matrix.

Multiple functions of mitochondria require feedback mechanisms to reflect the functional status of mitochondria. For example, mitochondria can send retrograde signals to the cytoplasm by modulating calcium signaling, which depends on the mitochondrial electrochemical potential ($\Delta\Psi$) across the IM. Reactive oxygen species (ROS) can also play a role in retrograde signaling as a functional output of respiratory chain activity. In this review, we focus on retrograde signaling that directly involves mechanisms of protein transport into mitochondria. The necessity to import a majority of mitochondrial proteome from the cytoplasm exposes the organelle to a constant flux of precursors across their membranes, which can be interrupted as a result of mitochondrial dysfunction. Protein import into mitochondria acts as a signaling pathway to reflect the functional status of these organelles. We also discuss novel concepts of retrograde trafficking of mitochondrial proteins and signaling through factors that are encoded in the mitochondrial genome.

2. Major protein import pathways to mitochondria

2.1. Translocase of the outer membrane as a common entry gate

The synthesis of mitochondrial proteins in the cytosol requires a mechanism that can organize the transfer of precursor proteins across the OM. All canonical import pathways require the presence of Tom40 protein, which constitutes a channel of the translocase of the outer membrane (TOM) complex [3–6] (Fig. 1). Apart from the channel itself, the TOM complex contains a number of receptor proteins, such as Tom20, Tom22, and Tom70, that participate in recognition of the incoming precursor proteins. Import through the TOM complex depends on interactions between precursor proteins with receptor domains that are exposed by subunits of the TOM complex. The receptors bind the precursor with increasing affinity as it gradually translocates across the OM [4,5,7]. In addition to the channel and receptors, the TOM complex contains accessory subunits Tom5, Tom6, and Tom7 that are responsible for assembly and stability of the complex [4,5,8]. The TOM complex contains three Tom40 subunits, but it can dynamically exchange with a dimeric form, which is presumably an assembly intermediate [9]. Active TOM complexes are located in discrete subdomains of the OM that are close to openings of cristae in the IM, known as cristae junctions [10].

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http://dx.doi.org/10.1016/j.bbamcr.2016.10.019

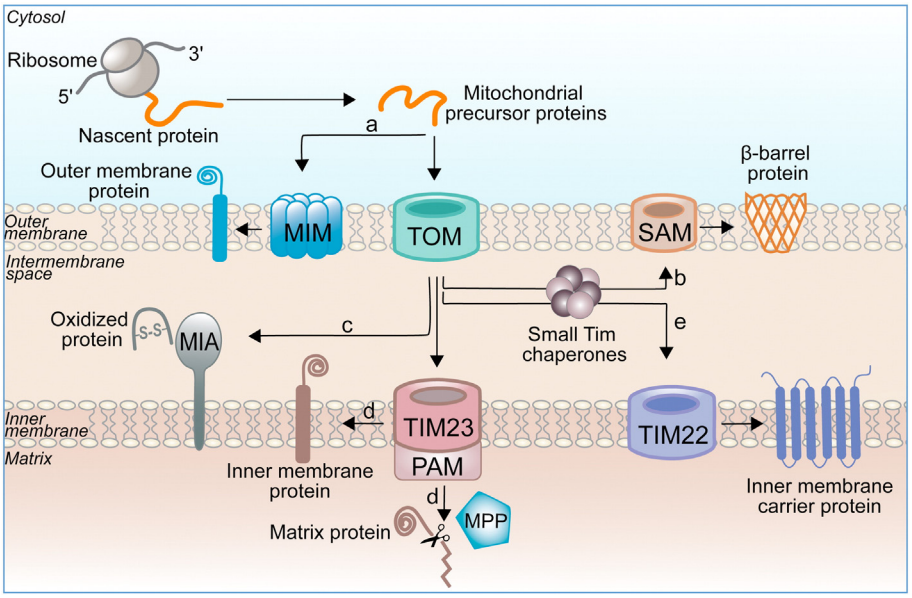

Fig. 1. Protein transport pathways into mitochondria. Mitochondrial proteins are synthesized on the cytosolic ribosomes and delivered as precursor forms to the translocase of the outer membrane (TOM), to be directed to their final mitochondrial destinations with assistance from specific sorting machineries. a) Precursors with α-helical transmembrane segments are directed to the insertase of the mitochondrial outer membrane (MIM) for assembly. b) β-barrel precursors are transported to the sorting and assembly machinery (SAM) by the small Tim chaperone proteins. c) Cysteine-rich precursor proteins are guided into the IMS via the mitochondrial intermembrane space assembly (MIA) pathway. d) Precursors with cleavable N-terminal presequences are directed to the matrix and IM via TIM23. TIM23 is associated with the presequence translocase-associated motor (PAM) and transfers precursor proteins into the matrix in an ATP-dependent manner. Upon delivery into the matrix, the presequences are recognized and cleaved by mitochondrial processing peptidase (MPP). **e)** Hydrophobic precursors of carrier proteins with internal targeting signals are transported through the IMS by small TIM chaperone complexes that deliver precursors to the TIM22 complex. The membrane insertion via the TIM23 and TIM22 requires the electrochemical potential ($\Delta\Psi$) across the IM.

Certain classes of precursor proteins have long been known to differentially interact with the TOM complex. Precursor proteins that are targeted to mitochondria by the cleavable mitochondrial targeting signal, called a presequence, are first recognized by Tom20 before they interact with Tom22. Hydrophobic precursors of carrier proteins that contain an integral targeting signal require the presence of the Tom70 receptor. On their way through the channel of Tom40, both types of protein precursors interact with the pore, but the pattern of interacting residues of Tom40 is different for the presequence and carrier precursors [9,11].

Serving as the main entry gate for proteins, the TOM complex is a potential target for cytosolic factors that regulate the import of precursor proteins into mitochondria. In fact, the TOM complex contains around 30 phosphorylation sites, the majority of which localize to cytosolic domains of receptor proteins [12]. In *Saccharomyces cerevisiae*, two cytosolic kinases phosphorylate subunits of the TOM complex and apparently affect the functions of the TOM complex in opposite ways. Casein kinase 2 (CK2) promotes the biogenesis of the TOM complex by phosphorylating the Tom22 precursor to control its import into the OM and promote the assembly of Tom20 with the complex. Conversely, protein kinase A (PKA) impairs the assembly of the TOM complex through phosphorylation of the Tom40 precursor to inhibit its import [13]. PKA also targets the Tom70 receptor subunit and decreases the import of carrier precursors under fermentative conditions when the demand for oxidative phosphorylation is reduced. In higher eukaryotes, the tethering of PKA to mitochondria has been described, but its involvement in the regulation of the TOM complex is unknown [14].

2.2. Biogenesis of outer membrane proteins

Integral proteins of the OM are incorporated into the membrane following several routes, depending on their topology. Many OM proteins, known as signal-anchored proteins (e.g., Tom20 and Tom70), are anchored in the membrane by an N-terminal α-helix and expose the C-terminal part to the cytosol. From a topological point of view, these proteins do not require full translocation through the membrane. In yeast, precursors of signal-anchored proteins are imported by the mitochondrial import (MIM) complex, which consists of Mim1 and Mim2 proteins [15–19] (Fig. 1). An exceptional signal-anchored protein, OM45, reveals an inverted topology in which the bulk of the protein mass is exposed to the IMS [20,21]. In this case, the precursor first translocates through the TOM complex into the IMS in a Tim23-dependent manner and then assembles into the membrane with a help of the MIM complex. The MIM complex also participates in the biogenesis of multispanning proteins, such as Ugo1, but in this case the import of precursors also requires the Tom70 receptor [22,23]. Another example of an OM multispanning protein is Mcp3. Similar to OM45, Mcp3 first translocates to the IMS with the help of Tim23 before it is integrated to the OM by the MIM complex [24]. In contrast to fungi, no orthologues of either Mim1 or Mim2 have been identified in metazoans to date; therefore, the mechanism of the biogenesis of signal-anchored and multispanning proteins remains elusive.

Many OM proteins are integrated into the membrane by a transmembrane domain that is located at the C-terminus, referred to as tail-anchored (TA) proteins [4,25]. Among them are proteins (e.g., Mff, Fis1 and members of the Bcl-2 family) that are directly involved in the control of the functional state of mitochondria or of the mitochondrial pathway of apoptosis as well as proteins that are involved in mitochondrial dynamics [26–28]. Some TA proteins participate in coordination of mitochondria motility with Ca^{2+} signals or organize signaling platforms [29,30]. Several aspects of the biogenesis of TA proteins remain unknown. In particular, targeting mitochondrial TA proteins to the OM requires a specific mechanism that can differentiate them from TA proteins that are targeted to the endoplasmic reticulum (ER) [31]. An appropriate lipid composition of the OM appears to promote their targeting; at least in the case of some mitochondrial TA proteins, this is sufficient for their incorporation into the membrane [32,33]. The proper localization of mitochondrial TA proteins is safeguarded by proteolysis both before and after their insertion into the membrane [34–36].

The OM is exceptional among other eukaryotic membranes because it contains a conserved class of proteins that is characterized by a β-

barrel fold, sharing ancestry with proteins of Gram-negative bacteria [3,5,37]. In yeast, the biogenesis of β-barrel proteins requires the sorting and assembly (SAM) complex (Fig. 1). It is composed of the β-barrel protein Sam50, which integrates precursors into the membrane, and subunits Sam35 and Sam37, which participate in substrate recognition and complex stability. The precursors of β-barrel proteins cross the OM using Tom40 channel. They are received in the IMS by small Tim chaperones that prevent their aggregation and ascertain their safe passage to the SAM complex that incorporates the precursors into the membrane [38,39]. The biogenesis of β-barrel proteins also depends on Mic60 protein, a core subunit of the mitochondrial contact site and cristae organizing system (MICOS) complex, which physically interacts with the SAM complex [40]. The SAM complex also contains an accessory subunit, Mdm10, which might play a regulatory role. Apart from the interaction with the SAM complex, Mdm10 is involved in mitochondria-ER contact sites that are defined in yeast by the ER-mitochondria encounter structure (ERMES) complex [41].

2.3. Mitochondrial intermembrane space import and assembly pathway

Small soluble proteins of the IMS are imported by the mitochondrial IMS import and assembly (MIA) pathway, which consists of two components in yeast: Mia40 and Erv1 [42–47] (Fig. 1). In higher eukaryotes, orthologues of MIA components are known as MIA40/CHCHD4 and ALR, respectively [47–53]. Canonical substrates of the MIA pathway are small Tim chaperones (e.g., Tim9) and assembly factors of the respiratory complexes (e.g., Cox17). They are characterized by conserved twin Cx_3C or Cx_9C motifs. Substrates of the MIA pathway can translocate through the OM via channels formed by Tom40, which is not in complex with other canonical subunits of TOM like Tom22 [54]. Interestingly a similar route of entry to mitochondria has been proposed for another protein destined to the IMS, apocytochrome *c* [55]. After translocation through the Tom40 channel Mia40 interacts with precursor proteins both by covalent disulfide bond with one of the cysteine residues of Cx_3C or Cx_9C motifs and by non-covalent interactions [46,56–58]. Subsequently, Mia40 introduces intermolecular disulfide bridges to trigger the precursor folding [59,60]. Oxidative folding executed by the MIA pathway prevents the leakage of mature proteins from the IMS. The MIA pathway is linked to the respiratory chain by cytochrome *c*, which via Erv1 that reoxidizes Mia40, receives electrons that are derived from the oxidized substrate protein. Consequently, the efficiency of the MIA pathway correlates with the functional state of the respiratory chain [61–63]. Redox state of the MIA pathway is also regulated by IMS glutathione pool, which is connected with the cytosol pool via porins in the OM [64]. In addition to canonical IMS substrates, MIA participates in the biogenesis of other proteins, such as the IM translocases Tim17 and Tim22, DNA repair enzyme APE1, mitochondrial ribosome subunit Mrp10 and IMS protease Atp23 [65–70]. In addition to its involvement in the import of protein precursors MIA influences functioning of mitochondrial calcium uniporter (MCU) by the control of MICU1-MICU2 heterodimer formation [71].

2.4. TIM23 pathway

The majority of mitochondrial precursors use the presequence translocase of the inner membrane (TIM23) pathway for import and sorting in mitochondria [5,6,72,73] (Fig. 1). These precursors share a structural feature, known as the presequence, which consists of an amphipathic helix at the N-terminus that is formed by a stretch of 30-80 amino acids. Presequence-containing precursor proteins can be sorted to either the matrix or the IM, depending on secondary signals downstream from the presequence. After at least partial translocation across the OM, precursor proteins are recognized by the TIM23 translocase in the IMS, and the positively charged presequence initiates electrophoretic translocation through the IM that is promoted by $\Delta\Psi$. In the matrix, the presequence is excised by mitochondrial processing peptidase (MPP). Thus, mature proteins are shorter than their corresponding precursors.

TIM23 is a multi-subunit complex that is localized in the IM. It has a highly dynamic structure that adapts to different types of precursors and reacts with conformational modifications to a changing environment. Precursors that contain solely the presequence are translocated to the matrix through a mechanism that consumes energy. It requires engagement of the presequence translocase-associated motor (PAM) complex, which binds to the TIM23 complex and catalyzes the translocation of the entire protein through TIM23 [6,74,75]. The key subunit of the PAM complex, chaperone Hsp70, binds with the precursor in cycles, consequently promoting directional translocation through the TIM23 complex at the expense of ATP hydrolysis. The activity of the PAM complex and its association with the TIM23 complex are regulated by accessory subunits that, similar to Hsp70, can undergo cyclic binding to the complex [5,6,72,76]. Tim44 is a subunit of the PAM complex that is peripherally associated with the TIM23 complex. Tim44 binds the incoming protein precursor to coordinate its translocation and cyclic activity of the PAM complex [77–79].

The second type of precursors contains a hydrophobic region downstream from the presequence, which can form a transmembrane domain. This domain, known as a "stop-transfer" signal, promotes the lateral release of the protein from the TIM23 complex to the IM. For this mode of TIM23 activity, the PAM complex is not required. Instead, lateral sorting by the TIM23 complex requires the presence of Tim21 and Mgr2 [72,77,80–82].

Continuity of the IM is crucial for the maintenance of $\Delta\Psi$, a key attribute of mitochondria. The TIM23 complex forms a large channel that is capable of translocating long protein chains. An interesting line of investigation is determining the mechanisms that control the permeability of TIM23 and the influence that $\Delta\Psi$ has on the complex. The TIM23 channel is kept sealed by interactions with Tim50 and becomes more prone to opening in the presence of the presequence [83]. In the presence of $\Delta\Psi$, the opening of the channel is regulated by interactions between Tim23, Tim50, and the presequence. In addition opening of Tim23 channel depends on secondary structure of Tim17 that contains a disulfide bond [69,70]. Fluctuations of $\Delta\Psi$ promote large modifications of the structure of the TIM23 complex. Disruptions of $\Delta\Psi$ trigger structural rearrangements in the transmembrane domains of Tim23 and modify interactions between Tim17 and Tim23 (i.e., core subunits of TIM23 that form the channel) [84]. In summary, the TIM23 translocase is a dynamic complex that is characterized by the architectural rearrangements in a $\Delta\Psi$-dependent manner in response to precursor proteins.

2.5. TIM22 pathway

TIM22 translocase mediates the import and insertion of multi-pass transmembrane proteins into the IM [3,85–88] (Fig. 1). The most prominent substrates of TIM22 are mitochondrial metabolite carriers, such as adenosine diphosphate ADP/ATP carriers, the phosphate carrier, and the dicarboxylate carrier [89,90]. In addition to mitochondrial carriers, proteins that are constituents of the IM translocases are also transported via the TIM22 pathway, including Tim22 and two homologue proteins, Tim17 and Tim23 [3,88]. In contrast to mitochondrial precursor proteins with the cleavable presequence, carrier proteins contain several internal import signals. Hydrophobic carrier precursors are prevented from aggregation or premature folding by cytosolic Hsp70 and Hsp90 chaperones [91]. Once the precursors reach the TOM complex, they are recognized by Tom70 and passed to the Tom40 channel in a process that requires ATP. Transport from the TOM complex to the IM is facilitated by small Tim chaperones that are located in the IMS. In yeast, the imported proteins are associated with Tim9-Tim10 or Tim8-Tim13 and delivered to TIM22 with the assistance of Tim9-Tim10-Tim12 [87]. In higher eukaryotes, the biogenesis of precursors is facilitated by at least three different complexes that are formed by TIMM8A, TIMM9, TIMM10A, TIMM10B and TIMM13 [47,92–94]. Similar to precursors that are transported via

the TIM23 pathway, carrier proteins require $\Delta\Psi$ to be integrated into the IM. Tim22 forms two functionally voltage-activated pores and the insertion process ends when a properly membrane-inserted carrier is assembled into the functional dimer [95]. In yeast, the core component of the TIM22 complex is formed by the central pore-forming subunit Tim22 together with Tim54, Tim18, and Sdh3. Interestingly, Tim22 possesses stabilizing intramolecular disulfide bonds, an evolutionary conserved feature [66,70,96]. Tim54 has a receptor role, with its C-terminal domain exposed to the IMS where it interacts with Tim9-Tim10-Tim12 via peripherally attached Tim12. Tim18 stabilizes TIM22 but does not directly participate in protein integration into the IM. Interestingly, Tim18 is homologous to Sdh4, which is a membrane integral subunit of complex II (succinate dehydrogenase) [97,98]. Within complex II, Sdh4 strongly interacts with Sdh3, which has also been identified as a subunit of the TIM22 complex where it interacts with Tim18. This finding suggests the co-evolution of mitochondrial protein import machineries and respiratory chain complexes. In contrast to other translocase complexes, TIM22 is poorly conserved between yeast and metazoans as it shares only one common core subunit Tim22. A recently discovered mammalian subunit TIM29 shares no homology with yeast proteins [99,100].

3. Proteolysis and degradation of mitochondrial proteins

Mitochondrial proteolytic systems fall into two general classes: ATP-dependent and -independent proteases [101–103]. ATP-dependent proteases belong to the AAA+ superfamily and share a conserved oligomeric structure with a ring-like ATPase domain and barrel-shaped proteolytic chamber. Members of this class are present in the matrix (e.g., CLPP and LONP1/Pim1) and IM (e.g., *m*-AAA and *i*-AAA, exposed to the matrix and IMS, respectively). ATP-independent proteases belong to various classes and are present in the matrix (e.g., MPP), IM (e.g., HTRA2, IMS processing peptidase (IMP), OMA1/Oma1, and PARL/Pcp1), and IMS (e.g., Atp23). Mitochondrial proteases are involved in the maturation of proteins and quality control of misfolded or damaged proteins.

3.1. Maturation of mitochondrial proteins

To acquire full functionality, many mitochondrial proteins require additional steps of maturation that can involve proteolytic processing, the introduction of prosthetic groups, and assembly into protein complexes [102,104]. The majority of mitochondrial proteins contain a cleavable presequence that needs to be removed from the mature protein by MPP upon arrival to the matrix. In some proteins, proteolysis by MPP exposes destabilizing amino acids at the N-terminus according to the "N-end rule", which in yeast are consecutively excised by such peptidases as Icp55 and Oct1 [105]. Similarly, proteins that are encoded by mtDNA undergo limited proteolysis on the N-terminus by aminopeptidase Map1 to remove methionine. The presequence is degraded to amino acids by the presequence peptidase Mop112.

The IMS has another class of proteins that require further proteolytic processing. These proteins are imported via the TIM23 pathway and are laterally sorted to the IM. To complete their maturation, they require excision of the transmembrane domain. Cleavage can be performed by different proteases, depending on the substrate protein. In yeast, the processing of cytochrome *b2* involves IMP, whereas other proteins (e.g., Ccp1 and Mgm1) are processed by rhomboid protease Pcp1. Some target proteins have a complex pattern of processing, such as mammalian Mgm1 orthologue OPA1 [106–108]. OPA1, similar to Mgm1, is engaged in the fusion of mitochondria, which is fine-tuned by the balance between long (membrane) and short (soluble) forms of OPA1. The processing of OPA1 is orchestrated by YME1L of the AAA+ family and the metalloprotease OMA1, which differentially target various splicing isoforms of OPA1. Interestingly, both proteases are engaged in the complex processing of OPA1 and undergo reciprocal control to adequately tune the expression of different forms of OPA1 to the energetic state of the cell [109].

Several known mitochondrial proteins require processing before they can be assembled with destined complexes. In yeast the Mgr2 subunit of TIM23 translocase is targeted to mitochondria by a non-canonical C-terminal sequence and integrated to the IM by two transmembrane domains [110]. The proper assembly of TIM23 translocase requires excision of the C-terminal domain by IMP. Another example is yeast mitochondrial ribosome subunit bL32m (previously known as MrpL32 [111]), which is targeted to mitochondria by the N-terminal sequence excised the *m*-AAA protease upon import to the matrix [112]. The proteolytic maturation of MrpL32 is required for its proper integration into the mitochondrial ribosome.

3.2. Quality control of mitochondrial proteins

Protein turnover in mitochondria requires quality control systems to degrade damaged or misfolded proteins. In contrast to the cytosol, mitochondria lack an autonomous system of conjugation-based protein degradation, such as the ubiquitin-proteasome system (UPS), although recent studies have reported the presence of ubiquitinated species in the mitochondrial matrix [113]. Misfolded proteins or proteins that fail to assemble into the destined complexes are recognized by AAA+ proteases. For example, pairs of small Tim chaperones form hetero-oligomeric complexes that shepherd hydrophobic precursor proteins on their way through the IMS. When the folding of one of the Tim chaperones is impaired, Yme1 protease (i.e., a yeast orthologue of human *i*-AAA protease) degrades both partner proteins [114]. A similar mechanism regulates the turnover of Ups1 and Ups2 proteins that are responsible for the biogenesis of mitochondrial phospholipids [115]. In the matrix, LONP1/Pim1 and *m*-AAA proteases have been implicated in the clearance of misfolded, unassembled, and oxidatively damaged proteins [116–118].

The proteasome degrades some mitochondrial proteins although mitochondria do not contain the UPS. Since the large majority of mitochondrial proteins are produced in the cytosol, they can be subject to proteasomal degradation. A fraction of newly synthesized MIA pathway-dependent precursors is degraded in the cytosol before they reach the IMS [119]. Similarly, defects in import trigger the accumulation of precursors of the presequence import pathway in the cytosol [120]. Activity of the UPS toward mitochondrial proteins is not limited to degradation prior to their import. The turnover of some integral proteins of the OM involves the UPS in a process that is known as mitochondria-associated degradation (MAD) [121,122]. The degradation of membrane proteins by the UPS requires their extraction from the OM, which is effectuated by a member of AAA+ family - Cdc48 in yeast and p97/VCP in higher eukaryotes [123–125]. Cdc48/p97/VCP also participates in the pathway of degradation of ER proteins, namely the extraction of proteins from the ER lumen for degradation (ERAD) pathway. Specificity toward mitochondrial proteins is controlled by interacting partners of ATPase, which recognize ubiquitinated membrane proteins [126]. Interestingly, the UPS is also involved in the degradation of IMS proteins, which retrotranslocate to the cytosol. In yeast, metabolic adaptation to fermentative growth conditions involves the reduction and unfolding of small soluble proteins of the IMS. These proteins are released to the cytosol through TOM and are degraded by the proteasome [127].

4. Mitochondrial protein biogenesis in retrograde signaling

Mitochondrial fitness requires efficient coordination between nuclear and mitochondrial genomes to avoid the accumulation of unassembled precursor proteins and consequently proteotoxic stress. In the course of evolution, several mechanisms developed that employ the biogenesis and transport of mitochondrial proteins to propagate information about mitochondrial fitness and proteostasis to the cytoplasm

and nucleus. In this chapter, we discuss examples of retrograde signaling from mitochondria that is involved in the preservation of functional mitochondria.

4.1. Signaling proteotoxic stress in mitochondria: UPRmt

Mitochondria are subject to the constant flux of precursor proteins that are encoded by nuclear DNA that need to reach mitochondria and mature to properly sustain numerous mitochondrial functions. The accumulation of misfolded or damaged proteins in mitochondria poses a threat to mitochondrial proteostasis. The perturbation of protein biogenesis in mitochondria triggers a broad transcriptional program in the nucleus, known as the mitochondrial unfolded protein response (UPRmt), which was originally identified in mammalian cells and is conserved in nematodes, flies, and mammals [128–130] (Fig. 2). Among proteins that are upregulated during the UPRmt are multiple mitochondrial chaperone proteins that are responsible for protein synthesis and degradation and counteract the deleterious effects of mitochondrial proteotoxic stress [131,132]. The machinery that is involved in the UPRmt has been studied intensively in nematodes (Fig. 2). In these animals, the UPRmt requires the activity of two mitochondrial proteins: the matrix protease CLPP-1 and the ABC transporter of IM HAF-1 [133,134]. In isolated mitochondria, the degradation of matrix proteins by CLPP-1 leads to the accumulation of peptides that are released from mitochondria by HAF-1. Both the protease and the transporter are required for activation of the UPRmt. Consequently, downstream events are triggered by peptides outside mitochondria, but the release of peptides has only been examined *in organello*. Moreover, unclear are which peptides are critical for activating downstream factors of the UPRmt and whether there is any selectivity in this process. Nematodes have at least two systems that receive information from mitochondria and control transcription in the nucleus [102]. ATFS-1 is a transcription factor that presents a bimodal distribution in the cell. It possesses two targeting sequences: the presequence that targets it to mitochondria at the N-terminus and a nuclear localization signal at the C-terminus [131]. Under basal conditions, the import to mitochondria prevails, and ATFS-1 is rapidly degraded in the mitochondrial matrix by the Lon protease. During mitochondrial stress, however, it translocates to the nucleus where it binds to promoters of the regulated genes [135]. The mechanism that controls the differential localization of ATFS-1 is not fully understood. One proposal is that it depends on a general decrease in mitochondrial import that is induced by peptides that are released by HAF-1 [131]. This view is supported by the fact that induction of the UPRmt by inhibiting mitochondrial import does not require the presence of HAF-1 [136]. In the nucleus, ATFS-1 controls the expression of multiple genes by both direct binding to regulatory elements in the promoters and indirect interactions. Interestingly, ATFS-1 controls broad adaptation of the cell to mitochondrial stress, in which it binds not only to promoters of genes that are directly involved in the control of proteotoxic stress in mitochondria but also enzymes of metabolic pathways, such as glycolysis and TCA. Transcriptomic analysis suggests that ATFS-1 promotes glycolysis and represses the expression of TCA enzymes and oxidative phosphorylation machinery (OXPHOS) proteins [135].

A second branch of the UPRmt involves DVE-1, UBL-5, and LIN-65, which reside in the cytosol under basal conditions but can accumulate in the nucleus during mitochondrial stress [133,137,138]. Similar to ATFS-1, the exact mechanism of signaling between mitochondria and these factors is unknown, but genetic interactions place DVE-1 downstream from CLPP-1. UBL-5 is considered to participate in the same pathway because it coexists in a common complex with DVE-1. LIN-65 is required for the nuclear localization of DVE-1, but the nuclear localization of LIN-65 itself occurs partially independently from CLPP-1 activity. Recent studies show that DVE-1 and LIN-65 participate in the reorganization of chromatin in the nucleus [138,139]. The execution of the transcriptional response in the UPRmt requires specific rearrangements in methylation patterns of histones, which lead to general condensation of the chromatin with separated low-density loci. DVE-1 colocalizes with low-density chromatin in a LIN-65-dependent manner. The human orthologue of DVE-1, SATB2, has been implicated in the structural organization of active chromatin [140,141]. The UPRmt has been proposed to trigger the rewiring of chromatin, characterized by the focal stabilization of active chromatin that is controlled by DVE-1. The epigenetic pathway, therefore, may prepare a structural framework for the transcriptional factor ATFS-1, which regulates the activity of downstream genes. This interpretation is supported by the fact that ATFS-1 depletion does not inhibit the translocation of LIN-65 to the nucleus.

Similar to nematodes, mammalian cells also activate specific transcriptional programs in response to mitochondrial stress, including the

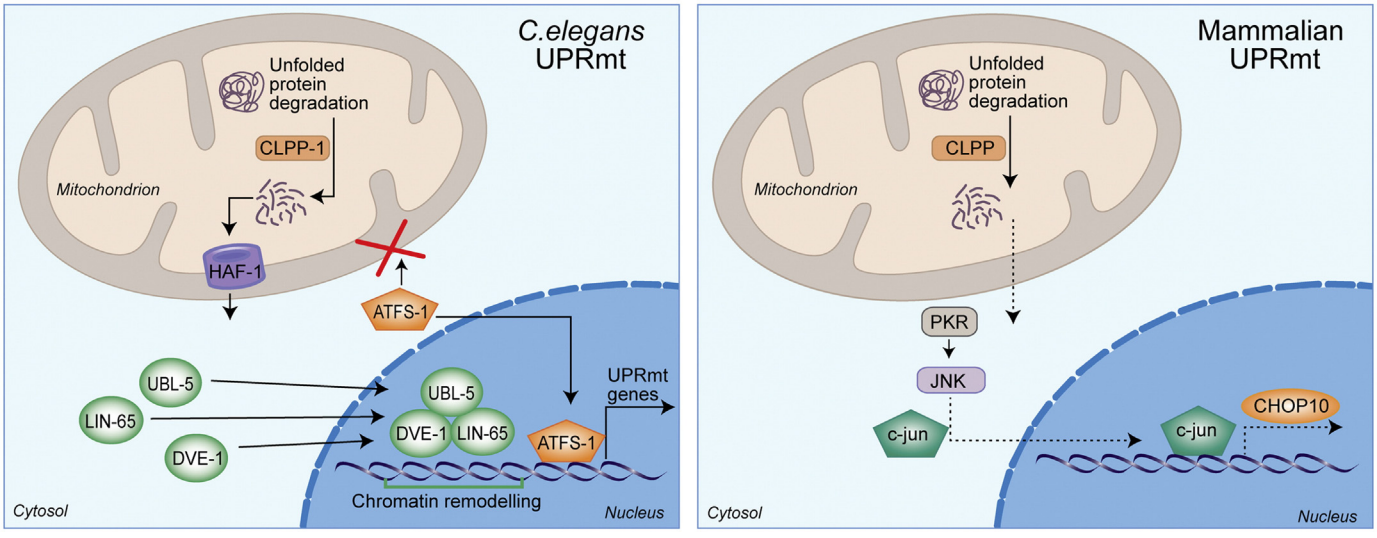

Fig. 2. The mitochondrial unfolded protein response (UPRmt). UPRmt in *C. elegans*: Unfolded proteins in the mitochondrial matrix are processed by the mitochondrial CLPP-1 protease and released by the HAF-1 transporter in the IM. The activities of protease and transporter are needed for the activation of UPRmt. UPRmt involves action of ATFS-1 which is normally imported into mitochondria and degraded. However, during mitochondrial stress, when the import efficiency is reduced, ATFS-1 accumulates in the cytosol and translocases to the nucleus where it promotes the transcription of mitochondrial proteins. Additional mechanism of UPRmt involves cytosolic DVE-1, UBL-5 and LIN-65 which translocate to the nucleus during mitochondrial stress. These proteins are involved in an epigenetic pathway of chromatin remodeling. UPRmt in mammals: Unfolded proteins inside mitochondria are processed by the mitochondrial CLPP peptidase and released via unknown pathway to the cytosol. Additional UPRmt mechanisms may involve PRK and JNK kinases that phosphorylate c-Jun resulting in its translocation to the nucleus and activation of CHOP10, transcription factor that participates in transcription of mitochondrial proteins.

greater expression of mitochondrial chaperones and proteases [142] (Fig. 2). Moreover, the involvement of mitochondrial proteases in communicating mitochondrial stress appears to be conserved, in which the activity of matrix protease CLPP is required for the induction of the UPRmt in some mammalian models [132]. Nevertheless, our understanding of the mammalian UPRmt is still elusive. For example, the involvement of CLPP has been recently questioned in DARS2-defficient mice, in which the expression of typical UPRmt markers occurred independently from the presence of the protease [143]. Moreover, unclear is the way in which mitochondria propagate information about such stress. Some light is being shed by the types of experimental manipulations that have been proven to successfully induce the UPRmt, including protein aggregation in the matrix, the abolition of mitochondrial translation, and the impairment of protein import [132,144,145]. These triggers should induce the accumulation of misfolded proteins in the matrix either directly or indirectly by introducing an imbalance between proteins that are encoded by nuclear and mitochondrial genomes, which could be degraded by CLPP to produce signaling peptides. Recently the transcription factor ATF5 was postulated to fulfill a similar role to ATFS-1 in mammals as it displays a dual localization to mitochondria and the nucleus and it regulates expression of several UPRmt-induced genes [146]. Alternatively, transmission of stress signals to the nucleus could depend on PKR and JNK kinases [147,148]. PKR is a kinase that participates in the antiviral innate response, but currently unknown is the way in which it is activated by mitochondrial stress. Interestingly, the antiviral innate response requires a signalosome that is orchestrated by the OM protein MAVS [149]. Both proteins have been implicated in common signaling pathways. Therefore, a tempting speculation is that MAVS may link PKR to intra-mitochondrial events. In this context, it is intriguing that mtDNA stress that is elicited by TFAM depletion potentiates the innate antiviral response [150].

The mechanisms that are responsible for activation of the transcriptional program in the nucleus are far less understood in mammals than in nematodes. PKR has been suggested to phosphorylate JNK2, which in turn activates c-Jun, a subunit of AP-1 transcription factor [147,148]. The activation of AP-1 is a first step in the transcriptional activation of UPRmt genes because it induces the expression of a secondary transcription factor, CHOP10. CHOP10-responsive elements are localized to multiple genes that are involved in the UPRmt, such as HSP60 or CLPP although it must be stressed these elements are not exclusive to UPRmt induced genes and other factors may be required to specifically activate this subset of genes [144,151]. Mammalian mitochondria appear to also possess alternative pathways of stress communication to the nucleus that are CHOP10-independent and require ROS that are produced by dysfunctional mitochondria [152–154].

Apart from the nuclear transcriptional program, mitochondrial stress also activates autonomous mitochondrial responses to restore proteostasis [155]. These authors used chemical inhibition of mitochondrial chaperone TRAP1 and protease LONP1 to evaluate the global response of the transcriptome and proteome to acute mitochondrial proteotoxic stress. In addition to the well-established activation of mitochondrial chaperones, they observed a decrease in the expression of MRPP3, a nuclease that is involved in the processing of pre-RNA in mitochondria. Accordingly, pre-RNA processing and mitochondrial translation were attenuated following TRAP1 inhibition. The inhibition of mitochondrial translation can serve as a protective mechanism to restore the balance between the mitochondrial proteome of nuclear and mitochondrial origin. Similarly, in nematodes, the accumulation of mtDNA-encoded transcripts during mitochondrial stress is prevented by a direct association between ATFS-1 and mtDNA [135].

The UPRmt has a prominent influence on the entire organism. One of the most notable effects of mitochondrial stress is its influence on aging in *Caenorhabditis elegans*. The induction of mitochondrial stress in the early stage of nematode development increases the lifespan of the adult animal [156]. The persistent characteristic of this phenomenon suggests a sustained adaptive response of the organism. This view is corroborated by a recent discovery that nuclear mechanisms of the UPRmt involve the epigenetic orchestration of chromatin [138,139]. A similar phenotype of an increased longevity can be also induced by activation of mitophagy [157]. However, activation of the UPRmt can oppose mitophagy and by these means can promote the propagation of deleterious copies of mtDNA [158].

4.2. Signaling mislocalization of mitochondrial proteins: UPRam

Recently, another aspect of mitochondrial signaling has been recognized in yeast, which in contrast to UPRmt regulates proteostasis in the cytoplasm [120,130,159]. Defective protein import to mitochondria leads to cellular dysfunction and eventually cell death, demonstrated by the fact that multiple subunits of mitochondrial translocases are essential for life. Surprisingly, mild impairment in the same mitochondrial import evokes a protective program referred to as unfolded protein response activated by mistargeting of proteins (UPRam), which involves activation of the proteasome [120] (Fig. 3). An increase in mitochondrial precursor protein expression in the cytoplasm promotes the assembly of the proteasome through Irc25 and Poc4, components of the proteasome assembly chaperone complex (Fig. 3). UPRam can be induced by either a general impairment of mitochondrial import via the MIA and TIM23 pathways or the overexpression of mitochondrial precursor proteins. The spectrum of proteasome activators appears to be rather broad, in which precursors of matrix, IM, and IMS proteins are capable of increasing proteasomal activity even if they lack sequences that target them to mitochondria. Regardless of the mechanism that triggers the accumulation of precursor proteins in the cytoplasm, the propensity of mislocalized mitochondrial proteins to stimulate proteasomal activation raises the possibility of activating the protective program of UPRam in the absence of general dysfunction of mitochondria. Although the mechanistic aspects of this signaling pathway are still unclear, a tempting speculation is that UPRam may be triggered not only by proteins that fail to be imported but also by peptides and proteins that escape from mitochondria. This is particularly intriguing in context of soluble proteins of the IMS that retrotranslocate to the cytosol due to reduction and metabolic rewiring of mitochondria in yeast [127].

The presence of UPRam in higher eukaryotes has not been verified, but proteasomal activation appears to be involved in the response to some types of mitochondrial proteotoxic stresses in mammalian cells [152]. Moreover, some responses to mitochondrial proteotoxic stress can occur independently from the UPRmt. For example, HAF-1 in

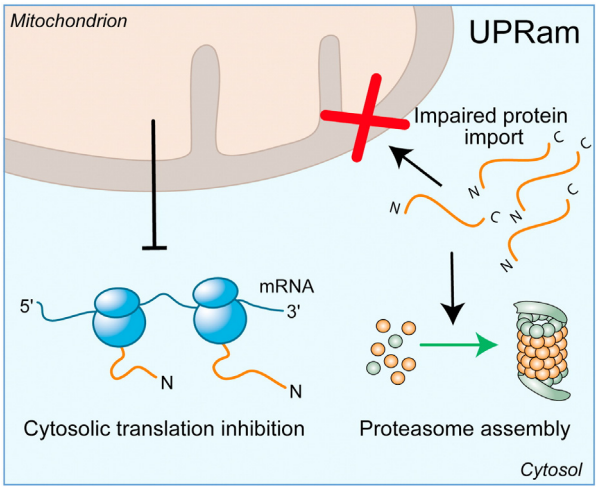

Fig. 3. Consequences of defective protein import into mitochondria. Increased levels of mistargeted precursor proteins in the cytosol trigger unfolded protein response activated by mistargeting of proteins (UPRam) resulting in more efficient proteasomal assembly and increased protein degradation. Mitochondria defective in protein import signal to cytosolic translation causing its pausing.

C. elegans is not required for chaperone expression that is induced by impairing TIM23-dependent mitochondrial import [131,136].

Defects in mitochondria are accompanied by the inhibition of cytosolic translation [120,130,159] (Fig. 3). In contrast to proteasomal activation, a decrease in translation occurs independently from the accumulation of mitochondrial precursor proteins in the cytoplasm and likely requires another yet unknown pathway of stress signaling from mitochondria. The attenuation of translation is elicited by the stabilization of Nog2, which prevents export of the 60S ribosome subunit from the nucleus, resulting in a lower number of active 80S ribosomes and polysomes. Together with an increase in proteasomal activity, this mechanism diminishes protein load in mitochondria and promotes proteostasis.

The attenuation of translation is beneficial and it may even extend the life span of organisms [160]. For example, a reduction of cytosolic translation increases longevity in nematodes and preserves mitochondria from degeneration [160–162]. A general attenuation of translation is accompanied by the selective translation of specific mRNAs that are involved in the stress response [163]. Such a mechanism enables the cell to concentrate its efforts on proteins that are essential for supporting cell survival. A similar phenomenon, induction of a specific translational program, may accompany dysfunction of mitochondria.

4.3. Induction of mitophagy by Parkin/PINK1

Mitochondria-specific autophagy, also known as mitophagy, serves as a quality control mechanism that removes damaged mitochondria from the cell [164–166]. In higher eukaryotes, the key proteins that constitute mitochondrial quality control in mitophagy are the mitochondrial kinase PINK1 and cytosolic E3 ubiquitin ligase Parkin. Mutations in these genes lead to early-onset Parkinson's disease [167]. Under normal, steady-state conditions, PINK1 is almost undetectable in cells as it undergoes mitochondrial processing, which finally leads to degradation via the proteasome. The PINK1 precursor, via its N-terminal signal sequence, is targeted to the TOM complex and then to the TIM23 translocase where it is cleaved by MPP and PARL [168,169]. The N-terminally processed form of PINK1 does not accumulate in mitochondria and undergoes degradation in the cytosol by the proteasome [168, 170] (Fig. 4a). With no PINK1 present, Parkin remains inactive in the cytosol, and mitophagy is repressed in cells with functional mitochondria. However, when $\Delta\Psi$ is disrupted by mitochondria-depolarizing agents, OXPHOS inhibitors, or environmental stresses, PINK1 processing by PARL is blocked, causing the accumulation of uncleaved PINK1 at the OM [171,172]. This recruits Parkin and eventually leads to the elimination of dysfunctional mitochondria via mitophagy.

Interestingly, a small subunit of the TOM complex, TOMM7, has been shown to be essential for stabilizing PINK1 on the outer mitochondrial membrane following mitochondrial depolarization [173]. Significantly less endogenous PINK1 accumulates following mitochondrial depolarization in TOMM7 knockdown cells. Additionally, when $\Delta\Psi$ was dissipated, radiolabeled PINK1 that was imported *in vitro* into TOMM7 knockout mitochondria failed to associate with the TOM complex. At the same time, the import of proteins into the matrix was unaffected by TOMM7 knockdown. This indicates that rather than the generalized protein import the function of TOMM7 may be more specific to catch and trap PINK1 and possibly other proteins. The loss of $\Delta\Psi$ has been known as a common mechanism through which different mitochondrial stresses induce PINK1 accumulation. However, recently unfolded proteins that are present in the matrix as a result of LONP1 protease deletion have also been shown to stabilize PINK1 on energetically healthy mitochondria [174]. LONP1 protease is known to be involved in the degradation of misfolded proteins, but the mechanism by which this protein stabilizes PINK1 without depolarizing the IM is still unknown. The authors postulated that the accumulation of unfolded proteins in mitochondria can act as a physiological trigger of mitophagy.

PINK1 promotes Parkin E3 ligase activity and the subsequent recruitment to mitochondria through a dual regulatory mechanism: (*i*) it phosphorylates Parkin at Ser65 in the N-terminal ubiquitin-like (UBL) domain [175–178], and (*ii*) PINK1 phosphorylates conserved Ser65 in ubiquitin [179–181] (Fig. 4b). This dual phosphorylation of Parkin at Ser65 and ubiquitin at Ser65 yields maximal Parkin activity. Phosphoubiquitin has been shown to bind to Parkin to activate its ligase activity by releasing Parkin's autoinhibitory domain, which exposes the ubiquitin ligase active site and allows the ubiquitination of mitochondrial proteins [182,183]. Once Parkin is recruited to the mitochondria, it dramatically alters the ubiquitination status of the mitochondrial proteome, which appears to trigger the recruitment of autophagy-related proteins to

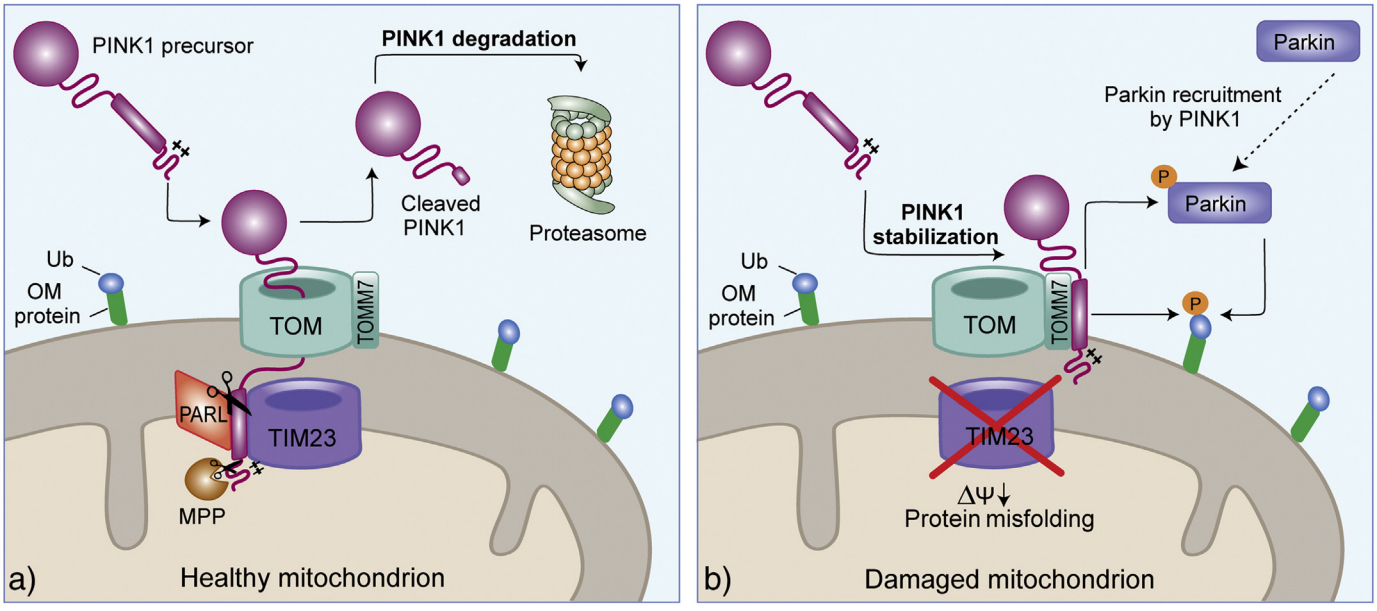

Fig. 4. PINK1/Parkin mediated mitophagy. a) In healthy mitochondrion full-length PINK1 is imported via TOM complex to the inner mitochondrial membrane (IM) where it is processed by the MPP and PARL proteases. This two-step processing generates a N-terminal cleaved form of PINK1 which is degraded by the proteasome. b) In depolarized mitochondria, the import to the IM is inhibited and PINK1 accumulates at the outer mitochondrial membrane (OM). TOMM7 is necessary for PINK1 stabilization after mitochondrial depolarization. PINK1 recruits and then phosphorylates both Parkin and ubiquitin (Ub) linked to OM proteins, providing and amplifying the signal for mitophagy.

mitochondria and activate autophagosome assembly [184–186] (Fig. 4b). A recent study by Richard Youle's group proposed a novel model in which PINK1 can induce mitophagy even in the absence of Parkin, which acts only to amplify the mitophagy signal [187]. In this model PINK1-generated phospho-ubiquitin plays a primary role in mitophagy by the recruitment of mitochondrial primary autophagy receptors (i.e., NDP52 and optineurin). More recently, optineurin has been shown to be phosphorylated by Tank-binding kinase 1 (TBK1), which then promotes ubiquitin chain binding and efficient PINK1-driven and Parkin-independent mitophagy [188,189].

Parkin and PINK1 are undoubtedly central proteins that are involved in mitophagy clearance, and recent genome-wide RNAi screens have also identified several other important PINK1 and Parkin regulators. Among these, sterol regulatory element binding protein 1 (SREBF1)-dependent lipid synthesis may be a key factor in PINK1 OM stabilization [190]. However, further work is needed to clarify the ways in which alterations in the lipidome alter PINK1 dynamics. Accumulating evidence suggests that the roles of PINK1 and Parkin in the cell are certainly not restricted to protein turnover during mitophagy. For example, PINK1 and Parkin may control mitochondrion quality in response to oxidative stress by modulating the biogenesis of mitochondrial-derived vesicles (MDVs) that bud off mitochondria and are targeted to the lysosomes [191]. Interestingly, PINK1 and Parkin have also been shown to play roles in selective cell death, in which damaged mitochondria are retained, independent of mitophagy [192].

5. Nuclear-mitochondrial dual localization of proteins

Some mitochondrial proteins may localize to other compartments of the cell. One of the best described examples of this phenomenon is yeast fumarase, which is present in the mitochondria, cytosol and nucleus, where it participates in DNA repair [193]. Similarly, in metazoans many enzymes that are involved in DNA maintenance, such as TIN2, TERT, RECQL4 and APE1, have bimodal mito-nuclear localization to support the stability of nuclear and mitochondrial genomes (discussed in [194]). The bimodal localization of other proteins serves as a signal to modify transcriptional programs in the nucleus. In this review, we focus on one recently described mitochondrial complex and three proteins that have been implicated in the control of transcription in the nucleus (Fig. 5).

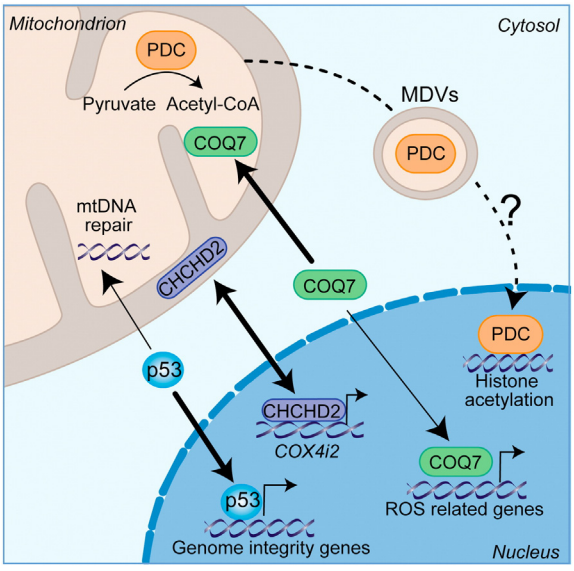

Fig. 5. Nuclear-mitochondrial dual localization of proteins and mitochondrial-derived peptides (MDPs). PDC (pyruvate dehydrogenase complex), COQ7 (Coenzyme Q biosynthesis protein 7) and CHCHD2 predominantly localize to mitochondria are also implicated in the control of the transcription in the nucleus. PDC in the mitochondrial matrix catalyzes transition of pyruvate to acetyl-CoA whereas in the nucleus participates in acetylation of histones. Potentially PDC may be delivered to the nucleus via vesicular pathway analogical to mitochondrial-derived vesicles (MDVs). COQ7 participates in the ubiquinone biosynthetic pathway in the mitochondrion however, a small pool translocates to the nucleus in response to the production of ROS by mitochondria. CHCHD2 in the mitochondria directly binds to the cytochrome *c* oxidase complex regulating its activity. Upon the transport to the nucleus which is stimulated by induction of apoptosis with UV or reduction of oxygen concentration CHCHD2 orchestrates expression of *COX4i2* gene. Tumor suppressor protein p53 in addition to nuclear functions localizes to mitochondria where participates in the repair of mtDNA.

5.1. Pyruvate dehydrogenase complex

The pyruvate dehydrogenase complex (PDC) is a metabolic mitochondrial complex that is localized to the matrix and catalyzes the transition of pyruvate to acetyl-CoA in the process of pyruvate decarboxylation (Fig. 5). Structurally, PDC is composed of six types of proteins with different enzymatic activities, forming a large multisubunit complex that reaches a mass of 9 MDa [195]. A recent report described the accumulation of PDC in the nucleus in the S-phase of the cell cycle where it participated in histone acetylation [196]. Intriguingly, the authors suggested synchronized translocation of the complex from the matrix to the nucleus. This was corroborated by the fact that PDC subunits that were found in the nucleus were devoid of presequences, suggesting that the proteins interacted with MPP prior to nuclear translocation. Moreover, the accumulation of PDC in the nucleus did not involve *de novo*-synthesized proteins, indicated by its resistance to the translation inhibitor cycloheximide. The possibility of the export of PDC from mitochondria is challenging because translocases that are located in the IM require unfolded substrates and transfer proteins unidirectionally. Alternatively, the PDC may leave mitochondria using a vesicular pathway that is analogous to MDVs [197]. In fact, MDVs can carry matrix material, and the presence of PDC subunits in MDVs was previously reported [191,198]. MDVs can segregate their cargo to peroxisomes or lysosomes, and this pathway has been recently implicated in the delivery of mitochondrial antigens to MHC Class I [199]. The assumption that an analogous pathway directs the PDC to the nucleus is highly speculative, although the involvement of endosomes in protein delivery to the nucleus has been recently described [200].

5.2. COQ7

COQ7 is a conserved enzyme of the ubiquinone synthesis pathway, which contains a cleavable presequence that targets it to the matrix [201]. It is conserved between nematodes and mammals, and it has been implicated in the control of the lifespan in these two groups [202,203]. The parallel nuclear localization of COQ7 and CLK-1 (i.e., a nematode orthologue) has been recently characterized [204] (Fig. 5). In this case, the mechanism of nuclear accumulation does not involve initial import to mitochondria because nuclear forms of COQ7 and CLK-1 are longer than their mitochondrial counterparts, suggesting the lack of MPP processing. The sequence, which targets mouse COQ7 to the nucleus, was identified upstream from the MPP cleavage site. Under basal conditions, nuclear and mitochondrial import may compete for the full-length protein, and the final balance depends on the effectiveness of both processes. Following oxidative stress, nuclear localization prevails, which can be a derivative of impairments in mitochondrial import pathways by ROS. In the nucleus, COQ7 binds to chromatin and changes the expression patterns of several genes that are related to mechanisms of defense against ROS (Fig. 5). Intriguingly, it has a negative effect on the expression of genes that participate in the UPRmt [204]. This effect could explain the increased longevity phenotype in worms and mice that lack CLK-1/COQ7 because the mechanisms of the UPRmt should be upregulated in this genetic background. The dependence of CLK-1/COQ7 localization on oxidative stress also suggests that it can participate in crosstalk between ROS and the UPRmt.

5.3. CHCHD2

CHCHD2 is a human orthologue of yeast Mix17 and is implicated in the regulation of oxidative phosphorylation and apoptosis [205,206]. Similar to its yeast orthologue, CHCHD2 contains a conserved twin Cx_9C motif and is imported by the MIA pathway, indicated by the blockade of its accumulation in mitochondria by the ALR inhibitor MitoBlock6 [52,207]. Moreover, CHCHD2 contains a predicted signal sequence, and rare variants of this sequence are associated with Parkinson's disease [208]. The function of CHCHD2 in mitochondria is connected to the cytochrome *c* oxidase complex through direct binding and regulating its activity. Intriguingly, CHCHD2 also displays nuclear activity as a transcription factor, regulating the expression of a tissue-specific isoform of COX4i2 that is prevalently expressed in the lungs [209] (Fig. 5). Together with two other transcription factors (i.e., RBPJ and CXXC5), it orchestrates the expression of *COX4i2* via interactions with a regulatory element in its promoter in response to oxygen concentration. The activity of the promoter and presence of CHCHD2 in the nucleus increase in response to reduction of oxygen concentration (from 20% to 4%). The translocation of CHCHD2 to the nucleus can also be stimulated by the induction of apoptosis by ultraviolet light, although its activity as a transcription factor in this case has not been verified [206]. A tempting speculation is that the concentration of oxygen, which is a final acceptor of electrons that are delivered by the MIA pathway, can control the distribution of CHCHD2 in the cell and consequently modify the expression of COX4i2 in specific tissues.

5.4. p53

p53 is a tumor suppressor, which acts as a transcription factor that is involved in the control of cell cycle and genome integrity and is one of the most frequent targets for mutations in cancer-related malignancies. One of the most prominent roles of p53 is induction of cell cycle arrest in response to various cellular stresses. Oxidative stress, UV irradiation and other noxious stimuli lead to accumulation of p53 in the nucleus and transcriptional induction of p21, which triggers cell cycle arrest via interactions with cyclins giving the cell an opportunity to repair DNA [210]. Unsuccessful DNA repair leads to the programmed cell death, in which p53 participates as an activator of OM permeabilization via its interactions with Bcl2 family proteins. A recent report demonstrated that p53 is present in the mitochondrial matrix where it supports mtDNA integrity [211,212]. Import of p53 is supported by the MIA pathway but the machinery, which drives translocation of p53 across the IM, is not known. In this respect mitochondrial import of p53 resembles biogenesis of APE1 in mitochondria, which also participates in DNA maintenance in the nucleus and mitochondria [68]. Interestingly, the transcriptional activity of p53 is controlled by MIA40, which limits its translocation to the nucleus upon induction of oxidative stress. Similar to CHCHD2 translocation, the MIA pathway may compete for p53 and thereby regulate the nuclear activity of the transcription factor (Fig. 5). Interestingly, the overexpression of MIA40 positively correlates with the severity of several tumors [213]. It is tempting to speculate that the MIA pathway may regulate the localization of p53 in cancer cells to abolish its control over the integrity of nuclear DNA.

6. Mitochondrial-derived peptides

An interesting trait of the UPRmt in *C. elegans* is the cell-non-autonomous characteristic of adaptations. Proteotoxic stress that is elicited in a single tissue in a worm can induce the UPRmt in another tissue and extend the lifespan of the organism as a whole [214]. This discovery led to the concept of a "mitokine" (i.e., an extracellular signal that can be delivered to different parts of the body to synchronize the response to stress). The mitokine theory has received support from recent experiments in mammals, which identified mitochondrial-derived peptides (MDPs). MDPs are peptides that are encoded in the mitochondrial genome by short open reading frames (ORFs) that are located in genes for 12S and 16S rRNA [215]. To date, several sequences of MDPs have been identified, including humanin, small humanin-like peptides, and the mitochondrial ORF within the 12S rRNA c (MOTS-c), which vary in length from 16 to 38 amino acids [215]. The main concern regarding the mitochondrial origin of MDPs is a process known as nuclear mitochondrial DNA transfer (NUMT). Over millions of years of co-evolution, NUMT resulted in the presence of MDP paralogs in the nuclear genome. In some cases, it is difficult to verify whether a certain peptide is translated from nuclear or mitochondrial mRNA or at the mitochondrial or cytosolic ribosomes. The location of MDP translation appears to be peptide-specific. For example, rat humanin is translated by mitochondrial ribosomes, whereas human MOTS-c undergoes translation in the cytosol [216,217]. The mechanisms that participate in the export of peptides and/or mRNA that encode MDPs are unknown. MDPs are relatively short, with an average mass in the range of 2000-3000 Da, which is slightly more than the size of peptides that are released from yeast mitochondria by the ABC transporter Mdl1 [116]. This suggests a specialized export pathway. Similarly, the release of RNA from mitochondria was also described, although the mechanisms that underlie this phenomenon are still unknown [218,219].

Regardless of the exact mechanism of MDP biogenesis, these peptides appear to have a profound effect on the cell and entire organism. For example, humanin exerts cytoprotective effects in numerous models of pathological conditions, both in cultured cells and in living animals [220]. MDPs can be released from the cell to the plasma and function as paracrine or endocrine factors. Interestingly, the plasma levels of humanin correlate with the physiological state of the organism and can decrease during aging of humans and mice.

A recent report by Lee at al. demonstrated a comprehensive approach to characterize the influence of MOTS-c on metabolic homeostasis at the cellular and organismal levels [216]. In cultured cells, MOTS-c inhibited the methionine-folate cycle, which led to the accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide and activation of adenosine monophosphate-activated protein kinase signaling. Consequently, glucose metabolism was shifted to glycolysis and the pentose phosphate pathway. These modifications had a prominent effect on glucose homeostasis at the organismal level. Repeated intraperitoneal administration of MOTS-c in mice resulted in an increase in insulin sensitivity in skeletal muscles, which prevented the development of the deleterious consequences of high-fat-diet intake, such as obesity and insulin resistance [216].

MDPs meet the criteria of a "mitokine" because their biogenesis is linked to mitochondria, and they have a paracrine and endocrine impact on the whole organism. MPDs could function as a feedback signal from actively transcribing mitochondria to synchronize metabolism at the systemic level.

7. Conclusions and perspectives

Mitochondria are vital component of the eukaryotic cell and their role in cellular signaling is well established. In this review we focus on the specific aspect of mitochondrial retrograde signaling, which involves the mechanisms of mitochondrial protein biogenesis. Our understanding of these processes improved substantially over the recent years. The spectrum of programs, which mitochondria can activate, ranges from organellar to global rearrangements of metabolism on the organismal level. This apparent complexity raises a question about hierarchy and coordination between various modes of mitochondrial signaling. For example, mitophagy and UPRam respond to such closely correlated triggers like invalid protein import and accumulation of mitochondrial precursors in the cytosol. Similarly, accumulation of unfolded proteins in the mitochondrial matrix activates UPRmt and mitophagy. It is intriguing to which extent these mechanisms overlap in terms of triggers and outputs, and how efficiently they can be manipulated. These questions may have long-awaited practical implications given a longevity phenotype of

animals, in which a mild mitochondrial dysfunction activates homeostatic mechanisms.

Another interesting question is the role of bimodal localization of mitochondrial proteins, which according to the estimates from yeast may concern a significant fraction of mitochondrial proteome [221]. Our understanding of these processes is still rudimental. Most likely, the translocation of these proteins involve non-canonical pathways. Future studies may reveal novel mechanisms, which allow facultative localization in or out of mitochondria.

Mitochondrial retrograde signaling may involve peptides encoded in the mtDNA. The concept of mitochondrialy encoded factors, which eventually act as endocrine regulators, is fascinating and opens a new field of possible regulatory mechanisms at the organismal level. However, it needs to be said that there is no mechanistic model to explain how transcripts or peptides leave mitochondria so far and without this critical step the mitochondrial origin of MOTS-c or humanin may be questioned.

We would like to conclude with a notion that mechanisms of mitochondrial biogenesis, once thoroughly characterized in yeast, do not stop to surprise and to bring thrilling developments, which hopefully will improve the understanding of ourselves.

Conflict of interest

The authors declare no conflict of interest or any commercial associations.

Transparency document

The Transparency Document associated with this article can be found, in online version.

Acknowledgements

Publication financed from the Ministerial funds for science within Ideas Plus program 000263 in 2014-2017 and from the funds of National Science Centre, Poland, within the projects 2015/18/A/NZ1/00025, 2015/19/B/NZ3/03272 and 2016/20/S/NZ1/00423.

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