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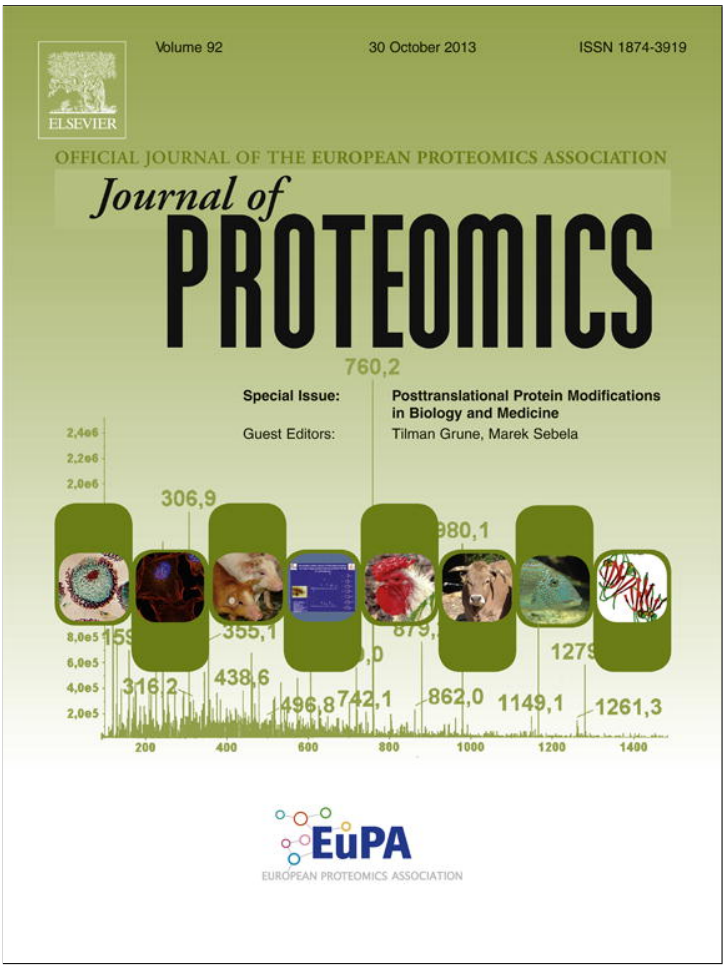

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Review

Oxidative stress and mitochondrial protein quality control in aging☆

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ABSTRACT

Mitochondrial protein quality control incorporates an elaborate network of chaperones and proteases that survey the organelle for misfolded or unfolded proteins and toxic aggregates. Repair of misfolded or aggregated protein and proteolytic removal of irreversibly damaged proteins are carried out by the mitochondrial protein quality control system. Initial maturation and folding of the nuclear or mitochondrial-encoded mitochondrial proteins are mediated by processing peptidases and chaperones that interact with the protein translocation machinery. Mitochondrial proteins are subjected to cumulative oxidative damage. Thus, impairment of quality control processes may cause mitochondrial dysfunction. Aging has been associated with a marked decline in the effectiveness of mitochondrial protein quality control. Here, we present an overview of the chaperones and proteases involved in the initial folding and maturation of new, incoming precursor molecules, and the subsequent repair and removal of oxidized aggregated proteins. In addition, we highlight the link between mitochondrial protein quality control mechanisms and the aging process.

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Contents

Abbreviations: mtPQC, mitochondrial protein quality control; TCA, tricarboxylic acid; mtDNA, mitochondrial DNA; TPR, tetratricopeptide repeat; Hsp, heat-shock protein; TOM, translocase of the outer membrane; TIM, translocase of the inner membrane; MTS, mitochondrial targeting signal; PAM, presequence translocase associated motor; IMS, intermembrane space; MPP, mitochondrial processing peptidase; IMP, inner membrane peptidase; AAA, ATPases Associated with various cellular activities; FtsH, Filament forming temperature sensitive; Clp, caseinolytic protease; RNAi, RNA interference; UPR^{mt}, mitochondrial unfolded protein response.

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1. Introduction

Mitochondria are key determinants of life and death in eukaryotic cells. They participate in, or directly execute, a number of essential cellular processes such as, ATP production through the TCA cycle and oxidative phosphorylation, β-oxidation of lipids, iron/sulfur cluster biogenesis, calcium buffering, apoptosis regulation, etc. All these functions are carried out by a limited number of proteins (~1500 in humans) that are, in their majority, encoded by nuclear genes. Mitochondrial proteins are targeted either post-translationally or co-translationally to the specific mitochondrial sub-compartment where they are destined. Mitochondria are also responsible for the generation of deleterious highly reactive free radicals. Lesions in mitochondrial or nuclear DNA, or the mitochondrial proteins themselves can cause pronounced mitochondrial dysfunction, which has been linked in several cases to aging and disease. To safeguard against such faults, cells evolved quality control mechanisms that continuously survey for dysfunctional mitochondrial components and ensure their repair or degradation and removal. The surveillance mechanisms that control the quality and functionality of mitochondria are crucial for the survival and integrity of the cell and the whole organism.

The first line of defense against mitochondrial dysfunction includes the mitochondrial chaperones and proteases. However, already in the cytosol, a nascent polypeptide chain destined for mitochondria needs to bind a cytosolic chaperone to remain in an unfolded import-competent conformation. Translocation across the outer and inner mitochondrial membranes is mediated by conserved protein complexes that function in close collaboration with mitochondrial chaperones [1]. After they have reached their destination mitochondrial precursors usually undergo proteolytic maturation catalyzed by soluble and membrane-bound processing peptidases. Final folding and assembly in the native functional form are facilitated by highly conserved chaperone systems. Given that mitochondria are oxidatively challenged, mitochondrial proteins are commonly subjected to oxidative damage. Chaperones and proteases involved in the repair and/or removal of damaged proteins are part of the mitochondrial protein quality control mechanisms (mtPQC) [2].

When the load of protein damage exceeds the capacity of the above protein quality control systems, other organelle quality control mechanisms may intervene to overcome the burden of accumulating misfolded or misassembled proteins. Mitochondrial fusion and fission are such processes that facilitate the mixing and redistribution of mitochondrial components, allowing functional repair mechanisms of one organelle to act on damaged components of another. However, when damage accumulates beyond the threshold capacity of these mechanisms, the damaged organelle is flagged for destruction. It is first cut off and isolated from the rest of the mitochondrial network and then removed through a specific autophagic process called mitophagy. Mitochondrial dynamics and mitophagy are important mechanisms of organelle quality control that act in concert with the protein translocation and protein quality control machineries to regulate proteostasis and mitochondrial function [3]. Disruption of these processes leads to mitochondrial stress, which has been closely associated with the aging process [4]. In this review we focus on the network of chaperones and proteases that facilitate mitochondrial protein maturation, folding and quality control, highlighting their involvement in combating oxidative stress and aging.

2. Mitochondrial protein import and maturation

The vast majority of mitochondrial proteins are imported into mitochondria in an unfolded conformation, through conserved membrane-bound translocases that reside on the outer and inner mitochondrial membranes. The preproteins destined to mitochondria are translated by either free cytosolic or mitochondria-associated ribosomes. The transport to the final destination as well as the final folding and assembly into the functional form are mediated by a pool of chaperones and proteolytic enzymes (Tables 1 and 2).

2.1. *Chaperones*

Precursors translated by free ribosomes travel through the aqueous cytosol bound to the cytosolic chaperones Hsp70 and Hsp90 [5]. These chaperones shield the hydrophobic segments of the polypeptides, to keep them in an unfolding conformation and protect them from aggregation. Cytosolic chaperones bind to tetratricopeptide (TPR) motifs of the mitochondrial surface receptors and this interaction prepares the receptors for the subsequent binding of their cargo [5,6]. In yeast and mammals the mitochondrial outer membrane receptors are subunits of the Translocase of the Outer Membrane (TOM) complex, namely Tom20, Tom22 and Tom70 [1,7]. Precursors with N-terminal targeting sequences are preferentially bound by the cytosolic Hsp70 and delivered to Tom20 and Tom22 [8,9]. Mammalian Hsp90, together with Hsp70,delivers precursors to the Tom70 receptor, which recognizes internal targeting signals in membrane-targeted precursors [5].

About half of mRNAs encoding for mitochondrial proteins are enriched in the vicinity of mitochondria. The peptides produced are translocated co-translationally [10]. In mammals, ribosomes bind to mitochondria via protease-sensitive components on the mitochondrial surface [11]. Factors that control the preferential, asymmetric localization of mRNAs close to mitochondria include; i) the translational machinery, ii) the mitochondrial targeting signal (MTS) located in the N terminus of newly synthesized polypeptides, as well as two elements in their coding region, iii) the outer membrane receptor Tom20

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Table 1 – Mitochondrial chaperones and chaperone-like proteins. Co-chaperones are separated from their cognate chaperones by a slash. Subunits of protein complexes are separated by a dash. The sub-mitochondrial localization and function of each protein is indicated.

Saccharomyces cerevisiae	*Homo sapiens*	Localization	Function
Ssc1/Pam18	HSPA9/DNAJC19	Matrix	Protein import and folding
Ssc1/Mdj1	HSPA9/TID1		
Ssq1/Jac1	-/DNAJC20	Matrix	Assembly of iron/sulfur clusters into proteins
Hsp60/Hsp10	HSPD1/HSPE1	Matrix	Folding and assembly of newly imported mitochondrial proteins
Hsp78	-	Matrix	Disaggregation activity
Mcx1	CLPX	Matrix	Unknown
Tim9-Tim10	TIMM9-TIMM10	Intermembrane space	Chaperoning of unfolded hydrophobic precursors through the intermembrane space
Tim8-Tim13	TIMM8A-TIMM13	Intermembrane space	Chaperoning of unfolded hydrophobic precursors through the intermembrane space
Mia40	CHCHD4	Intermembrane space	Oxidative protein folding of cysteine-rich substrates
Ccs1	CCS	Intermembrane space	Copper chaperone of SOD1
Cox11	COX11	Inner membrane	Copper delivery to CcO
Sco1	SCO1	Inner membrane	Copper delivery to Cco
–	SCO2	Inner membrane	Copper delivery to Cco
Cox17	COX17	Intermembrane space	Copper delivery to Sco1 and Cox11
Cox19	COX19	Intermembrane space	Unknown
Cox23	COX23	Intermembrane space	Unknown
Pet191	COA5	Inner membrane	Unknown

and iv) the RNA-binding protein Puf3 [12–15]. Mitochondria-destined proteins translated in the vicinity of the organelle are further divided into two groups, those that depend on the mitochondrial RNA-binding protein Puf3p for their import and those that do not [14,15]. It has been suggested that asymmetric localization of mRNA is a mechanism that allows cells to respond promptly to environmental cues by adapting the rates of mitochondrial protein import and biogenesis [16]. In addition, it ensures that precursor peptides do not become misfolded or aggregated, by facilitating their immediate delivery to mitochondria upon translation.

Delivery of the polypeptides to the receptors of the TOM complex initiates their traversal through the outer membrane and their targeting to a specific mitochondrial sub compartment. After crossing the TOM40 channel in the outer mitochondrial membrane, import into the matrix is mediated by the Translocase of the Inner Membrane, TIM23, and is driven by inner membrane potential and the multisubunit import motor PAM. This process is performed in a tightly regulated manner that keeps precursors in an unfolded conformation and ensures their proper folding and maturation as soon as they reach their destination [1]. Translocation is achieved through a sequence of binding events between the unfolded preprotein and specific subunits of the translocases of the inner and outer mitochondrial membranes. The increasing affinity of these interactions is thought to promote the translocation of the precursor molecule inwards [1,17,18].

Each one of the two aqueous mitochondrial subcompartments (matrix and intermembrane space), possesses its own pool of chaperones to assist the folding of incoming precursor molecules. The major chaperone of the matrix is the mitochondrial Hsp70 (mtHsp70, Ssc1 in yeast), a member of the Hsp70 family of chaperones, and a homolog of the bacterial DnaK (Table 1). The human homolog of mtHsp70 is commonly referred to as mortalin or Glucose-Regulated Protein 75 (GRP75). mtHsp70 is the central chaperone of the import motor PAM (Presequence translocase Associated Motor). Apart from mtHsp70, PAM contains the nucleotide release factor for mtHsp70, Mge1 (Mitochondrial GrpE-like protein 1), and four membrane bound co-chaperones [19–21]. The J-domain protein co-chaperone Pam18, which stimulates the ATPase activity of mtHsp70 [22,23], Pam16, a J-related protein that forms a complex with Pam18 and regulates its activity [24,25], the adaptor protein Tim44 that tethers mtHsp70 together with the J complex to the import channel Tim23 [26], and Pam17 that regulates the TIM23-PAM interaction. Polypeptides that arrive in the matrix are trapped by the mtHsp70 while still in transit [27]. The exact function of mtHsp70 during translocation is not completely understood. It has been suggested that trapping of incoming polypeptides by the mtHsp70 chaperone prevents their backward movement into the channel, while a conformational change of the chaperone, in conjunction with ATP hydrolysis, actively pulls the substrates across the inner membrane into the matrix [26,28]. This movement likely unfolds the C-terminal part of the precursor, which is still in the outer membrane, allowing its linearization, which is prerequisite for translocation through the outer and inner membrane channels [29,30].

Precursors of transmembrane mitochondrial proteins are typically polypeptides with multiple hydrophobic segments. These polypeptides need to travel through the aqueous intermembrane space in an unfolded state, suitable for delivery to the respective translocases. To avoid misfolding and aggregation upon their exit from the TOM channel, they are captured by oligomeric chaperone complexes that reside in the IMS. Two such complexes have been identified; the Tim9-Tim10 complex and the Tim8-Tim13 complex. The subunits of these complexes belong to the small Tim family of proteins, conserved from yeast to humans. Each complex comprises three molecules of each protein partner. Tim9 and Tim10 are essential for the survival of yeast cells, while Tim8 and Tim13 are dispensable.

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Table 2 – Mitochondrial proteases involved in protein maturation and quality control. The sub-mitochondrial localization and function of each protein is indicated. ND: not determined.

Saccharomyces cerevisiae	*Homo sapiens*	Localization	Function
Pim1	LONP1	Matrix	Quality control
–	CLPP	Matrix	Quality control, degradation of regulatory proteins
MPP (Mas1–Mas2	MPP (α-MPP–β-MPP)	Matrix	Processing of presequences
Oct1 (MIP)	MIPEP	Matrix	Octapeptide removal of MPP-processed substrates
Icp55	ND	Matrix	N′ amino acid removal from MPP-processed substrates
Cym1 (Mop112)	PREP	Matrix	Peptide/presequence degradation
m-AAA (Yta10-Yta12)	AFG3L2-SPG7	Inner membrane, substrate released in matrix	Quality control, processing, autocleavage
i-AAA (Yme1)	YME1L1	Inner membrane, substrate released in intermembrane space	Quality control
Pcp1	PARL	Inner membrane, intra-membrane cleavage	Processing
IMP (Imp1-Imp2)	IMMP1L-IMMP2L	Inner membrane, substrate released in intermembrane space	Processing of presequences
Oma1	OMA1	Inner membrane, substrate released in both sides of the membrane	Processing
ND	HtrA2 (Omi)	Intermembrane space	Quality control?
Atp23	XRCC6BP1	Intermembrane space	Processing and assembly of Atp6
Prd1	Neurolysin	Intermembrane space	Peptide degradation

The Tim9–Tim10 complex serves as a chaperone for the hydrophobic precursors of metabolite carriers and other inner membrane proteins as well as precursors of β-barrel proteins of the outer membrane [31–34]. The Tim8–Tim13 complex has a function similar to Tim9–Tim10 but different substrates [35,36]. All members of the small Tim family of proteins contain conserved twin cysteine motifs and form intramolecular disulfide bonds that drive their oxidative folding in vivo [32]. The mitochondrial intermembrane space possesses a catalytic machinery specific for the introduction of disulfides in such precursors. The central oxidoreductase of this machinery is Mia40 [37]. Except for the small Tim family of proteins numerous other substrates of this disulfide relay system have been identified so far. Recently it was shown that Mia40 also functions as a molecular chaperone, assisting α-helical folding of the internal targeting signal of its substrates and preventing their aggregation [38,39].

2.2. *Mitochondrial processing peptidases*

Mitochondria-targeted precursors carry sequences that serve as localization signals to each of the four mitochondrial subcompartments. These targeting signals can be classified into three different groups. The cleavable signal sequences that are located at the N terminal part of the precursor molecule and are often referred to as presequences, the non-cleavable internal signals, that are scattered in different parts of the precursor, and the signals that contain cysteine motifs [1]. Precursors that bear the cleavable presequences are typically targeted to the mitochondrial matrix, the inner membrane or the intermembrane space, and their translocation involves the translocases TOM and TIM23 of the outer and inner membrane respectively [40]. The non-mature form of the precursors targeted to the inner membrane or intermembrane space usually contains a hydrophobic sorting signal after the N terminal presequence that guides lateral sorting into the inner membrane. After incorporation of the preprotein into the membrane, one or two cleavage steps produce the mature form of the protein, which is either transmembrane or released into the intermembrane space, respectively [41,42]. These maturation steps are a prerequisite for the proper folding and stabilization of the protein, required for its function [43,44].

Several mitochondrial peptidases have been identified so far (Fig. 1; Table 2) [45]. Most presequences are removed in one cleavage event by the dimeric Mitochondrial Processing Peptidase (MPP), located in the mitochondrial matrix [46]. MPP is a heterodimeric zinc-metallopeptidase that consists of two essential and highly conserved subunits, α-MPP and β-MPP. The α-MPP subunit recognizes and binds the presequence, while subunit β-MPP is responsible the catalytic activity of the complex. In plants, MPP is membrane-bound and incorporated in the complex III of the respiratory chain, which exerts a dual function, presequence processing and electron transfer [47,48]. The general ATP-dependent protease m-AAA (see below) can also perform the first processing step on the incoming precursor of cytochrome c peroxidase (Ccp1) in yeast [41].

As noted above, many precursors need a second processing step to become fully mature. In some cases MPP carries out this sequential cleavage step to conclude maturation [49–51]. However, typically this second cleavage is the task of other processing peptidases that are either soluble or membrane-bound. These peptidases release their products in the matrix or the intermembrane space. The inner membrane peptidase (IMP) is an integral inner membrane hetero-oligomer that comprises two catalytic components of non-overlapping specificities, Imp1 and Imp2, and one non-catalytic component, Som1 [52–54]. The catalytic subunits of IMP face the intermembrane space, where their proteolytic products are released [42,55,56]. The octapeptidylaminopeptidase 1 (Oct1) is a soluble matrix metalloprotease, initially named MIP (Mitochondrial Intermediate Peptidase) [57]. Unlike IMP, which cleaves presequences independently of MPP, Oct1 functions only on MPP-processed substrates. Oct1 removes an octapeptide from the N-terminus of already processed precursors. Initially it was suggested that the

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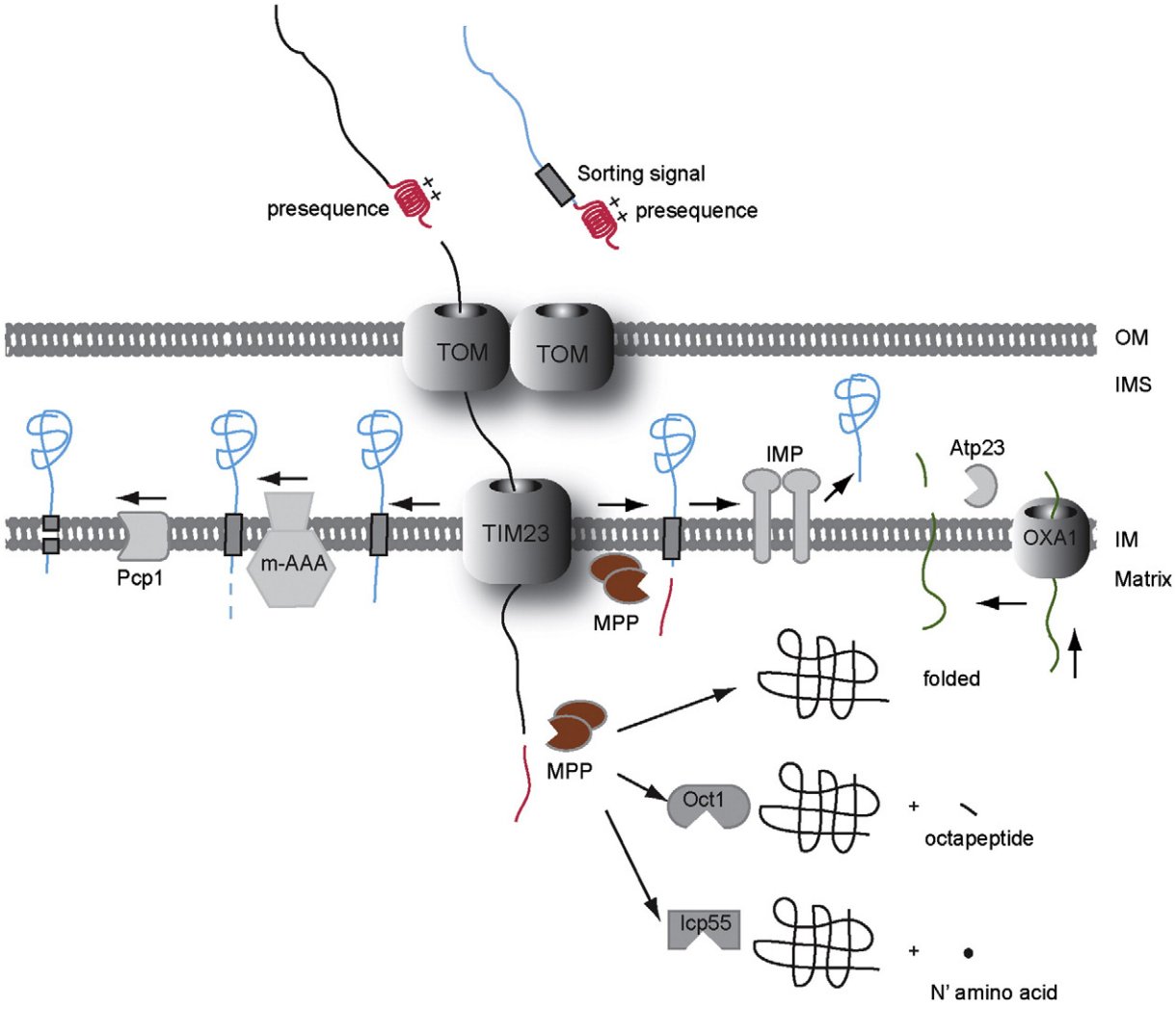

Fig. 1 – Processing peptidases. Mitochondrial precursors with cleavable N′ terminal presequences are imported in the matrix via the TOM and TIM23 translocases. While still in transit the N′ terminal presequence is cleaved by MPP. In several cases a second cleavage event takes place, which is mediated by peptidases such as IMP, MIP/Oct1 and Icp55. Precursors with bipartite signal peptides enter the organelle via translocases TOM and TIM23. When their hydrophobic sorting signal reaches the inner membrane, vectorial translocation stops and the protein is laterally sorted in the inner membrane. The N′ terminal presequence is cleaved by MPP. A second cleavage step by IMP in the intermembrane space releases the mature protein. An alternative processing pathway involves the m-AAA and Pcp1 proteases (for example in the case of cytochrome c peroxidase). Finally, mitochondrially encoded proteins are introduced in the inner membrane via Oxa1 (cytochrome oxidase activity 1) translocase. In the case of Atp6, maturation is achieved through processing by the Atp23 peptidase at the intermembrane space. The *Saccharomyces cerevisiae* nomenclature is used for the proteins shown here.

role of Oct1 is to remove the MPP cleavage site [58]. Recently, a systematic study of the sequences of all the known substrates of Oct1 revealed that they carry N-terminal destabilizing amino acids, according to the N-end rule of protein degradation [59]. Processing by Oct1 converts the destabilizing N-termini of preproteins into stable N-termini of mature proteins, thus, acting as a quality control mechanism for MPP-processed precursors [43]. Pcp1/PARL (Processing of cytochrome c peroxidase-Presenilin-associated rhomboid-like protease) is an integral inner membrane serine protease which belongs to the rhomboid-GlpG superfamily. Pcp1 is necessary for the second processing step in Ccp1 maturation, after the first cleavage by the m-AAA protease complex [41]. Moreover, Pcp1 is involved in the proteolytic maturation process of Mgm1/OPA1, an integral inner membrane protein facing the intermembrane space, required for mitochondrial fusion [60]. Therefore, Pcp1 is important for mitochondrial morphology and genome maintenance [61,62].

Atp23 is a conserved serine metallopeptidase of the intermembrane space with a dual function; it possesses a processing peptidase activity specifically for its substrate Atp6, and substrate-specific chaperone activity that promotes the assembly of Atp6 with the F1-Atp9 oligomeric subcomplex. Atp23was found to genetically interact with prohibitins [63]. Recently, a novel matrix aminopeptidase (Icp55; intermediate cleaving peptidase), involved in precursor processing has been identified [44]. Icp55 is peripherally attached to the inner membrane and cleaves single phenylalanine, tyrosine or leucine residues at the N-terminus of MPP-processed precursors [44].

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Nsf1 is the only known substrate, with Icp55 removing three amino acids from its N-terminus [64]. Similar to Oct1, the proposed role of Icp55 is the stabilization of the mitochondrial proteome.

Cleavage releases the polypeptide and presequence in an extended conformation. Accumulation of cleaved presequences may interfere with the inner membrane potential and inhibit the catalytic activity of MPP. Cleaved presequences, as well as protein degradation-derived peptides are extruded from the matrix into the intermembrane space and then diffused to the cytosol [65]. Peptide export is mediated by the mitochondrial ABC transporter Mdl1 (TAP in mammals, HAF-1 in *Caenorhabditis elegans*). Proteolytic breakdown of presequences or other small unfolded peptides is undertaken by oligopeptidases [66]. Two such oligopeptidases have been identified in mitochondria. PreP (Mop112/Cym1 in yeast) has homologs in yeast, plants and humans. Analysis of PreP homologs from *Saccharomyces cerevisiae*, *Arabidopsis thaliana* and humans revealed their functional conservation and their localization in the matrix [67]. The human homolog of PreP is an amyloid-beta-degrading enzyme, which was found to have reduced activity in brain mitochondria from Alzheimer's disease patients [68,69]. Saccharolysin (or Prd1) is a metallopeptidase that exerts similar functions in the mitochondrial intermembrane space, degrading peptides produced by the i-AAA protease [70].

3. Mitochondrial protein maintenance

Within the challenging mitochondrial environment mitochondrial proteins may experience misfolding or unfolding events that could lead to aggregation. Chaperones lie at the core of the repair mechanism. However, proteins that fail to mature, fold, or assemble properly as well as those that become irreversibly oxidized and denatured are degraded by mitochondrial proteases.

3.1. *Chaperones*

Besides its role in protein translocation, mtHsp70 is also one of the two major chaperones that assist protein folding into the matrix under normal conditions [71]. Both nascent polypeptides that have entered the matrix and stress-induced denatured proteins interact primarily with mtHsp70 to acquire their correct conformation [72]. This function of mtHsp70 relies on its ability to bind to hydrophobic segments of the substrate protein. This binding shields aggregation prone segments of the protein, while controlled release from the chaperone allows it to fold (or refold) to the mature and active conformation. When engaged in protein folding, mtHsp70 interacts with a different J-domain co-chaperone, the homolog of bacterial DnaJ and member of the Hsp40 protein family, Mdj1 (Mitochondrial DnaJ 1), as well as the nucleotide exchange factor Mge1 [73]. Lack of Mdj1p in *S. cerevisiae* mitochondria does not affect protein import but instead impairs folding of newly imported protein precursors, and refolding after incubation at elevated temperature [72]. The human homologs of Mdj1 and Mge1 are known as Tid1 and hMge1 respectively. The two spliced versions of Tid1, the long $Tid1_L$ and the short $Tid1_S$, have opposing effects on the regulation of apoptotic signals in the matrix [74]. Mdj1, similar to its cytosolic counterparts, targets protein substrates and stimulates the ATPase activity of mtHsp70, converting it to the ADP-bound form, which has high affinity for substrate. In *S. cerevisiae* mitochondria, a second member of the Hsp70 family, Ssq1, cooperates with the J-domain protein Jac1 to mediate iron homeostasis and promote the activity of iron/sulfur (Fe/S) cluster proteins [75,76]. Most higher eukaryotes employ one mtHsp70 which cooperates with a Jac1 homolog to promote Fe/S cluster protein maturation [76]. The human mitochondrial Hsp70 system, that comprises mortalin and Tid1, has the ability to function as a disaggregating machine, a capacity also shown for bacterial systems [77,78]. Folding and maintenance of the mtHsp70 itself is assisted by the chaperone Hep1, also known as Zim17, and Tim15 [79–81].

Folding of newly imported polypeptides in the matrix is often assisted by the Hsp60/Hsp10 chaperonin system [82,83]. Hsp60 is a eukaryotic homolog of the bacterial GroEL chaperone. Its characteristic structure dictates a mode of function different from other Hsp70 chaperones. Hsp60 oligomerizes into two stacked heptameric rings. Each ring forms a large internal cavity which accommodates unfolded polypeptides. The cofactor Hsp10 covers the opening of the ring. ATP hydrolysis induces a conformational change that renders the cavity more hydrophilic and allows the substrate to fold without interference from other protein factors. Substrate interaction with Hsp60 takes place after release from the mtHsp70, the first chaperone precursors meet during their crossing the inner membrane (Fig. 2) [84]. Loss of Hsp60 function leads to accumulation of protein aggregates in the matrix [82].

Severe stress conditions may cause denaturation of mitochondrial proteins to an extent beyond the capacity of chaperone repair systems. Unfolded proteins tend to form insoluble aggregates that generate an additional burden for the organelle. Many cell types contain specialized chaperones that are involved in reactivating these aggregates. These chaperones belong to the Heat-shock protein 100/Caseinolytic peptidase (Hsp100/Clp) subfamily of the AAA+ (ATPases Associated with various cellular activities) protein family and are essential for thermotolerance. They form a hexameric ring structure with an internal channel, and catalyze protein unfolding, disassembly and disaggregation in bacteria, plants, fungi [85]. For many of their disaggregating activities, these enzymes cooperate with the Hsp70 system forming a bi-chaperone network [85,86]. Hsp78 and Mcx1/ClpX, are two matrix-located, mitochondrial members of the Hsp100/Clp family. *S. cerevisiae* Hsp78 has been shown to oligomerize into hexameric ring-shaped complex in an ATP-dependent manner in vitro [87]. Hsp78 disaggregation activity depends on its interaction with Hsp70 system (Fig. 2) [88]. Although not essential for viability under normal conditions, Hsp78 is essential for respiratory competence and mitochondrial genome integrity under severe stress [89]. Deletion of the HSP78 gene significantly inhibits the ability to restore the fragmented mitochondrial network after heat stress [90]. It has been proposed that the disaggregation activity of Hsp78 is mediated mainly by maintaining mtHSP70 in a soluble and functional state, following heat stress [91]. Metazoan organisms appear to lack Hsp100 disaggregases. Their function is likely taken over by the Hsp70 system, possibly through interaction with members of the Hsp70-related protein family, Hsp110 [92,93]. Finally, it has been shown that Hsp78 is also involved in mitochondrial

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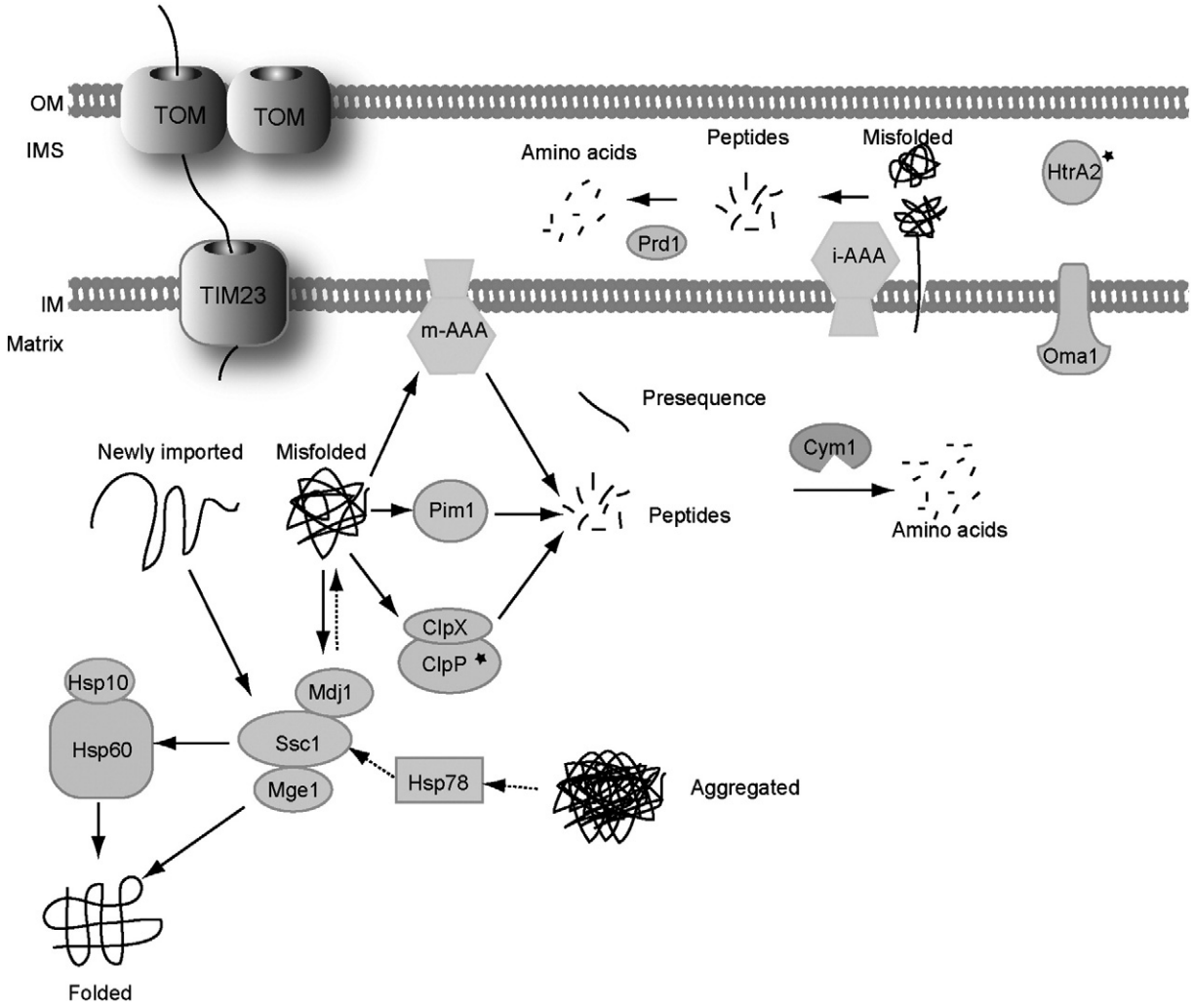

Fig. 2 – Mitochondrial protein quality control pathways. Newly imported or misfolded proteins in the matrix are treated by the main chaperone systems Ssc1 (mtHsp70) and Hsp60. Disaggregation is mediated by Ssc1 and Hsp78 chaperones. If proper folding is not achieved, degradation follows by the matrix quality control proteases Lon, ClpXP and m-AAA. The resulting peptides are further degraded to amino acids by the aminopeptidase Cym1. i-AAA protease degrades misfolded proteins in the intermembrane space. Peptides are further degraded by the oligopeptidase Prd1. The *Saccharomyces cerevisiae* nomenclature is used for the proteins shown here. Components that do not exist in *S. cerevisiae* are indicated with an asterisk (see text for details).

proteolysis mediated by the main matrix protease Lon/PIM1, a property that is absent from its bacterial and cytosolic counterparts [94].

3.2. *Metallochaperones*

Heavy metals like copper, zinc, manganese and iron are critical cofactors for a number of mitochondrial proteins, and assist their folding and/or their enzymatic activity. Although many metalloproteins may bind to more than one metal ions in vitro, they exhibit selectivity in vivo. This specificity is conferred by accessory proteins with chaperone-like activity known as metallochaperones, which bind and deliver the metal ion to the specific metalloprotein. Biogenesis of the cytochrome c oxidase (CcO) complex depends on a number of metallochaperones. In budding yeast delivery of redox-active copper in the subunits COX1 and COX2 of CcO is achieved by Cox11 and Sco1 metallochaperones respectively. Cox17 supplies both Cox11 and Sco1 [95,96]. In humans, a second Sco protein, Sco2, participates in metallation of CcO [97]. COX19, COX23 and PET191 are also copper binding proteins involved in CcO biogenesis but their role remains to be determined [98,99]. Sco proteins are redox regulated as they contain cysteine residues critical for their localization and metal coordination [100]. Mitochondrial import and maturation of the ROS-scavenger Cu, Zn-Superoxide Dismutase (SOD1) depends on the copper metallochaperone, CCS (Copper chaperone of the superoxide dismutase) [101]. In mammals, X-linked inhibitor of apoptosis (XIAP) has been identified as a novel substrate for CCS in the mitochondrial intermembrane space [102]. Mitochondrial metallochaperones, encoded by essential genes conserved in humans, ensure proper maturation and function of mitochondrial metalloproteins thereby protecting the organelle from oxidative damage.

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3.3. *Quality control proteases*

When refolding and reactivation of compromised proteins fails damaged polypeptides need to be removed from the organelle to prevent proteotoxic stress. Clearance of misfolded proteins is achieved through protein quality control mechanisms mainly involving proteolysis. Specialized proteolytic systems, related to those found in bacterial cells, exist in all the mitochondrial sub-compartments (Fig. 2; Table 2). Most mitochondrial proteases involved in removal of misfolded and aggregated proteins belong to the AAA+ protein family. These proteases assemble into homo-oligomeric ring-shaped complexes. The main mitochondrial protease of the matrix in yeast is a homolog of the bacterial Lon protease, also known as Pim1 (Proteolysis In Mitochondria 1). Lon family members are ATP-dependent serine proteases. Lon exhibits both chaperone and protease activities [103]. However, during its proteolytic function it collaborates with other mitochondrial chaperones, such as mtHsp70 and Hsp78 [94,104,105]. In budding yeast deletion of the PIM1 gene causes respiratory incompetence and loss of genome integrity [106]. Downregulation of human Lon in WI38 VA-13 human lung fibroblasts results initially in apoptotic, and finally in necrotic cell death, while cells that survive display abnormal mitochondrial morphology and shift to anaerobiosis [107]. It has been proposed that Lon/Pim1 acts as a quality control valve, degrading proteins that have escaped the refolding efforts of the mtHsp70 chaperone system [108]. Oxidized proteins often carry non-reversible covalent modifications and are primary substrates for Lon/Pim1 protease [108–111]. Moreover, Lon/Pim1 binds mitochondrial DNA preferentially in the heavy and light chain promoter regions required for transcription and replication, while it also interacts with DNA polymerase γ and the Twinkle helicase, both components of the mitochondrial nucleoids [112]. Recently, the Lon/Pim1 protease was shown to regulate mitochondrial DNA copy number and transcription by targeting the mitochondrial transcription factor A (TFAM) for degradation [113].

ClpP (caseinolytic protease) is a protease of the mitochondrial matrix that belongs to the Clp protein family. Unlike Lon protease, the ClpP complex consists of two different polypeptide chains assembled in oligomeric rings. The ClpXP holoenzyme consists of the ClpP protease that forms the catalytic core and the Mcx1 chaperone (Mitochondrial ClpX 1). Although ClpP is absent from yeast it has been found in mammalian mitochondria [114]. The structure of the human ClpP is similar to the bacterial homolog; it assembles into two face-to-face stacked heptameric rings with the proteolytic active sites exposed in the aqueous internal chamber [115]. The chaperone ClpX forms a hexameric ring-shaped structure and interacts with the ClpP complex to form the holoenzyme [116]. The ClpX chaperone initially recognizes, and then unfolds and translocates the substrates into the proteolytic chamber of ClpP [114]. The ClpP protease is a central component of the Mitochondrial Unfolded Protein Response (UPR^{mt}). Similar to the endoplasmic reticulum UPR, UPR^{mt} is a signal transduction pathway that senses accumulation of misfolded or aggregated proteins within the mitochondrial matrix and relays signals to the nucleus for transcriptional activation of repair mechanisms. ClpP is also a target of UPR^{mt}, since upon activation of the pathway its expression is increased [117,118]. ClpP deficiency results in attenuation of the whole pathway.

Apart from the soluble Lon and ClpXP proteolytic machines, mitochondria possess a third class of AAA proteases that are incorporated in the inner mitochondrial membrane, and their targets are primarily membrane-bound proteins. They belong to the FtsH (Filament forming temperature sensitive) protease family. Members of this family exist in bacteria, fungi, plants and animals. At least two members of this family are present in mitochondria; m-AAA and i-AAA, which expose their catalytic sites in the matrix and intermembrane space respectively [119,120]. The m-AAA protease in yeast is a hexamerichetero–oligomeric complex that consists of two subunits namely Yta10 (Afg3) and Yta12 (Rca1) [121]. However, mammalian mitochondria incorporate different isoenzymes of m-AAA proteases that assemble into homo- or hetero-oligomeric complexes [122]. As mentioned above, m-AAA are not only quality control proteases involved in the degradation of misfolded polypeptides but also have a role in specific processing of certain substrates, such as the ribosomal protein MrpL23, cytochrome c peroxidase and the dynamin-like GTPase involved in mitochondrial fusion OPA1 [41,123,124]. Mammalian m-AAA is involved in the proteolytic maturation of its own subunits, Afg3L1, Afg3L2 and paraplegin after cleavage by MPP. i-AAA protease is the only known ATP-dependent protease that faces the intermembrane space and is a hexameric homo-oligomeric complex of Yme1 (yeast mitochondrial escape 1) subunits. The human homolog YME1L1 is required for the proteolytic clearance of non-assembled respiratory chain complex subunits, for cristae morphogenesis and for apoptotic resistance in human embryonic kidney 293 cells [125]. Recently Yme1 was found to participate in the clearance of incompletely oxidized, misfolded proteins of the small Tim family [126]. Yme1 participates in the import and maturation of Polynucleotide Phosphorylase in the mitochondrial intermembrane space independently of its proteolytic activity [127]. Both AAA proteases also display a chaperone activity and are involved in the assembly of the respiratory complexes [128–130].

Another inner mitochondrial membrane protease is OMA1, a homolog of the HtpX protease of the *Escherichia coli* plasma membrane. OMA1 is a metallopeptidase whose catalytic center faces the matrix and has overlapping activities with m-AAA [131–134]. The intermembrane space of mammalian mitochondria contains a soluble homo-trimeric serine protease, HtrA/Omi. HtrA is apparently absent in yeast cells and worms but shares homology with the Deg proteins of bacteria and plants. Mammalian HtrA2 is strongly induced upon stress. Although its function is still obscure, it has been suggested that it participates in protein quality control at the mitochondrial intermembrane space by clearing excess misfolded proteins [135].

4. Oxidative stress challenges mitochondrial proteostasis during aging

Mitochondria are the primary generator of reactive oxygen species (ROS) in the cell. ROS are produced by incomplete reduction of oxygen with electrons that have leaked from respiratory chain complexes. ROS can damage several macromolecules such as proteins, lipids and DNA. Extensive oxidative damage has been linked with premature aging and development of age-related diseases. Post-mitotic cells accumulate mutations

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as a function of age. The rate of accumulation increases at higher temperatures [136]or under oxidative stress [137]. Accumulation of mtDNA mutations in mice that express an error-prone version of PolgA leads to reduced lifespan and early development of age-associated phenotypes [138]. Targeting DNA repair enzymes such as8-oxoguanine DNA glycosylase (OGG1) in mitochondria enhances mtDNA repair and cellular viability [139]. However, an age-dependent decline in the import efficiency of mitochondrial proteins required for repair of oxidatively damaged mtDNA has been reported [140]. These enzymes become stuck in the outer membrane of liver mitochondria of aged mice, in the unprocessed precursor form. By contrast, none remains in precursor form in mitochondria from young mice. These findings indicate that impairment of DNA repair enzyme import, and the resulting accumulation of mtDNA oxidative damage is a consequence of aging and also contributes to senescent decline.

Conversely, oxidative stress has been shown to inhibit protein import into mitochondria [141], although the exact mechanism of this inhibition remains elusive. Defects in mitochondrial protein translocation often lead to premature aging. Mice with only one intact copy of TIM23 gene show reduced lifespan [142]. In contrast, overexpression of mtHsp70 extends lifespan in *C. elegans* and normal human cells, while RNAi knockdown leads to shorter lifespan and progeria-like phenotypes in nematodes [143,144]. Proteomic analysis revealed reduced levels of mortalin in brain tissues from Parkinson's disease patients [145]. Mortalin is a major target for oxidation, with the oxidized form being more abundant in brains from older compared with young mice [146]. Mitochondria derived from mice homozygous for a mutation in the *immp2l* gene, encoding a subunit of the mitochondrial inner membrane peptidase, generate elevated levels of superoxide. These mutant animals manifest many age-associated phenotypes [147].

Mitochondrial proteins are primarily subjected to oxidative stress. Oxidatively damaged proteins may become inactivated, lose their proper tertiary structure and potentially form toxic aggregates within the organelle. Oxidative modifications cause reversible or irreversible damage to proteins. Reversible modifications occur in the sulfur-containing amino acids, cysteine and methionine. Mitochondria have a limited ability to repair some of the oxidized proteins using the antioxidant systems of thioredoxin/thioredoxin reductase, glutaredoxin/glutathione/glutathione reductase and methionine sulfoxide reductase [148]. Irreversibly oxidized proteins need to be removed by degradation and this is mediated, as already mentioned, mainly by the Lon/Pim1 protease in the matrix. Enzymes that carry oxidation-sensitive prosthetic groups are primary targets for oxidation and thus degradation by Lon [149]. Lon activity assayed by the release of fluorescent peptides significantly decreases in 27-month-old compared with 10-month-old rats, while oxidized proteins accumulated in the matrix of older animals [150]. Furthermore, Lon protein levels are reduced in skeletal muscles of aged versus young mice [151]. Overexpression of Lon in the fungal aging model *Podospora anserina* leads to both lifespan and health span extension. Transgenic strains display reduced oxidized protein load, reduced production of hydrogen peroxide and higher resistance to oxidative stress [152]. It has been proposed that Lon itself is a redox-regulated enzyme complex, since oxidative stress caused by exogenous addition of the oxidant peroxynitrite in isolated rat brain mitochondria markedly decreases Lon activity whereas subsequent treatment with reduced glutathione can partly restore this effect [153].

Mitochondrial protein import and protein quality control machineries may also act synergistically to initiate signal transduction pathways that regulate critical aspects of organellar quality control [154–156]. This is achieved in part by acting on regulatory proteins that target mitochondria and relay signals to the cytosol and/or the nucleus of the cell. In nematodes, the bZip transcription factor ATFS-1 (Activating Transcription Factor associated with Stress 1) targets both the nucleus and mitochondria. Under normal conditions, it translocates to the mitochondrial matrix, where it is degraded by the Lon protease. Under mitochondrial stress conditions, it accumulates in the nucleus due to the reduced efficiency of the mitochondrial import machinery [155]. When in the nucleus, ATFS-1 activates the UPR^{mt} pathway. Interestingly, it was shown that perturbation of the misfolded protein load in the mitochondria of one tissue signals the activation of stress response in a different tissue. This adaptive response has been implicated in lifespan extension observed in long-lived *C. elegans* mitochondrial mutants [157].

Mutations in PINK1 (PTEN induced putative kinase 1), a serine/threonine kinase, have been linked to the pathogenesis of recessive familial early-onset Parkinson's disease [158]. Pink1 is rapidly imported and degraded in healthy mitochondria. Pink1 processing is mediated by MPP, PARL, m-AAA and ClpXP proteases [154,156]. According to the current model, PINK1 is no longer imported to mitochondria upon mitochondrial depolarization. Instead, it accumulates on the outer mitochondrial membrane and recruits Parkin to promote elimination of the dysfunctional organelle by mitophagy [159,160]. These findings emphasize the regulatory role of mitochondrial protein quality control components in the crosstalk between mitochondria and cellular physiology, and implicate them in aging and senescent decline.

5. Concluding remarks

Accelerated accumulation of damage under conditions of environmental and genetic stress has been associated with short lifespan and incidence of age-related phenotypes. Organisms have evolved elaborate systems to restore or remove damaged macromolecules under normal and stress conditions. Mitochondrial protein translocation and quality control pathways regulate sorting, folding and assembly of protein precursors as well as refolding and removal of damaged material. Their role, within the oxidatively challenged mitochondrial environment, is critical. Mitochondrial proteins are primary targets for oxidation, whereas mitochondrial proteome imbalance causes further exacerbation of oxidative stress. During the last decade, multiple components of the mtPQC systems have been identified and characterized. These studies have provided novel insights into mechanistic aspects of mitochondrial quality control systems and established them as significant contributors to aging and age-related diseases. While substantial progress has been made, we have only just begun to unravel the diverse contributions of mtPQC mechanisms in the complex interplay between mitochondria and other cellular compartments. Moreover, information on the involvement of mitochondria in non-cell autonomous signal

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transduction between cells or tissues and the nature of these signals remains limited. Further study of mtPQC mechanisms and their interconnections will be the focus of future research efforts and should yield important information relevant to organismal health and longevity.

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REFERENCES

[1] Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell 2009;138:628–44.
[2] Voos W. Chaperone-protease networks in mitochondrial protein homeostasis. Biochim Biophys Acta 2012;1833:388–99.
[3] Baker MJ, Tatsuta T, Langer T. Quality control of mitochondrial proteostasis. Cold Spring Harb Perspect Biol 2011;3.
[4] Baker BM, Haynes CM. Mitochondrial protein quality control during biogenesis and aging. Trends Biochem Sci 2011;36: 254–61.
[5] Young JC, Hoogenraad NJ, Hartl FU. Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. Cell 2003;112:41–50.
[6] Li J, Qian X, Hu J, Sha B. Molecular chaperone Hsp70/Hsp90 prepares the mitochondrial outer membrane translocon receptor Tom71 for preprotein loading. J Biol Chem 2009;284: 23852–9.
[7] Terada K, Kanazawa M, Yano M, Hanson B, Hoogenraad N, Mori M. Participation of the import receptor Tom20 in protein import into mammalian mitochondria: analyses *in vitro* and in cultured cells. FEBS Lett 1997;403:309–12.
[8] Abe Y, Shodai T, Muto T, Mihara K, Torii H, Nishikawa S, et al. Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20. Cell 2000;100: 551–60.
[9] Brix J, Dietmeier K, Pfanner N. Differential recognition of preproteins by the purified cytosolic domains of the mitochondrial import receptors Tom20, Tom22, and Tom70. J Biol Chem 1997;272:20730–5.
[10] Marc P, Margeot A, Devaux F, Blugeon C, Corral-Debrinski M, Jacq C. Genome-wide analysis of mRNAs targeted to yeast mitochondria. EMBO Rep 2002;3:159–64.
[11] MacKenzie JA, Payne RM. Ribosomes specifically bind to mammalian mitochondria via protease-sensitive proteins on the outer membrane. J Biol Chem 2004;279: 9803–10.
[12] Garcia M, Delaveau T, Goussard S, Jacq C. Mitochondrial presequence and open reading frame mediate asymmetric localization of messenger RNA. EMBO Rep 2010;11:285–91.
[13] Eliyahu E, Pnueli L, Melamed D, Scherrer T, Gerber AP, Pines O, et al. Tom20 mediates localization of mRNAs to mitochondria in a translation-dependent manner. Mol Cell Biol 2010;30: 284–94.
[14] Garcia-Rodriguez LJ, Gay AC, Pon LA. Puf3p, a Pumilio family RNA binding protein, localizes to mitochondria and regulates mitochondrial biogenesis and motility in budding yeast. J Cell Biol 2007;176:197–207.
[15] Gerber AP, Herschlag D, Brown PO. Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. PLoS Biol 2004;2: E79.
[16] Devaux F, Lelandais G, Garcia M, Goussard S, Jacq C. Posttranscriptional control of mitochondrial biogenesis: spatio-temporal regulation of the protein import process. FEBS Lett 2010;584:4273–9.
[17] Chacinska A, Rehling P, Guiard B, Frazier AE, Schulze-Specking A, Pfanner N, et al. Mitochondrial translocation contact sites: separation of dynamic and stabilizing elements in formation of a TOM–TIM-preprotein supercomplex. EMBO J 2003;22:5370–81.
[18] Komiya T, Rospert S, Koehler C, Looser R, Schatz G, Mihara K. Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis. EMBO J 1998;17: 3886–98.
[19] Dekker PJ, Pfanner N. Role of mitochondrial GrpE and phosphate in the ATPase cycle of matrix Hsp70. J Mol Biol 1997;270:321–7.
[20] Miao B, Davis JE, Craig EA. Mge1 functions as a nucleotide release factor for Ssc1, a mitochondrial Hsp70 of *Saccharomyces cerevisiae*. J Mol Biol 1997;265:541–52.
[21] Yaguchi T, Aida S, Kaul SC, Wadhwa R. Involvement of mortalin in cellular senescence from the perspective of its mitochondrial import, chaperone, and oxidative stress management functions. Ann N Y Acad Sci 2007;1100:306–11.
[22] D'Silva PD, Schilke B, Walter W, Andrew A, Craig EA. J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. Proc Natl Acad Sci U S A 2003;100:13839–44.
[23] Truscott KN, Voos W, Frazier AE, Lind M, Li Y, Geissler A, et al. A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria. J Cell Biol 2003;163:707–13.
[24] Kozany C, Mokranjac D, Sichting M, Neupert W, Hell K. The J domain-related cochaperone Tim16 is a constituent of the mitochondrial TIM23 preprotein translocase. Nat Struct Mol Biol 2004;11:234–41.
[25] Mokranjac D, Bourenkov G, Hell K, Neupert W, Groll M. Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. EMBO J 2006;25:4675–85.
[26] Krayl M, Lim JH, Martin F, Guiard B, Voos W. A cooperative action of the ATP-dependent import motor complex and the inner membrane potential drives mitochondrial preprotein import. Mol Cell Biol 2007;27:411–25.
[27] Erbse A, Mayer MP, Bukau B. Mechanism of substrate recognition by Hsp70 chaperones. Biochem Soc Trans 2004;32:617–21.
[28] Becker D, Krayl M, Strub A, Li Y, Mayer MP, Voos W. Impaired interdomain communication in mitochondrial Hsp70 results in the loss of inward-directed translocation force. J Biol Chem 2009;284:2934–46.
[29] Lim JH, Martin F, Guiard B, Pfanner N, Voos W. The mitochondrial Hsp70-dependent import system actively unfolds preproteins and shortens the lag phase of translocation. EMBO J 2001;20:941–50.
[30] Voisine C, Craig EA, Zufall N, von Ahsen O, Pfanner N, Voos W. The protein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70. Cell 1999;97:565–74.
[31] Curran SP, Leuenberger D, Oppliger W, Koehler CM. The Tim9p–Tim10p complex binds to the transmembrane domains of the ADP/ATP carrier. EMBO J 2002;21:942–53.
[32] Lu H, Allen S, Wardleworth L, Savory P, Tokatlidis K. Functional TIM10 chaperone assembly is redox-regulated *in vivo*. J Biol Chem 2004;279:18952–8.

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[33] Vasiljev A, Ahting U, Nargang FE, Go NE, Habib SJ, Kozany C, et al. Reconstituted TOM core complex and Tim9/Tim10 complex of mitochondria are sufficient for translocation of the ADP/ATP carrier across membranes. Mol Biol Cell 2004;15:1445–58.
[34] Webb CT, Gorman MA, Lazarou M, Ryan MT, Gulbis JM. Crystal structure of the mitochondrial chaperone TIM9.10 reveals a six-bladed alpha-propeller. Mol Cell 2006;21:123–33.
[35] Beverly KN, Sawaya MR, Schmid E, Koehler CM. The Tim8–Tim13 complex has multiple substrate binding sites and binds cooperatively to Tim23. J Mol Biol 2008;382:1144–56.
[36] Hoppins SC, Nargang FE. The Tim8–Tim13 complex of *Neurospora crassa* functions in the assembly of proteins into both mitochondrial membranes. J Biol Chem 2004;279: 12396–405.
[37] Chacinska A, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuan Szklarz LK, Schulze-Specking A, et al. Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. EMBO J 2004;23:3735–46.
[38] Banci L, Bertini I, Cefaro C, Cenacchi L, Ciofi-Baffoni S, Felli IC, et al. Molecular chaperone function of Mia40 triggers consecutive induced folding steps of the substrate in mitochondrial protein import. Proc Natl Acad Sci U S A 2010;107:20190–5.
[39] Weckbecker D, Longen S, Riemer J, Herrmann JM. Atp23 biogenesis reveals a chaperone-like folding activity of Mia40 in the IMS of mitochondria. EMBO J 2012;31:4348–58.
[40] van der Laan M, Hutu DP, Rehling P. On the mechanism of preprotein import by the mitochondrial presequence translocase. Biochim Biophys Acta 1803;2010:732–9.
[41] Esser K, Tursun B, Ingenhoven M, Michaelis G, Pratje E. A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1. J Mol Biol 2002;323:835–43.
[42] Gasser SM, Ohashi A, Daum G, Bohni PC, Gibson J, Reid GA, et al. Imported mitochondrial proteins cytochrome b2 and cytochrome c1 are processed in two steps. Proc Natl Acad Sci U S A 1982;79:267–71.
[43] Vogtle FN, Prinz C, Kellermann J, Lottspeich F, Pfanner N, Meisinger C. Mitochondrial protein turnover: role of the precursor intermediate peptidase Oct1 in protein stabilization. Mol Biol Cell 2011;22:2135–43.
[44] Vogtle FN, Wortelkamp S, Zahedi RP, Becker D, Leidhold C, Gevaert K, et al. Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. Cell 2009;139:428–39.
[45] Mossmann D, Meisinger C, Vogtle FN. Processing of mitochondrial presequences. Biochim Biophys Acta 1819;2012:1098–106.
[46] Witte C, Jensen RE, Yaffe MP, Schatz G. MAS1, a gene essential for yeast mitochondrial assembly, encodes a subunit of the mitochondrial processing protease. EMBO J 1988;7:1439–47.
[47] Braun HP, Schmitz UK. Are the 'core' proteins of the mitochondrial bc1 complex evolutionary relics of a processing protease? Trends Biochem Sci 1995;20:171–5.
[48] Glaser E, Dessi P. Integration of the mitochondrial-processing peptidase into the cytochrome bc1 complex in plants. J Bioenerg Biomembr 1999;31:259–74.
[49] Branda SS, Cavadini P, Adamec J, Kalousek F, Taroni F, Isaya G. Yeast and human frataxin are processed to mature form in two sequential steps by the mitochondrial processing peptidase. J Biol Chem 1999;274:22763–9.
[50] Cavadini P, Adamec J, Taroni F, Gakh O, Isaya G. Two-step processing of human frataxin by mitochondrial processing peptidase. Precursor and intermediate forms are cleaved at different rates. J Biol Chem 2000;275:41469–75.
[51] Schmidt B, Wachter E, Sebald W, Neupert W. Processing peptidase of *Neurospora* mitochondria. Two-step cleavage of imported ATPase subunit 9. Eur J Biochem 1984;144:581–8.
[52] Esser K, Pratje E, Michaelis G. SOM 1, a small new gene required for mitochondrial inner membrane peptidase function in *Saccharomyces cerevisiae*. Mol Gen Genet 1996;252: 437–45.
[53] Jan PS, Esser K, Pratje E, Michaelis G. Som1, a third component of the yeast mitochondrial inner membrane peptidase complex that contains Imp1 and Imp2. Mol Gen Genet 2000;263:483–91.
[54] Nunnari J, Fox TD, Walter P. A mitochondrial protease with two catalytic subunits of nonoverlapping specificities. Science 1993;262:1997–2004.
[55] Daum G, Gasser SM, Schatz G. Import of proteins into mitochondria. Energy-dependent, two-step processing of the intermembrane space enzyme cytochrome b2 by isolated yeast mitochondria. J Biol Chem 1982;257:13075–80.
[56] Esser K, Jan PS, Pratje E, Michaelis G. The mitochondrial IMP peptidase of yeast: functional analysis of domains and identification of Gut2 as a new natural substrate. Mol Genet Genomics 2004;271:616–26.
[57] Kalousek F, Isaya G, Rosenberg LE. Rat liver mitochondrial intermediate peptidase (MIP): purification and initial characterization. EMBO J 1992;11:2803–9.
[58] Isaya G, Kalousek F, Fenton WA, Rosenberg LE. Cleavage of precursors by the mitochondrial processing peptidase requires a compatible mature protein or an intermediate octapeptide. J Cell Biol 1991;113:65–76.
[59] Varshavsky A. The N-end rule pathway and regulation by proteolysis. Protein Sci 2011;20:1298–345.
[60] Wong ED, Wagner JA, Gorsich SW, McCaffery JM, Shaw JM, Nunnari J. The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria. J Cell Biol 2000;151:341–52.
[61] Herlan M, Vogel F, Bornhovd C, Neupert W, Reichert AS. Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. J Biol Chem 2003;278:27781–8.
[62] McQuibban GA, Saurya S, Freeman M. Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. Nature 2003;423:537–41.
[63] Osman C, Wilmes C, Tatsuta T, Langer T. Prohibitins interact genetically with Atp23, a novel processing peptidase and chaperone for the F1Fo-ATP synthase. Mol Biol Cell 2007;18: 627–35.
[64] Naamati A, Regev-Rudzki N, Galperin S, Lill R, Pines O. Dual targeting of Nfs1 and discovery of its novel processing enzyme, Icp55. J Biol Chem 2009;284:30200–8.
[65] Young L, Leonhard K, Tatsuta T, Trowsdale J, Langer T. Role of the ABC transporter Mdl1 in peptide export from mitochondria. Science 2001;291:2135–8.
[66] Augustin S, Nolden M, Muller S, Hardt O, Arnold I, Langer T. Characterization of peptides released from mitochondria: evidence for constant proteolysis and peptide efflux. J Biol Chem 2005;280:2691–9.
[67] Alikhani N, Berglund AK, Engmann T, Spanning E, Vogtle FN, Pavlov P, et al. Targeting capacity and conservation of PreP homologues localization in mitochondria of different species. J Mol Biol 2011;410:400–10.
[68] Alikhani N, Ankarcrona M, Glaser E. Mitochondria and Alzheimer's disease: amyloid-beta peptide uptake and degradation by the presequence protease, hPreP. J Bioenerg Biomembr 2009;41:447–51.
[69] Alikhani N, Guo L, Yan S, Du H, Pinho CM, Chen JX, et al. Decreased proteolytic activity of the mitochondrial amyloid-beta degrading enzyme, PreP peptidasome, in Alzheimer's disease brain mitochondria. J Alzheimers Dis 2011;27:75–87.
[70] Kambacheld M, Augustin S, Tatsuta T, Muller S, Langer T. Role of the novel metallopeptidase Mop112 and saccharolysin for the complete degradation of proteins

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residing in different subcompartments of mitochondria. J Biol Chem 2005;280:20132–9.
[71] Kang PJ, Ostermann J, Shilling J, Neupert W, Craig EA, Pfanner N. Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature 1990;348:137–43.
[72] Rowley N, Prip-Buus C, Westermann B, Brown C, Schwarz E, Barrell B, et al. Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding. Cell 1994;77:249–59.
[73] Liu Q, Krzewska J, Liberek K, Craig EA. Mitochondrial Hsp70 Ssc1: role in protein folding. J Biol Chem 2001;276:6112–8.
[74] Syken J, De-Medina T, Munger K. TID1, a human homolog of the *Drosophila* tumor suppressor l(2)tid, encodes two mitochondrial modulators of apoptosis with opposing functions. Proc Natl Acad Sci U S A 1999;96:8499–504.
[75] Kim R, Saxena S, Gordon DM, Pain D, Dancis A. J-domain protein, Jac1p, of yeast mitochondria required for iron homeostasis and activity of Fe–S cluster proteins. J Biol Chem 2001;276:17524–32.
[76] Vickery LE, Cupp-Vickery JR. Molecular chaperones HscA/Ssq1 and HscB/Jac1 and their roles in iron–sulfur protein maturation. Crit Rev Biochem Mol Biol 2007;42: 95–111.
[77] Iosefson O, Sharon S, Goloubinoff P, Azem A. Reactivation of protein aggregates by mortalin and Tid1—the human mitochondrial Hsp70 chaperone system. Cell Stress Chaperones 2012;17:57–66.
[78] Ben-Zvi A, De Los Rios P, Dietler G, Goloubinoff P. Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual Hsp70 chaperones. J Biol Chem 2004;279:37298–303.
[79] Sichting M, Mokranjac D, Azem A, Neupert W, Hell K. Maintenance of structure and function of mitochondrial Hsp70 chaperones requires the chaperone Hep1. EMBO J 2005;24:1046–56.
[80] Blamowska M, Neupert W, Hell K. Biogenesis of the mitochondrial Hsp70 chaperone. J Cell Biol 2012;199:125–35.
[81] Sanjuan Szklarz LK, Guiard B, Rissler M, Wiedemann N, Kozjak V, van der Laan M, et al. Inactivation of the mitochondrial heat shock protein zim17 leads to aggregation of matrix hsp70s followed by pleiotropic effects on morphology and protein biogenesis. J Mol Biol 2005;351:206–18.
[82] Ostermann J, Horwich AL, Neupert W, Hartl FU. Protein folding in mitochondria requires complex formation with Hsp60 and ATP hydrolysis. Nature 1989;341:125–30.
[83] Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, Neupert W, et al. Mitochondrial heat-shock protein Hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature 1989;337:620–5.
[84] Manning-Krieg UC, Scherer PE, Schatz G. Sequential action of mitochondrial chaperones in protein import into the matrix. EMBO J 1991;10:3273–80.
[85] Doyle SM, Wickner S. Hsp104 and ClpB: protein disaggregating machines. Trends Biochem Sci 2009;34:40–8.
[86] Lee S, Sowa ME, Choi JM, Tsai FT. The ClpB/Hsp104 molecular chaperone—a protein disaggregating machine. J Struct Biol 2004;146:99–105.
[87] Krzewska J, Konopa G, Liberek K. Importance of two ATP-binding sites for oligomerization, ATPase activity and chaperone function of mitochondrial Hsp78 protein. J Mol Biol 2001;314:901–10.
[88] Krzewska J, Langer T, Liberek K. Mitochondrial Hsp78, a member of the Clp/Hsp100 family in *Saccharomyces cerevisiae*, cooperates with Hsp70 in protein refolding. FEBS Lett 2001;489:92–6.
[89] Schmitt M, Neupert W, Langer T. The molecular chaperone Hsp78 confers compartment-specific thermotolerance to mitochondria. J Cell Biol 1996;134:1375–86.
[90] Lewandowska A, Gierszewska M, Marszalek J, Liberek K. Hsp78 chaperone functions in restoration of mitochondrial network following heat stress. Biochim Biophys Acta 2006;1763:141–51.
[91] von Janowsky B, Major T, Knapp K, Voos W. The disaggregation activity of the mitochondrial ClpB homolog Hsp78 maintains Hsp70 function during heat stress. J Mol Biol 2006;357:793–807.
[92] Rampelt H, Kirstein-Miles J, Nillegoda NB, Chi K, Scholz SR, Morimoto RI, et al. Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. EMBO J 2012;31:4221–35.
[93] Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. EMBO J 2006;25:2519–28.
[94] Rottgers K, Zufall N, Guiard B, Voos W. The ClpB homolog Hsp78 is required for the efficient degradation of proteins in the mitochondrial matrix. J Biol Chem 2002;277:45829–37.
[95] Horng YC, Cobine PA, Maxfield AB, Carr HS, Winge DR. Specific copper transfer from the Cox17 metallochaperone to both Sco1 and Cox11 in the assembly of yeast cytochrome C oxidase. J Biol Chem 2004;279:35334–40.
[96] Robinson NJ, Winge DR. Copper metallochaperones. Annu Rev Biochem 2010;79:537–62.
[97] Horng YC, Leary SC, Cobine PA, Young FB, George GN, Shoubridge EA, et al. Human Sco1 and Sco2 function as copper-binding proteins. J Biol Chem 2005;280:34113–22.
[98] Barros MH, Johnson A, Tzagoloff A. COX23, a homologue of COX17, is required for cytochrome oxidase assembly. J Biol Chem 2004;279:31943–7.
[99] Rigby K, Zhang L, Cobine PA, George GN, Winge DR. Characterization of the cytochrome c oxidase assembly factor Cox19 of *Saccharomyces cerevisiae*. J Biol Chem 2007;282:10233–42.
[100] Leary SC. Redox regulation of SCO protein function: controlling copper at a mitochondrial crossroad. Antioxid Redox Signal 2010;13:1403–16.
[101] Banci L, Bertini I, Cantini F, Kozyreva T, Massagni C, Palumaa P, et al. Human superoxide dismutase 1 (hSOD1) maturation through interaction with human copper chaperone for SOD1 (hCCS). Proc Natl Acad Sci U S A 2012;109:13555–60.
[102] Brady GF, Galban S, Liu X, Basrur V, Gitlin JD, Elenitoba-Johnson KS, et al. Regulation of the copper chaperone CCS by XIAP-mediated ubiquitination. Mol Cell Biol 2010;30:1923–36.
[103] Rep M, van Dijl JM, Suda K, Schatz G, Grivell LA, Suzuki CK. Promotion of mitochondrial membrane complex assembly by a proteolytically inactive yeast Lon. Science 1996;274:103–6.
[104] Voos W. Mitochondrial protein homeostasis: the cooperative roles of chaperones and proteases. Res Microbiol 2009;160: 718–25.
[105] Savel'ev AS, Novikova LA, Kovaleva IE, Luzikov VN, Neupert W, Langer T. ATP-dependent proteolysis in mitochondria. m-AAA protease and PIM1 protease exert overlapping substrate specificities and cooperate with the mtHsp70 system. J Biol Chem 1998;273:20596–602.
[106] Van Dyck L, Pearce DA, Sherman F. PIM1 encodes a mitochondrial ATP-dependent protease that is required for mitochondrial function in the yeast *Saccharomyces cerevisiae*. J Biol Chem 1994;269:238–42.
[107] Bota DA, Ngo JK, Davies KJ. Downregulation of the human Lon protease impairs mitochondrial structure and function and causes cell death. Free Radic Biol Med 2005;38:665–77.
[108] Bender T, Lewrenz I, Franken S, Baitzel C, Voos W. Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol Biol Cell 2011;22:541–54.
[109] Bota DA, Davies KJ. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. Nat Cell Biol 2002;4:674–80.

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[110] Bayot A, Gareil M, Rogowska-Wrzesinska A, Roepstorff P, Friguet B, Bulteau AL. Identification of novel oxidized protein substrates and physiological partners of the mitochondrial ATP-dependent Lon-like protease Pim1. J Biol Chem 2010;285:11445–57.
[111] Major T, von Janowsky B, Ruppert T, Mogk A, Voos W. Proteomic analysis of mitochondrial protein turnover: identification of novel substrate proteins of the matrix protease pim1. Mol Cell Biol 2006;26:762–76.
[112] Liu T, Lu B, Lee I, Ondrovicova G, Kutejova E, Suzuki CK. DNA and RNA binding by the mitochondrial lon protease is regulated by nucleotide and protein substrate. J Biol Chem 2004;279:13902–10.
[113] Matsushima Y, Goto Y, Kaguni LS. Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). Proc Natl Acad Sci U S A 2010;107:18410–5.
[114] Truscott KN, Bezawork-Geleta A, Dougan DA. Unfolded protein responses in bacteria and mitochondria: a central role for the ClpXP machine. IUBMB Life 2011;63:955–63.
[115] Kang SG, Maurizi MR, Thompson M, Mueser T, Ahvazi B. Crystallography and mutagenesis point to an essential role for the N-terminus of human mitochondrial ClpP. J Struct Biol 2004;148:338–52.
[116] Kang SG, Ortega J, Singh SK, Wang N, Huang NN, Steven AC, et al. Functional proteolytic complexes of the human mitochondrial ATP-dependent protease, hClpXP. J Biol Chem 2002;277:21095–102.
[117] Haynes CM, Petrova K, Benedetti C, Yang Y, Ron D. ClpP mediates activation of a mitochondrial unfolded protein response in *C. elegans*. Dev Cell 2007;13:467–80.
[118] Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ. A mitochondrial specific stress response in mammalian cells. EMBO J 2002;21:4411–9.
[119] Gerdes F, Tatsuta T, Langer T. Mitochondrial AAA proteases—towards a molecular understanding of membrane-bound proteolytic machines. Biochim Biophys Acta 1823;2012: 49–55.
[120] Tatsuta T, Langer T. AAA proteases in mitochondria: diverse functions of membrane-bound proteolytic machines. Res Microbiol 2009;160:711–7.
[121] Arlt H, Tauer R, Feldmann H, Neupert W, Langer T. The YTA10-12 complex, an AAA protease with chaperone-like activity in the inner membrane of mitochondria. Cell 1996;85:875–85.
[122] Koppen M, Metodiev MD, Casari G, Rugarli EI, Langer T. Variable and tissue-specific subunit composition of mitochondrial m-AAA protease complexes linked to hereditary spastic paraplegia. Mol Cell Biol 2007;27:758–67.
[123] Nolden M, Ehses S, Koppen M, Bernacchia A, Rugarli EI, Langer T. The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. Cell 2005;123:277–89.
[124] Ishihara N, Fujita Y, Oka T, Mihara K. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J 2006;25:2966–77.
[125] Stiburek L, Cesnekova J, Kostkova O, Fornuskova D, Vinsova K, Wenchich L, et al. YME1L controls the accumulation of respiratory chain subunits and is required for apoptotic resistance, cristae morphogenesis, and cell proliferation. Mol Biol Cell 2012;23:1010–23.
[126] Baker MJ, Mooga VP, Guiard B, Langer T, Ryan MT, Stojanovski D. Impaired folding of the mitochondrial small TIM chaperones induces clearance by the i-AAA protease. J Mol Biol 2012;424:227–39.
[127] Rainey RN, Glavin JD, Chen HW, French SW, Teitell MA, Koehler CM. A new function in translocation for the mitochondrial i-AAA protease Yme1: import of polynucleotide phosphorylase into the intermembrane space. Mol Cell Biol 2006;26:8488–97.
[128] Arlt H, Steglich G, Perryman R, Guiard B, Neupert W, Langer T. The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease. EMBO J 1998;17:4837–47.
[129] Leonhard K, Stiegler A, Neupert W, Langer T. Chaperone-like activity of the AAA domain of the yeast Yme1 AAA protease. Nature 1999;398:348–51.
[130] Francis BR, Thorsness PE. Hsp90 and mitochondrial proteases Yme1 and Yta10/12 participate in ATP synthase assembly in *Saccharomyces cerevisiae*. Mitochondrion 2011;11:587–600.
[131] Kaser M, Kambacheld M, Kisters-Woike B, Langer T. Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. J Biol Chem 2003;278:46414–23.
[132] Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, et al. Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol 2009;187:1023–36.
[133] Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Bliek AM. Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J Cell Biol 2009;187: 959–66.
[134] Khalimonchuk O, Jeong MY, Watts T, Ferris E, Winge DR. Selective Oma1 protease-mediated proteolysis of Cox1 subunit of cytochrome oxidase in assembly mutants. J Biol Chem 2012;287:7289–300.
[135] Radke S, Chander H, Schafer P, Meiss G, Kruger R, Schulz JB, et al. Mitochondrial protein quality control by the proteasome involves ubiquitination and the protease Omi. J Biol Chem 2008;283:12681–5.
[136] Garcia AM, Calder RB, Dolle ME, Lundell M, Kapahi P, Vijg J. Age- and temperature-dependent somatic mutation accumulation in *Drosophila melanogaster*. PLoS Genet 2010;6: e1000950.
[137] Hegde ML, Mantha AK, Hazra TK, Bhakat KK, Mitra S, Szczesny B. Oxidative genome damage and its repair: implications in aging and neurodegenerative diseases. Mech Ageing Dev 2012;133:157–68.
[138] Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 2004;429:417–23.
[139] Dobson AW, Xu Y, Kelley MR, LeDoux SP, Wilson GL. Enhanced mitochondrial DNA repair and cellular survival after oxidative stress by targeting the human 8-oxoguanine glycosylase repair enzyme to mitochondria. J Biol Chem 2000;275:37518–23.
[140] Szczesny B, Hazra TK, Papaconstantinou J, Mitra S, Boldogh I. Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases. Proc Natl Acad Sci U S A 2003;100:10670–5.
[141] Wright G, Terada K, Yano M, Sergeev I, Mori M. Oxidative stress inhibits the mitochondrial import of preproteins and leads to their degradation. Exp Cell Res 2001;263:107–17.
[142] Ahting U, Floss T, Uez N, Schneider-Lohmar I, Becker L, Kling E, et al. Neurological phenotype and reduced lifespan in heterozygous Tim23 knockout mice, the first mouse model of defective mitochondrial import. Biochim Biophys Acta 2009;1787:371–6.
[143] Yokoyama K, Fukumoto K, Murakami T, Harada S, Hosono R, Wadhwa R, et al. Extended longevity of *Caenorhabditis elegans* by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett 2002;516:53–7.
[144] Kimura K, Tanaka N, Nakamura N, Takano S, Ohkuma S. Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in *Caenorhabditis elegans*. J Biol Chem 2007;282:5910–8.

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[145] Jin J, Hulette C, Wang Y, Zhang T, Pan C, Wadhwa R, et al. Proteomic identification of a stress protein, mortalin/mthsp70/GRP75: relevance to Parkinson disease. Mol Cell Proteomics 2006;5:1193–204.
[146] Choi J, Forster MJ, McDonald SR, Weintraub ST, Carroll CA, Gracy RW. Proteomic identification of specific oxidized proteins in ApoE-knockout mice: relevance to Alzheimer's disease. Free Radic Biol Med 2004;36:1155–62.
[147] George SK, Jiao Y, Bishop CE, Lu B. Mitochondrial peptidase IMMP2L mutation causes early onset of age-associated disorders and impairs adult stem cell self-renewal. Aging Cell 2011;10:584–94.
[148] Ugarte N, Petropoulos I, Friguet B. Oxidized mitochondrial protein degradation and repair in aging and oxidative stress. Antioxid Redox Signal 2010;13:539–49.
[149] Bender T, Leidhold C, Ruppert T, Franken S, Voos W. The role of protein quality control in mitochondrial protein homeostasis under oxidative stress. Proteomics 2010;10:1426–43.
[150] Bakala H, Delaval E, Hamelin M, Bismuth J, Borot-Laloi C, Corman B, et al. Changes in rat liver mitochondria with aging. Lon protease-like reactivity and N(epsilon)-carboxymethyllysine accumulation in the matrix. Eur J Biochem 2003;270:2295–302.
[151] Bota DA, Van Remmen H, Davies KJ. Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. FEBS Lett 2002;532:103–6.
[152] Luce K, Osiewacz HD. Increasing organismal healthspan by enhancing mitochondrial protein quality control. Nat Cell Biol 2009;11:852–8.
[153] Stanyer L, Jorgensen W, Hori O, Clark JB, Heales SJ. Inactivation of brain mitochondrial Lon protease by peroxynitrite precedes electron transport chain dysfunction. Neurochem Int 2008;53:95–101.
[154] Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. EMBO Rep 2012;13:378–85.
[155] Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 2012;337:587–90.
[156] Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol 2010;191:933–42.
[157] Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 2011;144:79–91.
[158] Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–60.
[159] Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol 2010;8:e1000298.
[160] Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 2010;189:211–21.