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The proteasome: friend and foe of mitochondrial biogenesis

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Most mitochondrial proteins are synthesized in the cytosol and subsequently translocated as unfolded polypeptides into mitochondria. Cytosolic chaperones maintain precursor proteins in an import-competent state. This post-translational import reaction is under surveillance of the cytosolic ubiquitin-proteasome system, which carries out several distinguishable activities. On the one hand, the proteasome degrades nonproductive protein precursors from the cytosol and nucleus, import intermediates that are stuck in mitochondrial translocases, and misfolded or damaged proteins from the outer membrane and the intermembrane space. These surveillance activities of the proteasome are essential for mitochondrial functionality, as well as cellular fitness and survival. On the other hand, the proteasome competes with mitochondria for nonimported cytosolic precursor proteins, which can compromise mitochondrial biogenesis. In order to balance the positive and negative effects of the cytosolic protein quality control system on mitochondria, mitochondrial import efficiency directly regulates the capacity of the proteasome *via* transcription factor Rpn4 in yeast and nuclear respiratory factor (Nrf) 1 and 2 in animal cells. In this review, we provide a thorough overview of how the proteasome regulates mitochondrial biogenesis.

Keywords: aging; mitochondria; mitochondria-associated degradation; mitoprotein-induced stress response; proteasome; protein degradation; protein quality control; Rpn4; ubiquitin

Eukaryotic cells evolved from a merger of two initially independent prokaryotic cells. During the past 1.5 billion years of eukaryotic evolution, mitochondria and the 'extramitochondrial space' of our cells developed from a bacterium that resided in the inside of an archaeon [1–3]. This biparental origin is obvious from many duplicated structures that are still present in eukaryotes. An example are the two types of ribosomes, one archaea derived in the cytosol and one bacteria derived in the matrix of mitochondria [4]. For respiring organisms, both translation systems are essential. Still, the individual workload of the two ribosomes is extremely different as, while the cytosolic translation system produces many thousand different products, mitochondrial ribosomes only synthesize a small handful of proteins [5]. Apparently, nature maintained both translation systems, but during evolution, the former evolved into the predominant general

Abbreviations
DUB, deubiquitinating enzyme; ER, endoplasmic reticulum; ERAD, ER-associated degradation; IMS, intermembrane space; MAD, mitochondria-associated degradation; mitoCPR, mitochondrial compromised protein import response; mitoTAD, mitochondrial protein translocation-associated degradation; mPOS, mitochondrial precursor overaccumulation stress; MTS, mitochondrial targeting sequence; TIM, translocase of the inner membrane; TOM, translocase of the outer membrane; UPRam, unfolded protein response activated by mistargeting of proteins; UPS, ubiquitin-proteasome system.

protein-producing machine, whereas the latter became a highly specialized device that only has relevance for mitochondria.

The same evolutionary trajectory is apparent for the protein degradation machineries: The proteasome in the cytosol and nucleus originated from the archaeal ancestor, whereas the mitochondrial protease systems, the LON/Pim1 and ClpXP proteases of the matrix and the AAA proteases of the inner membrane are of bacterial origin [6–8]. Since mitochondria do not contain proteasomes, it was assumed that the degradation of mitochondrial proteins is exclusively carried out by mitochondrial proteases. There is no doubt that the mitochondrial quality control system is crucial to maintain mitochondrial proteostasis and for the dynamic adaptation of the mitochondrial proteome to changing metabolic conditions [9–12]. Nevertheless, studies over the past decade have shown that the ubiquitin-proteasome system (UPS) of the cytosol is of pivotal relevance for the surveillance of the mitochondrial proteome [13–17]. Particularly important in this context are the degradation of cytosolic mitochondrial precursor proteins and that of mitochondrial surface proteins, the so-called mitochondria-associated degradation (MAD), by the proteasome. In respect to their mechanisms and components, both processes are often indistinguishable.

Mitochondrial biogenesis follows different pathways

Mitochondria consist of between 800 and 1500 different proteins [18–22]. Only a very small number of proteins is encoded by the mitochondrial genome: 13 in humans and 8 in baker's yeast, which lacks the complex I subunits, whereas all other mitochondrial proteins are synthesized on cytosolic ribosomes. For simplicity, we refer henceforth to the cytosolic forms of all of these proteins as precursors (even though many of these proteins lack presequences). Arguably, all of these proteins contain targeting signals that are recognized by receptors on the mitochondrial surface, and use the help of translocases to be imported into mitochondria [23]. The translocase of the outer membrane (TOM) complex [24,25] serves as a general entry gate for mitochondrial proteins from where proteins can be handed over to the translocase of the inner membrane (TIM) complexes and other import components. In terms of their targeting routes and signals, different groups of mitochondrial proteins can be distinguished (for overview, see ref. [26]).

Proteins of the matrix and inner membrane use the TIM23 or matrix-targeting pathway (Fig. 1A). These proteins are synthesized with an N-terminal matrix-targeting signal (MTS or presequence) [20,27,28]. They are imported through the TOM complex of the outer membrane and the TIM23 complex of the inner membrane [29]. The membrane-associated import motor drives protein translocation into the matrix by use of ATP hydrolysis. The matrix processing peptidase (MPP) and other matrix proteases remove the presequences and thereby initiate protein folding of the mature proteins.

The TIM22 or carrier pathway (Fig. 1B) mediates the import reaction of hydrophobic carriers and other inner membrane proteins [30]. Carriers lack presequences but contain (often multiple and redundant) internal targeting signals. Following translocation through the TOM pore, carriers are bound by specific chaperones in the IMS, called small Tim complexes, and inserted into the inner membrane by the TIM22 translocase [31,32]. The mitochondrial steps of the carrier pathway are well characterized *in vitro* but the early, premitochondrial reactions are completely unknown, despite the fact that carriers are highly relevant and among the most abundant mitochondrial proteins.

Many proteins of the intermembrane space (IMS) use a distinct targeting route referred to as the MIA pathway (Fig. 1C). Whereas the import of matrix proteins and of carriers is energized by the membrane potential of the inner membrane, the import of many IMS proteins is driven by their oxidative folding mediated by the oxidoreductase Mia40 (also called CHCHD4 in humans) [33–35]. Mia40 substrates are short proteins (mostly in the range between 70–120 residues) with distinct patterns of cysteine residues [36,37]. In addition to these three pathways, there are additional less-characterized routes that facilitate protein insertion into the outer or inner membrane [26].

We know only little about the kinetics of the import reactions. The clients of Mia40 appear to remain in the cytosol for considerable time (several minutes) before they find their way through the TOM pore [38,39]. In contrast, proteins with presequences presumably are imported rapidly (within seconds) after their synthesis and some might even engage the TOM complex before translation is completed [40–43].

The ubiquitin-proteasome system

The ubiquitin-proteasome system (UPS) plays an important role in cellular protein quality control [44,45]. It mediates targeted protein degradation and is a key element in the maintenance of protein homeostasis (proteostasis). The UPS detects and degrades

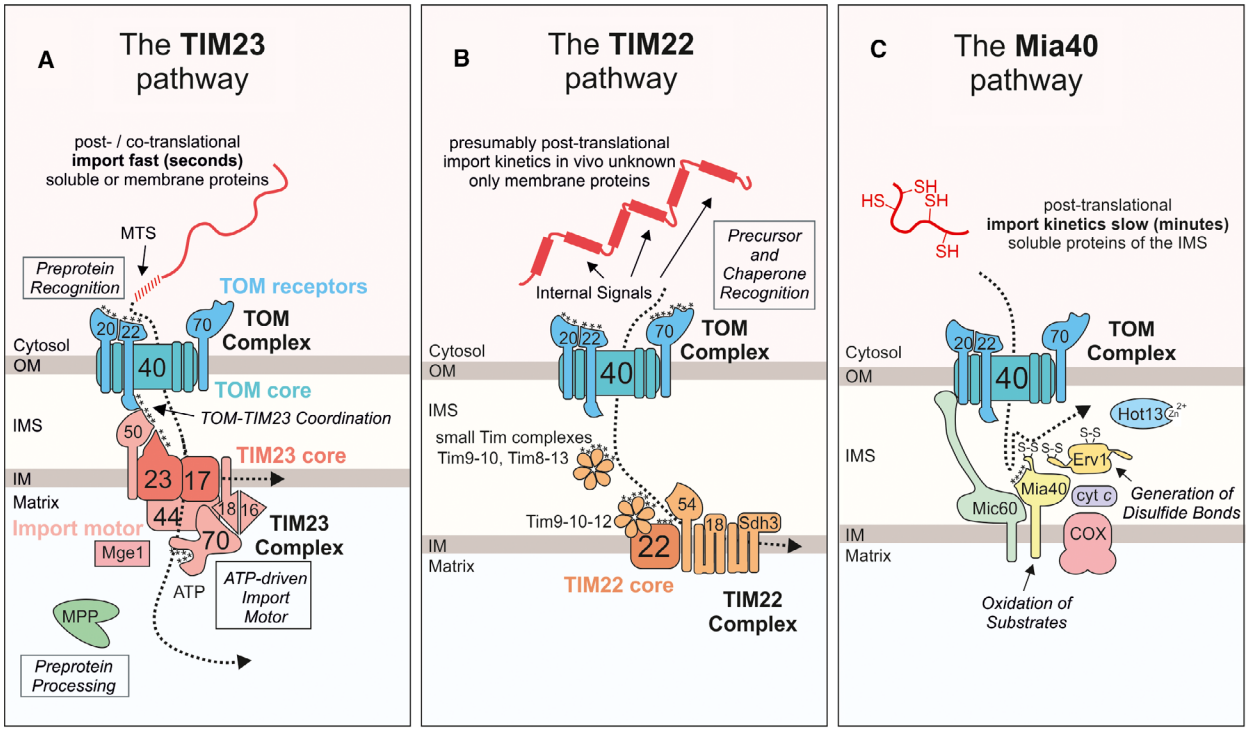

Fig. 1. The three main import pathways of yeast mitochondria. Precursor proteins embark on different pathways, which direct them into the different mitochondrial subcompartments. (A) The TIM23 pathway. Most proteins of the matrix and inner membrane are made with an N-terminal matrix-targeting sequence (MTS), which directs them through protein-conducting channels in the TOM and TIM23 complexes of the outer and inner membrane, respectively. Binding sites on several subunits of the import machinery direct them into mitochondria (indicated by asterisks). In the matrix, several processing peptidases remove and degrade the MTS. (B) The TIM22 pathway. This pathway directs polytopic membrane proteins into the inner membrane. Multiple internal targeting sequences direct these proteins *via* the TOM and TIM22 complexes into the inner membrane. (C) The MIA40 pathway. Cysteine-containing IMS proteins follow this import route, which involves oxidative folding of the cargo protein. Mia40 substrates do not bind to mitochondrial surface receptors with detectable affinity, and they can stay in the cytosol for many minutes before they are taken up into mitochondria.

misfolded, defective, or superfluous proteins, thereby regulating the cellular proteome in response to metabolic, developmental, or stress signals.

Protein modification by ubiquitination

Ubiquitin is a highly conserved protein of 76 residues that is covalently attached *via* its C terminus to lysine residues of target proteins including already protein-bound ubiquitins, thereby forming ubiquitin chains of different lengths. This reaction is catalyzed by a cascade of enzymes: A ubiquitin-activating enzyme (E1, Uba1 in yeast) forms a thioester with ubiquitin and transfers it *via* one of several cellular ubiquitin-conjugating enzymes (E2s) to the target protein. Substrate specificity is provided by ubiquitin ligases (E3s) which often bind E2s and target proteins simultaneously. Cells contain a large number of E3 ubiquitin ligases for various groups of substrates. Deubiquitylating enzymes (DUBs) antagonize ubiquitination enzymes, edit the linkages in the ubiquitin chain, and remove ubiquitin from proteins to rescue proteins from degradation.

Protein degradation by the proteasome

The 26S proteasome is a 2.5 MDa complex that consists of the barrel-shaped, catalytically active 20S core complex and 19S regulatory subunits, which seal the 20S core on one or both ends [44]. The 20S particle consists of four stacked rings: two inner rings of seven closely related β subunits and two outer rings of seven also closely related α subunits. Three β subunits are catalytically active (β_1 has caspase-like, β_2 has trypsin-like, and β_5 has chymotrypsin-like activity). The active sites are oriented toward the lumen of the 20S complex, and substrates need to be threaded into the barrel through a narrow opening. Some substrates are

degraded directly by the 20S core particle [46]. However, the insertion of most substrates is facilitated by the 19S particles which recognizes substrate proteins (often by affinity to their ubiquitin chains) and removes ubiquitin by built-in DUBs [47,48]. Its hexameric ATP-hydrolyzing AAA complex, formed by Rpt1-6, pushes substrates into the central cavity of the 20S complex, where they are shredded into small peptides [44,49].

The Cdc48/p97 unfoldase and other proteasome-assisting factors

Cdc48 (in yeast) or p97 or VCP (in mammals) is an essential and highly conserved cytosolic AAA ATPase that assists proteasomal degradation. Its function is important for the degradation of more 'tricky' proteasome substrates, such as subunits of multimeric complexes, membrane-embedded proteins, or polypeptides that are entangled into aggregates [50–53]. In order to interact with its many substrates, Cdc48/p97 employs substrate-specifying cofactors (sometimes also referred to as adaptors). Many of these contain a characteristic UBX (ubiquitin regulatory X) domain as well as different types of ubiquitin-binding domains [54]. Despite considerable differences in sequence and structure, many Cdc48/p97-binding regions bind to the same position of the AAA ATPase complex in a mutually exclusive fashion [55]. Thus, distinct populations of Cdc48/p97 complexes exist in cells, each specialized to mediate unfolding of a selective set of substrate proteins.

The function of Cdc48 is particularly well understood in the context of endoplasmic reticulum-associated degradation (ERAD). Several endoplasmic reticulum (ER) membrane proteins, including Doa10, Ubx2, and Hrd1, serve as adaptors which directly bind to Cdc48 to support its ATP-driven extraction of ER proteins [56].

The friendly proteasome: the UPS supports mitochondrial biogenesis

Mitochondrial biogenesis is under surveillance of the proteasome. In this context, three groups of relevant proteasome substrates can be distinguished: (a) cytosolic precursors that are on their way from the ribosome to the mitochondrial surface; (b) partially cytosol-exposed import intermediates that already engaged the import machinery; and (c) resident proteins of the mitochondrial surface (and the IMS) that are under surveillance of the cytosolic UPS and eventually degraded by the proteasome.

Degradation of nonimported precursors

The synthesis and import of presequence-containing mitochondrial precursor proteins is tightly coordinated. At normal growth conditions, no pools of non-imported precursors are detected [57–59]. Thus, presequence-containing precursors are either rapidly imported or, if import fails, rapidly degraded by the proteasome. Even though mitochondrial import occurs predominantly post-translationally, many studies presented convincing evidence that mRNAs encoding for mitochondrial proteins are specifically bound to the mitochondrial surface [40–43]. Yeast mitochondria even employ the outer membrane protein Puf3 as specific receptor to recruit specific mRNAs to their surface, and comparable RNA-binding proteins also exist on mammalian mitochondria [60–64]. Puf3 is not critical for protein import, but the local restriction of protein synthesis to the mitochondrial surface might prevent the proteasomal degradation of nascent precursor proteins.

A recent study suggests that mitochondria can modulate the binding as well as translation rates of specific mRNAs in dependence of metabolic conditions [41]. This points to an exciting mitochondria-controlled mRNA recruitment mechanism to determine the distance between ribosomes and the mitochondrial surface and thereby the exposure of precursors to the UPS in the cytosol.

Nonimported precursor proteins are rapidly degraded by the proteasome (Fig. 2A) [65–69]. When mitochondrial protein import is impaired, for example, by dissipation of the membrane potential, only few mitochondrial proteins accumulate in their precursor form. One example is the precursor of Hsp60 which gave rise to the hypothesis that this cytosolic precursor form might be functional [70,71]. Obviously, the individual stability of cytosolic precursors is variable. A systematic microscopy screen in yeast with C-terminally GFP-tagged precursors confirmed this heterogeneity and showed that several of the accumulating precursors enter the nucleus or associate with the ER surface [72]. This study also identified the three E3 ubiquitin ligases San1, Ubr1, and Doa10 as critical, but functionally redundant components for the proteasomal degradation of the precursor form of Ilv2 in the nucleus. The E3 ubiquitin ligases which specify mitochondrial precursors in the cytosol for degradation still await to be identified. Thus, it is conceivable that the intracellular spatial localization determines the stability of precursors: They might be 'safe' in proximity to mitochondria, but destined for degradation at other locations of the cell.

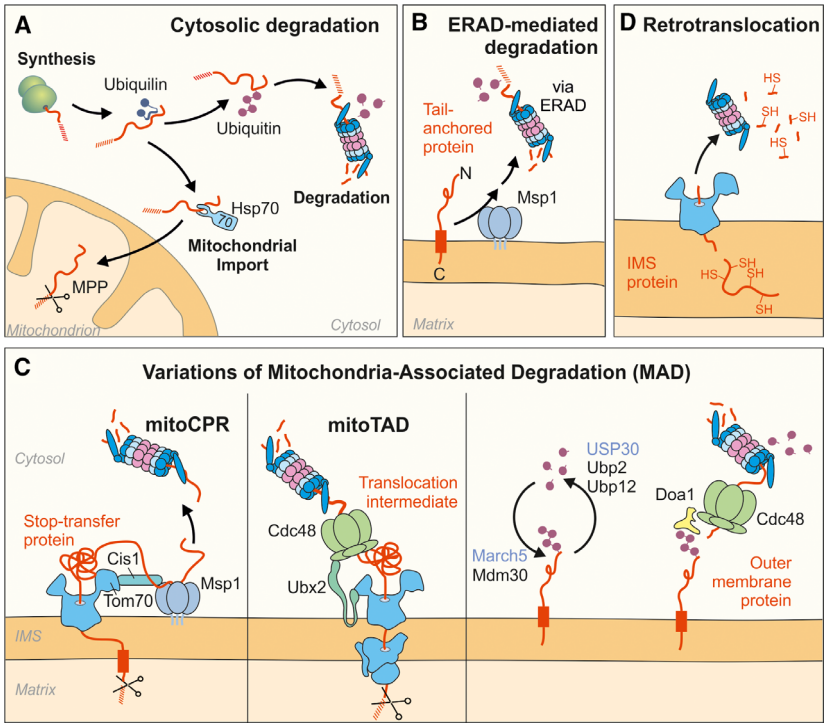

Fig. 2. Different functions of the proteasome in the context of mitochondrial biogenesis. (A) Mitochondria and the proteasome compete for cytosolic precursor proteins. Ubiquilins and chaperones influence this triage reaction. (B) Msp1 serves as extractor for tail-anchored proteins in the mitochondrial outer membrane. Some of its clients are degraded on the ER surface with the help of Doa10 by ERAD. (C) Different reactions of the mitochondria-associated degradation (MAD) of proteins were recently described. Msp1 can be recruited *via* Cis1 to Tom70 for extraction of arrested precursor proteins, in particular inner membrane proteins with stop-transfer sequences (also called cloggers). This process is referred to as mitoCPR. Ubx2 is located in the outer membrane and the ER membrane. For mitoTAD, the mitochondrial fraction connects the TOM complex to Cdc48 to extract and degrade stalled translocation intermediates. Mitochondrial surface proteins can be ubiquitinated and extracted from the surface by Cdc48 in MAD. Mdm30 in yeast and March5 in mammals facilitate ubiquitination, and Ubp2 and Ubp12 in yeast and USP30 in mammals the deubiquitination of mitochondrial outer membrane proteins. In yeast, Doa1 recruits Cdc48 to the mitochondrial surface. (D) The proteasome can also degrade some IMS proteins after their retrotranslocation through the protein-conducting channel of the TOM complex.

Removal of import intermediates

Tail-anchored proteins have C-terminal transmembrane domains which tether them to the membrane of the ER, of mitochondria, or of peroxisomes. These proteins have no 'canonical' N-terminal targeting sequences for these organelles but rather the hydrophobicity and the regions flanking their tail-anchor determine their target membrane [73,74]. Mitochondria recognize mistargeted tail-anchored proteins, that is, those made for peroxisomes or the ER, and employ the hexameric AAA protein Msp1 (called ATAD1 in mammals) to remove these from the membrane (Fig. 2B). After their ATP-driven extraction from the outer membrane [75], tail-anchored proteins have the chance to find their correct target membrane [76–78]. Alternatively, for example, in the case they are defective, they are degraded on the ER surface by ERAD after ubiquitination by the E3 ubiquitin ligase Doa10. In the absence of Doa10 (or its complex partners Ubc6, Ubc7, and Cue1), these tail-anchored proteins accumulate on the ER membrane [79,80].

The same Msp1 extractor also removes stalled translocation intermediates from mitochondria (Fig. 2C). Particularly, inner membrane proteins with N-terminal stop-transfer sequences (so-called bipartite targeting signals) are problematic and can serve as 'cloggers' of the TOM complex [81–83]. If such cloggers are overexpressed, yeast cells launch a response program called mitochondrial compromised protein import response (mitoCPR). mitoCPR induces the expression of Cis1 which connects the Msp1 extractor to Tom70 of the TOM complex [81]. The TOM-bound Msp1 serves as an unclogger that removes intermediates from the outer membrane and hands it over to the proteasome for degradation. However, the

mechanistic details of this process are still not clear. Interestingly, patients with a mutation in the human Msp1 homolog ATAD1 develop an encephalopathy that is caused by mitochondrial accumulation of a subunit of the AMPA receptor. This suggests that, in humans, ATAD1 also serves as an unclogger of stalled translocation intermediates [84].

The Cdc48/p97 complex supports Msp1/ATAD1 in protein extraction from the TOM complex [85]. Ubx2 is a Cdc48 adaptor that is present both on the ER (as an ERAD factor) and on the mitochondrial outer membrane (Fig. 2C). Ubx2 physically binds to the TOM complex and monitors protein translocation in a reaction called mitochondrial protein translocation-associated degradation (mitoTAD). Cdc48, together with its cofactors Npl4 and Ufd1, extracts translocation intermediates and targets them for proteasomal degradation. In contrast to mitoCPR, mitoTAD is not a response program to an exceptional stress situation but rather a permanent monitoring strategy to remove nonproductive import intermediates.

How import intermediates are removed from mammalian mitochondria is less well understood. In this context, the enzymes March5 and USP30 which are both tethered to the mitochondrial surface are relevant [86]: The E3 ubiquitin ligase March5 (also referred to as MITOL) adds ubiquitins onto precursor proteins which are reciprocally removed again by the DUB USP30. Ubiquitin prevents protein translocation and USP30 depletion therefore induces considerable import defects. Interestingly, these mutants also show reduced levels of TOM subunits, suggesting that the stalled intermediates induce the degradation of components of the import machinery. The absence of USP30 also results in the accumulation of PINK1 on the mitochondrial surface and induces autophagy of these import-compromised mitochondria [87–89]. It is likely that March5 and USP30 constitute a spillway mechanism that prevents overloading and jamming of the import machinery in mammalian cells.

Mitochondria-associated degradation (MAD)

MAD (also called outer mitochondrial membrane-associated degradation, OMMAD) is a quality control pathway for the surveillance and degradation of mitochondrial outer membrane proteins [90]. Conceptionally, it is similar to ERAD and MAD even shares some components with ERAD (Fig. 2C). In yeast, Doa1 was identified as Cdc48 cofactor that recruits the Cdc48-Ufd1-Npl4 complex to ubiquitinated mitochondrial outer membrane proteins, including Fzo1, Mdm34, Msp1, or Nde1 [91–94]. Ubiquitination of the yeast mitofusin Fzo1 (and other outer membrane proteins) is mediated by the E3 ubiquitin ligases Mdm30 and Rsp5, and reversed by the DUBs Ubp2 and Ubp12 [95,96]. This interplay controls the local abundance, distribution, and functional state of Fzo1 and thereby coordinates mitochondrial fusion [97].

In animals, the Fzo1-homologous mitofusins Mfn1 and Mfn2 are also degraded by MAD [98,99]. March5 ubiquitinates these mitofusins, as well as the mitochondrial dynamin-like protein Drp1 and the carrier-related outer membrane protein SLC25A46 (the homolog of the yeast protein Ugo1). March5-mediated ubiquitination thereby influences mitochondrial morphology as well as mitochondria-ER contact sites [100–103]. Interestingly, high levels of March5 also provide resistance against some cytosolic aggregates suggesting that the relevance of the quality control system on the mitochondrial surface is not restricted to mitochondrial processes [104,105].

It should be noted here that MAD is sometimes used to describe exclusively the degradation of mature resident outer membrane proteins, but sometimes also used as an umbrella term for the proteasomal degradation of mitochondrial proteins in general, so that mitoTAD and mitoCPR might be regarded as subcategories of MAD (Table 1).

Proteasomal degradation of mitochondrial proteins might not be restricted to proteins of the outer membrane. The proteasome also degrades IMS proteins that were exported from mitochondria in a process called retrotranslocation (Fig. 2D). *In vitro*, retrotranslocation can be induced by reduction in the disulfide bonds in Mia40 substrates [106,107]. Upon (partial) unfolding, these proteins enter the protein-conducting channel of the TOM complex from the inside from where they are extracted by the UPS in the cytosol.

The proteasome as competitor

The mass of mitochondria in cells can be highly variable. In some cell types such as human brown adipose tissue cells and respiring yeast cells, mitochondrial proteins can make up more than 30% of the entire protein mass [18,108–110]. When yeast cells switch from fermentation to respiration, the volume of mitochondria expands more than sevenfold within a rather short time [110]. How cells cope with this sudden burst of newly synthesized mitochondrial precursor proteins is unknown. However, there is evidence that precursors that accumulate in the cytosol are rapidly degraded by the proteasome [65–69]. Thus, at least under certain physiological conditions, the UPS competes with the

Table 1. Components involved in the proteasomal degradation of mitochondrial proteins.

Function	Yeast	Mammals	Comments
MAD, mitochondria-associated degradation			
mitoCPR, mitochondrial compromised protein import response			
Transcription factor	Pdr3		Transcription factor of pleiotropic drug resistance
Msp1 recruitment	Cis1		Linker of Msp1 and Tom70. Not expressed under nonstress conditions
AAA ATPase	Msp1	ATAD1	Outer membrane-bound extractor
mitoTAD, mitochondrial protein translocation-associated degradation			
E3 ubiquitin ligase		March5/MITOL	Outer membrane protein, not only for translocation intermediates
DUB		USP30	Outer membrane (and peroxisomal) protein
AAA ATPases	Cdc48	p97	Extractor, unfoldase
Cdc48 recruitment	Ubx2		Outer membrane and ER protein
Cdc48 cofactor	Ufd1	UFD1L	
Cdc48 cofactor	Npl4	NPL4	
Other factors			
E3 ubiquitin ligase	Mdm30		Outer membrane protein
E3 ubiquitin ligase	Rsp5		Essential protein with multiple functions
E3 ubiquitin ligase		Parkin	Accumulates on compromised mitochondria
DUB	Ubp2		Cytosolic ubiquitin protease that counteracts Rsp5
DUB	Ubp12		Cytosolic ubiquitin protease
DUB		USP30	Outer membrane (and peroxisomal) protein
Cdc48 recruitment	Vms1	ANKZF1/VMS1	Peptidyl hydrolase which also binds Cdc48-Npl4
Regulatory factors			
Transcription factor	Rpn4		Major regulator of proteasome expression
Transcription factor		Nrf1, Nrf2	Regulators of proteasome expression
Assembly factor	Poc4		Control factor for ribosome assembly

mitochondrial import machinery for the same pool of newly synthesized mitochondrial precursor proteins.

In mammalian cells, members of the ubiquilin protein family regulate the fate of precursors, either entry in a productive import pathway or degradation by the proteasome [111]. Ubiquilins bind precursor proteins, in particular those with hydrophobic stretches, prevent their aggregation, and promote their import into mitochondria. However, if the transfer to mitochondria does not occur rapidly, ubiquilins recruit E3 ligases to ubiquitinate their clients and thereby promote their degradation. Thus, ubiquilins serve as timers in the targeting reaction (Fig. 2A). In the absence of ubiquilins, such as in mutants, or if ubiquilins are sequestered by cytosolic polyglutamine aggregates, precursor proteins lose their import competence and become insoluble. Interestingly, if B cells lack their main ubiquilin protein Ubqln1, the accumulating precursor proteins do not impair mitochondrial functionality but induce arrest of the cell cycle [112]. Obviously, it is the predominant function of ubiquilins to prevent the toxicity in the cytosol and nucleus, whereas their relevance for mitochondrial protein targeting seems less immediate.

IMS proteins that are imported by the Mia40 pathway are apparently especially vulnerable for premature proteasomal degradation [38,39,106,113,114]. The individual stability of cytosolic precursors of Mia40 substrates differs considerably, and some even contain dedicated stabilizing sequences to prevent proteasomal degradation [68,114,115].

In certain cases, the proteasomal degradation of Mia40 substrates can compromise mitochondrial functionality. A recent study identified a mutated form of the human cytochrome *c* oxidase assembly factor 7 (COA7, also known as RESA1) as cause for mitochondrial leukoencephalopathy and complex IV deficiency [116–118]. COA7 is a Mia40 substrate and serves as assembly factor of complex IV. The import velocity of the mutant COA7 form found in patients is considerably reduced. As a consequence, the mutated COA7 is degraded in the cytosol by the proteasome before the proteins finds its way into the IMS. Surprisingly, inhibition of the proteasome restores the mitochondrial accumulation of the mutated COA7 and its function in complex IV assembly [65]. Thus, the respiration defect in this mutant is largely cured by suppression of proteasomal activity. Whether the inhibition of proteasomal degradation can serve as strategy to cure diseases that are associated with reduced mitochondrial import efficiency will have to be tested in the future.

Mitochondrial import regulates proteasomal capacity

A sophisticated response network adjusts the level of proteasomal activity to the performance of the mitochondrial protein import system. The accumulation of nonimported precursor proteins in the cytosol leads to proteotoxic stress and impairs cell growth. In the last years, several abbreviations for this situation and for the respective cellular responses were introduced. In particular, the *unfolded protein response activated by mistargeting of proteins* (UPRam) was introduced for a precursor-induced increase in the proteasome capacity and a simultaneous reduction in cytosolic protein synthesis [67,119]. The *mitochondrial precursor overaccumulation stress* (mPOS) program refers to the response of cells to the accumulation of toxic inner membrane carriers [69,120], and, as described above, mitoCPR describes the Cis1/Msp1-mediated extraction of stop-transfer proteins [81]. Despite all these different names, these stress reactions are presumably all elements of one overarching response referred to as the *mitoprotein-induced stress response* [82,121,122]. This response increases the capacity of the cytosolic UPS and induces the proteolytic degradation of proteins that are stuck in the TOM complex.

Detailed studies in yeast could elucidate the initial cascade of reactions induced by the sudden accumulation of mitochondrial precursor proteins [82]. These reactions are in part similar to the canonical heat-shock response [123] but also contain mitochondria-specific reactions. Under nonstress conditions (Fig. 3A), mitochondrial precursors are rapidly imported and interact only transiently with cytosolic chaperones. However, upon mitoprotein-induced stress conditions (Fig. 3B), the accumulation of precursor proteins sequesters cytosolic chaperones which activates the heat-shock factor Hsf1. As a consequence, Hsf1 induces the expression of chaperones and of the transcription factor Rpn4, which in turn stimulates the expression of proteasome subunits and other proteins

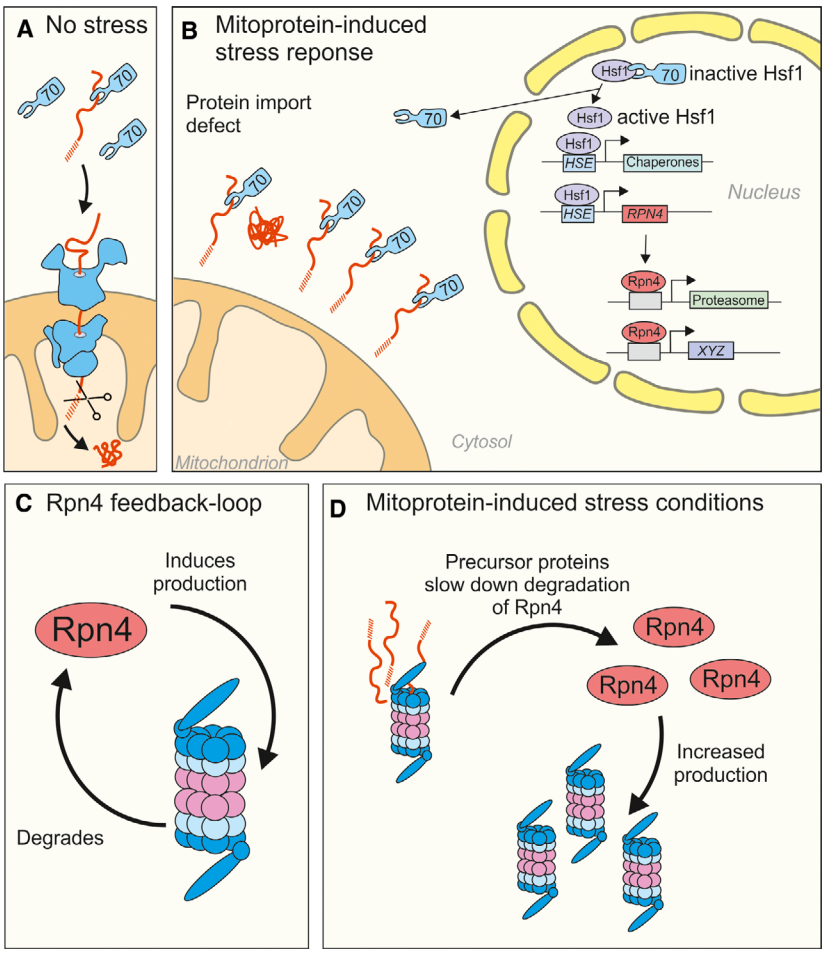

Fig. 3. Regulation of proteasomal capacity by the mitochondrial import performance. (A) Under nonstress conditions, mitochondrial precursors only transiently associate with cytosolic chaperones. (B) Accumulating precursor proteins sequester chaperones and thereby induce binding of the heat-shock factor Hsf1 to heat-shock elements in the genome. This induces chaperones and Rpn4, a transcription factor for the expression of the proteasome and many other proteins (here labeled as XYZ). (C) Rpn4 is controlled by a feedback loop. High proteasome levels lead to rapid Rpn4 degradation and, as a consequence, low levels of proteasome synthesis. Vice versa, low proteasome levels induce proteasome expression by Rpn4. (D) When proteasomal function is inhibited or overloaded, Rpn4 accumulates and induces the expression of proteasomal subunits. Mitochondrial precursors might induce Rpn4 stabilization although this interplay is not carefully studied yet.

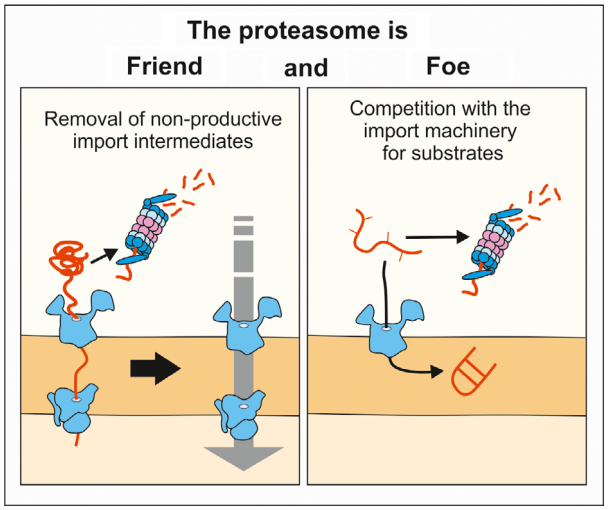

Fig. 4. The proteasome supports mitochondrial protein import, but also competes with it. The UPS removes missorted proteins or nonproductive import intermediates from the mitochondrial surface and thereby keeps the import machinery functional. However, precursors are also removed by the proteasome if import is slow. This competition might be particularly relevant for IMS proteins that are imported by the MIA pathway as these precursors explore the cytosol for several minutes before they cross the outer membrane.

of the UPS [124]. Rpn4 also induces the Pdr3 transcription factor leading to mitoCPR [82].

Rpn4 levels are controlled by an autoregulatory feedback loop: Since Rpn4 is quickly degraded by the proteasome, low proteasome levels increase Rpn4 amounts, whereas high proteasomal activity removes Rpn4 (Fig. 3C). It is therefore likely that the accumulation of cytosolic precursors that occupy the proteasome will directly increase Rpn4 independently of Hsf1 (Fig. 3D).

Mammalian cells lack homologs of Rpn4; however, the transcription factors Nrf1 and Nrf2 play comparable roles as major regulators of proteasome synthesis [45]. Nrf1 is bound to the ER surface and degraded by the proteasome in a p97-mediated reaction [125,126]. The relevance of mitochondrial precursors for Nrf1-mediated UPS induction was not analyzed in detail, but a recent study showed that brown adipose tissue cells depend on Nrf1 to adjust proteasome levels to different metabolic conditions, presumably as a consequence of mitoprotein-induced stress conditions [127].

The implications of mitochondria for the activation of Nrf2 are much better understood: In this case, the production of superoxide and hydrogen peroxide by mitochondria triggers Nrf2-mediated transcription of proteasomal subunits [128,129]. Under nonstress conditions, Nrf2 is ubiquitinated by Keap1 and rapidly degraded. Upon oxidative stress conditions, Keap1 is inactivated and Nrf2 escapes degradation and induces gene expression in the nucleus. Interestingly, not only proteasomal genes are under control of Nrf1 and Nrf2, but also components of the mitochondrial import machinery such as Tom20 [130]. This suggests that mammalian cells increase the protein import capacity of mitochondria together with that of the proteasome in order to prevent precursor degradation.

A comparable tight correlation of proteasome activation and modulation of mitochondrial import capacity was also recently discovered in yeast cells [131]. Here, the Rpn4-induced IMS protein Mix23 modulates mitochondrial import activity, but mechanistic details are still unclear.

In summary, high proteasome activity removes problematic proteins from the cytosol and thereby improves cytosolic proteostasis. However, at the same time, overactive proteasomes might jeopardize efficient import of (certain) mitochondrial precursors, which would put mitochondrial functionality at risk. Eukaryotic cells therefore employ a number of efficient mechanisms to precisely maintain the balance between the positive and negative effects of proteasomal activity.

Relevance for disease

The proteasome controls a wide range of vital processes, which include cell cycle, DNA repair, transcriptional regulation, signaling, trafficking, and apoptosis [132]. Given the essential nature of these functions and the central role of the proteasome in cellular quality control, it is no wonder that proteasomal dysfunction is associated with a plethora of pathological defects, particularly those associated with aging processes. During aging, proteasomal activity decreases and proteostasis declines [133]. Although this unfavorable development occurs in every aging individual, the consequences of proteotoxicity are particularly obvious and pronounced in the context of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease, or frontotemporal dementia. The common denominator of these pathologies is the abnormal accumulation of protein aggregates. In neurons of patients suffering from these diseases, oligomeric states of superoxide dismutase, Aβ, tau, α-synuclein, TDP-43, and other proteins, interfere with mitochondrial functionality. These aggregates can sequester chaperones, thereby preventing binding to precursor proteins [134,135] or directly associate with precursor proteins. For example, it was reported that oligomeric α-synuclein traps precursors of the mitochondrial protein

Hsp10 and thereby induces a Hsp60/Hsp10 chaperonin deficiency in mitochondria of aging neurons [136]. Aggregated proteins can also bind to the mitochondrial surface and block TOM receptors or other components of the import machinery [137–143]. Thus, clearance of aggregated proteins from neurons is crucial to maintain mitochondrial functionality.

The central relevance of the proteasome for aging cells is not limited to neurons, but also well established for the skeletal and cardiac muscle cells of. Owing to their permanent activity, their high energy consumption, and mechanical stress conditions, (cardio)myocytes are indeed particularly vulnerable to proteotoxic stress [13,144,145].

Interestingly, all cell types that are of particular relevance for aging have a high energy demand and a relatively large mitochondrial content. Since mitochondrial biogenesis strongly relies on surveillance by the proteasome, the decline in proteasomal activity with age presumably leads to devastating consequences such as the decline of mitochondrial functionality on the one hand and the decline of cellular proteostasis on the other. Obviously, these two hallmarks of aging are more closely related than previously expected.

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