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Interplay between the Ubiquitin Proteasome System and Mitochondria for Protein Homeostasis

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Abstract

Eukaryotic cells are subdivided into membrane-bound compartments specialized in different cellular functions and requiring dedicated sets of proteins. Although cells developed compartment-specific mechanisms for protein quality control, chaperones and ubiquitin are generally required for maintaining cellular proteostasis. Proteotoxic stress is signalled from one compartment into another to adjust the cellular stress response. Moreover, transport of misfolded proteins between different compartments can buffer local defects in protein quality control. Mitochondria are special organelles in that they possess an own expression, folding and proteolytic machinery, of bacterial origin, which do not have ubiquitin. Nevertheless, the importance of extensive cross-talk between mitochondria and other subcellular compartments is increasingly clear. Here, we will present local quality control mechanisms and discuss how cellular proteostasis is affected by the interplay between mitochondria and the ubiquitin proteasome system.

Introduction

In order to fulfil their biological function, proteins must fold into their native three-dimensional structures and organelles need to function properly. The factors controlling protein homeostasis processes are collectively termed the proteostasis network (Klaips *et al.*, 2018). In sum, cells must ensure either proper protein folding or -if this fails- undertake efficient elimination of malfunctioning proteins or damaged organelles. A prominent role in proteostasis is ensured by ATP-dependent cellular machineries dedicated to proper protein folding, called chaperones (Hartl *et al.*, 2011). In turn, the central proteolytic components of this network are the ubiquitin proteasome system (UPS), a soluble machinery, and the lysosomes, organelles that enclose peptidases with a powerful and non-specific lytic capacity (Bard *et al.*, 2018). Consequently, the UPS is the main proteolytic pathway of the cell for cytosolic substrates, being the lysosomes generally responsible for the clearance of membrane proteins, entire organelles and large protein aggregates (Kerscher *et al.*, 2006; Amm *et al.*, 2014). Both processes

rely on the ear-marking of the desired protein with ubiquitin. In addition, autophagy requires the ubiquitin-like proteins or Atg8(yeast)/LC3(mammals).

Organelles, despite being functional units with dedicated roles, must respond to their cellular environment. Mitochondria – the cellular energy powerhouse – are special semi-autonomous organelles, which evolved through a symbiotic event of alpha-proteobacterium at the origin of a eukaryotic cell (Zimorski *et al.*, 2014). Mitochondria possess an own DNA and the machineries allowing its replication, transcription and translation, originating from their bacterial ancestors, thus resembling the ones from free living prokaryotes (Falkenberg *et al.*, 2007; D'Souza and Minczuk, 2018). During evolution of this endosymbiotic process, most of the mitochondrial genetic information was transferred to the nucleus. This means that most proteins located at mitochondria need to be imported from the cytoplasm, rendering mitochondrial quality control processes fundamentally important for the biogenesis of this essential organelle (Pfanner *et al.*, 2019). Therefore, the presence at mitochondria of specific chaperones and proteases is not surprising. As mentioned, these are closely homologous to their bacterial relatives and make up for local protein quality control (Voos *et al.*, 2016). Perhaps for this reason, it was long assumed that mitochondrial proteostasis was ubiquitin-independent. Instead, it is now clear that mitochondrial stress is also engaging cytosolic and lysosomal proteostasis networks, including ubiquitin and the UPS but also Atg8/LC3 and the lysosomes (Germain, 2008; Escobar-Henriques and Langer, 2014; Topf *et al.*, 2016; Braun and Westermann, 2017; D'Amico *et al.*, 2017). The most prominent example is certainly the mitophagy process, where damaged mitochondria are selectively degraded (McWilliams and Muqit, 2017). Reciprocally, mitochondria also sense and regulate external stress, clearly impacting on cellular homeostasis and longevity and certainly relevant for neurodegeneration (Chung *et al.*, 2018; Guaragnella *et al.*, 2018; Ruan *et al.*, 2018).

In this review, we describe novel insights on how mitochondria crosstalk and bi-directionally cooperate with their cellular environment to deal with proteotoxic stress (see Fig. 12.1). After a general overview on quality control – cytosolic and mitochondrial – we then describe emerging cross-functional concepts. First, we focus on the role of mitochondria in coping with excessive cytosolic proteostasis. These findings illustrate the proteolytic power of mitochondria, which does not depend on ubiquitin. Second, we describe several cytosolic and ubiquitin-dependent pathways engaging on mitochondria. We present integrated cellular responses, requiring ubiquitin and the proteasome or the autophagic marker LC3 and lysosomes, which contribute to alleviate mitochondrial stress. Finally, we present the dual role of the peptidyl-tRNA hydrolase Vms1 (yeast)/ANKZF1 (mammals) in ribosomal quality control and mitochondrial proteostasis, being both processes regulated by ubiquitin.

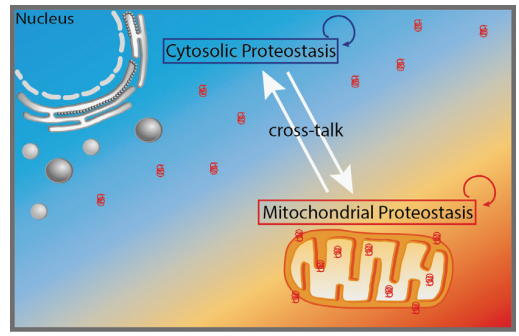

Figure 12.1 Crosstalk of cytosolic and mitochondrial proteostasis. Protein aggregates or malfunctional proteins activate different proteolytic pathways for their clearance. Mitochondria as well as the cytosol harbour their own proteolytic machineries providing clearance of damaged proteins. Nevertheless, an extensive interplay between mitochondria and their environment is key to maintain cellular homeostasis.

Principles of protein quality control – cytosolic and mitochondrial

Aberrant folding or unfolding does not only compromise the affected protein but is also accompanied with a great risk of disrupting the functionality of other proteins, by undergoing non-specific protein–protein interactions. Especially metastable proteins with disordered regions (up to 30% of the mammalian proteome) are prone to undergo unwanted interactions and form toxic protein aggregates, which are associated with

neurodegenerative diseases (Dunker *et al.*, 2008). This underlines the broad importance of proteome surveillance. In addition, the vast majority of proteins are synthesized by cytosolic ribosomes, followed by post- or co-translational transport of proteins to their final destination (Dudek *et al.*, 2013). Thus, the cytosolic quality control machineries are essential for the integrity of the entire cellular proteome.

Quality control components in the cytoplasm

Protein folding

Chaperones are central players in protein quality control, which support other proteins in acquiring their functional conformation, without usually being present in the final structure (Hartl, 1996). Unfolded proteins expose hydrophobic residues, normally buried inside their three-dimensional structure, being such non-native regions recognized by chaperones. Chaperones promote folding by ATP dependent cycles of binding and release of their substrate proteins, till they reach their native state. Thus, by assisting in protein folding, chaperones prevent unspecific interactions and protein aggregation and refold stress-denatured proteins. However, if encountering terminally misfolded proteins, chaperones also cooperate with proteolytic machineries in their degradation (Tyedmers *et al.*, 2010; Balchin *et al.*, 2016).

Hsp70 chaperones

Hsp70 chaperones and their co-factors constitute major components for protein quality control (Kityk *et al.*, 2015). Hsp70 binds to substrates in an open, ATP bound conformation. The substrate-binding pocket is closed on ATP hydrolysis and release of ADP results in substrate release. In addition to refolding soluble proteins, Hsp70 supports protein import into cellular compartments such as the endoplasmic reticulum and mitochondria, where the proteins have to pass membranes in an unfolded state, through an import channel (Craig, 2018). Moreover, Hsp70 support protein degradation machineries requiring soluble and at least partially unfolded proteins, like the 26S proteasome (Fernández-Fernández *et al.*, 2017). The binding of Hsp70 shields hydrophobic regions in non-native proteins, thereby preventing non-specific interactions, until proteins reach their native state and/or final destination.

The intrinsic activity of Hsp70 alone is low and therefore folding requires the help of additional factors. On the one hand, efficient Hsp70 function requires one of several structurally unrelated nucleotide exchange factors (NEFs), which promote the exchange between ADP and ATP. On the other hand, ATPase activity is stimulated by Hsp40 chaperones, also called J-proteins, which bind the substrates and deliver them to Hsp70, thus avoiding their aggregation. Therefore, substrate specificity of Hsp70 is mainly determined by Hsp40 chaperones (Kampinga and Craig, 2010). These often contain substrate-binding domains themselves and mediate the transfer of substrates to Hsp70, depending on their J-domain. In addition, several specialized Hsp40s lack a substrate-binding domain but localize Hsp70 within the cell to the vicinity of certain substrates. For instance, the Hsp40 Zuo1 targets cytosolic Hsp70 to the ribosomal exit tunnel to aid in folding of nascent proteins (Yan *et al.*, 1998; Gautschi *et al.*, 2001). In sum, Hsp40 chaperones, in conjunction with NEFs, are responsible for the versatile functions exerted by the Hsp70 system (Fig. 12.2).

Protein ubiquitination and turnover by the UPS

When proteins cannot reach their native conformation, due to mutations or exogenous stresses, they might interfere with the function or folding of other proteins, and thus have to be separated from the rest of the proteome. This can be achieved by sequestration into inclusions or through proteolytic breakdown (Fig. 12.2). The main machinery degrading soluble proteins, in the cytosol and in the nucleus, is the ubiquitin proteasome system (UPS) (Kerscher *et al.*, 2006; Amm *et al.*, 2014). Turnover of proteins generally requires them being tagged with ubiquitin, a highly conserved small protein of 76 aa. It occurs by the covalent attachment of ubiquitin to lysine residues in target proteins (termed ubiquitination). Substrate ubiquitination is mediated by an enzymatic cascade, involving an ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin protein ligases (E3). Specificity towards individual

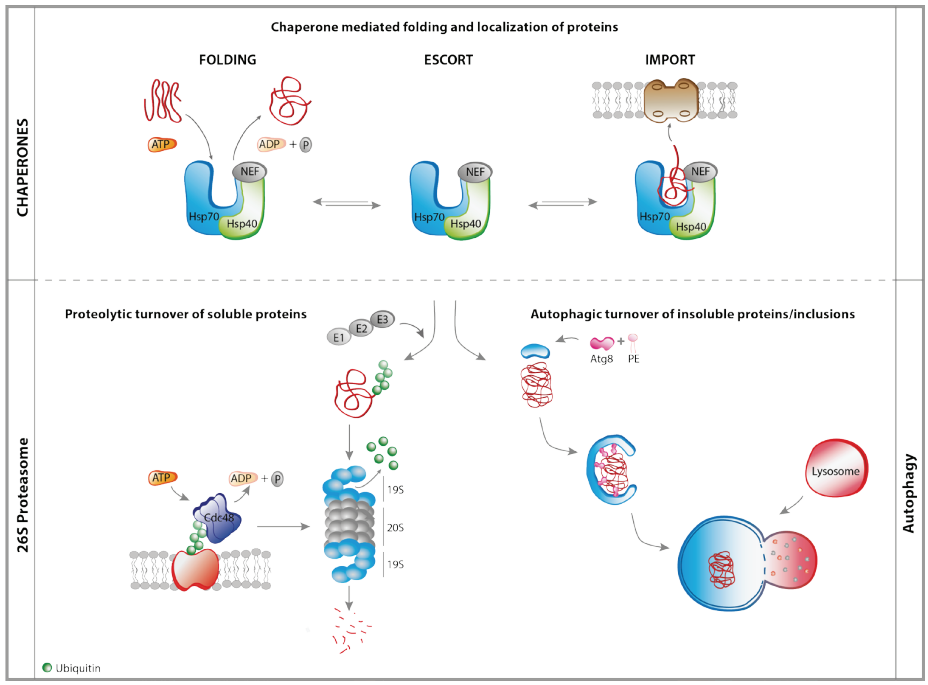

Figure 12.2 Chaperones, ubiquitin–proteasome system and autophagy. Different layers of quality control machineries maintain cellular protein homeostasis. As a first layer, the chaperone Hsp70 as well as its co-factors (Hsp40) and nucleotide exchange factors (NEF) mediate protein folding, escort target proteins to their destination and support import of nascent polypeptide chains into organelles. The ubiquitin proteasome system (UPS, 26S Proteasome, consisting of its 19S regulatory particle and the 20S proteolytic core) represents the second layer of protein quality control. Substrate proteins targeted with the small modifier ubiquitin (E1, E2 and E3 enzymes mediate covalent attachment of ubiquitin to the substrate) are degraded by the 26S proteasome, whereby ubiquitin itself is recycled. The accessibility of defective proteins to the 26S proteasome is supported by the AAA-ATPase Cdc48, which extracts ubiquitinated membrane proteins. As a third layer, protein aggregates/inclusion, organelles or pathogens are targeted to autophagy. This requires the engulfment by an autophagosome, which expands around the substrate by the lipidation of the ubiquitin-like modifier Atg8 with phosphatidylethanolamine (PE). Subsequently, the autophagosome fuses with a cellular lysosome, where final degradation occurs. (NEF, nucleotide exchange factor; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P, phosphate).

substrates, or target recognition, is mostly mediated by the E3 ligase enzymes. Ubiquitination can either result in the attachment of single ubiquitin moieties (mono-ubiquitination), or chains build on lysine residues within ubiquitin itself (poly-ubiquitination). The seven lysine residues of ubiquitin allow the formation of different types of ubiquitin-chains. Often, these serve as targets for different ubiquitin-linkage specific binding proteins, thereby dictating the downstream consequences of ubiquitination. Finally, ubiquitin is a reversible process, being cleaved by ubiquitin-specific peptidases called deubiquitinases (Komander and Rape, 2012). Ubiquitin chains formed via lysine 48 of ubiquitin are the canonical signal targeting proteins for degradation by the 26S proteasome. This protease complex degrades proteins into short peptides by multiple proteolytic activities within its barrel shaped 20S core particle, being its 19S particle responsible for regulatory functions (Fig. 12.2).

Role of Cdc48/p97/VCP

The 26S proteasome is only able to degrade soluble proteins. Thus, substrates bound to larger structures, such as protein complexes, or embedded into membranes, need to be extracted before degradation, with the help of accessory factors (Fig. 12.2). The main component of the UPS exerting this function

is the ATPase and ubiquitin-dedicated chaperone Cdc48 (p97/VCP in mammals). Cdc48 segregates proteins by an ATPase driven mechanism, thereby allowing proteolysis by the 26S proteasome. Cdc48 assembles with several ubiquitin-binding co-factors, which assist it in substrate recognition. Moreover, Cdc48 also directly binds ubiquitin-ligases and deubiquitinating proteins, thus acting as a general hub in ubiquitin related processes (van den Boom and Meyer, 2018).

Autophagy

In contrast to soluble proteins, larger structures are refractory to proteasomal turnover. Interestingly, proteasomes can be much smaller in size than protein aggregates. In fact, cryo-EM structures identified entire proteasomes incorporated into the structure of a sub-set of large protein aggregates (Guo *et al.*, 2018). While being consistent with biomedical data suggesting up to 50% proteasome entanglement in neurons, these structures also possibly explain reduced proteasomal activity in neurodegeneration (Pontano Vaites and Harper, 2018).

Protein aggregates can be targeted for degradation inside the lysosomes, organelles that are called vacuoles in yeast and plants (Khaminets *et al.*, 2016). In this process, termed macroautophagy (hereafter autophagy), substrates are engulfed by double-membrane bound autophagosomes, which subsequently fuse with the vacuole/lysosome (Fig. 12.2). Vacuoles are acidic compartments containing promiscuous proteolytic enzymes that then deconstruct the engulfed substrates. Similarly to the UPS substrates, which are ear-marked by ubiquitin, the autophagosome membrane is ear-marked by the small ubiquitin-like modifier Atg8 (LC3 in mammals). Atg8 is a cytosolic protein that gets covalently conjugated to the lipid phosphatidylethanolamine at the autophagosomal membrane, on induction of autophagy. In addition to aggregated proteins, a broad range of substrates can be targeted for turnover by autophagy, including entire organelles or pathogens. These pathways of selective autophagy utilize specific autophagy receptors, which characteristically have a dual organization, consisting of a substrate recognition domain and an Atg8 interacting motif. Therefore, autophagy receptors promote the engulfment of their substrates by bridging the autophagosomal membrane to the target substrates. For example, the selective turnover of mitochondria, or mitophagy, depends on the ubiquitination of a myriad of substrates at the mitochondrial outer membrane, which engage several autophagy receptors like Optineurin, NDP52 and p62 (Geisler *et al.*, 2010; Narendra *et al.*, 2010; Lazarou *et al.*, 2015; Khaminets *et al.*, 2016; McWilliams and Muqit, 2017). Moreover, similar to misfolded soluble substrates, modification by ubiquitin of protein aggregates targets them for degradation by selective autophagy, utilizing specific receptors containing ubiquitin-binding domains. For example, Hsp42 dependent aggregate formation has been shown to be required for the turnover of defective proteasome subunits by autophagy (Marshall *et al.*, 2016). This common feature of the UPS and selective autophagy ensures efficient degradation of aberrant proteins once they have been tagged for degradation (Lu *et al.*, 2017).

Protein inclusions

When efficient degradation fails, especially during acute stress or when the proteostasis network is perturbed, proteins are sequestered into inclusions, thereby minimizing their reactive surface compared to soluble proteins (Miller *et al.*, 2015; Sontag *et al.*, 2017). Such inclusions are often transient structures, which can either be resolved by disaggregating chaperones, or instead be degraded by selective autophagy, in case they persist in the cytoplasm.

General features of mitochondria

Mitochondrial functions

Mitochondria are central organelles of all eukaryotic cells, functioning as energy-converting powerhouses, metabolic factories and signalling centres (McBride *et al.*, 2006; Nunnari and Suomalainen, 2012). They are required for oxidative phosphorylation (OXPHOS), thus being known as the ATP powerhouse. In addition, mitochondria are key for many metabolic processes, like the synthesis of phospholipids (Silva Ramos *et al.*, 2016; Tatsuta and Langer, 2017). Moreover, the assembly of iron–sulfur-clusters (essential enzymatic cofactors) starts within mitochondria, reason why these organelles are essential for cellular viability (Braymer and Lill, 2017; Cardenas-Rodriguez *et al.*, 2018). Finally, mitochondria are active components of many

signalling pathways, such as programmed cell death, ageing, cellular differentiation and organism development (Green *et al.*, 2014; Kauppila *et al.*, 2017; Noguchi and Kasahara, 2018; Pallafacchina *et al.*, 2018; Paupe and Prudent, 2018; Zhang *et al.*, 2018).

Sub-compartmentalization of mitochondria

Mitochondria are bound by two separate membranes, the outer mitochondrial membrane and the inner mitochondrial membrane (Jakobs and Wurm, 2014; Schorr and van der Laan, 2018). The two compartments bound by these membranes are called intermembrane space and matrix. The inner membrane forms large invaginations, called cristae, harbouring the respiratory OXPHOS chain complexes. Moreover, the part of the inner membrane that lines parallel to the outer membrane is called inner boundary membrane. Finally, cristae and inner boundary membrane are connected at cristae junctions, and the outer membrane and the inner boundary membrane make close contacts, termed contact sites. Due to their high biosynthetic demands, mitochondria are extremely rich in proteins, many of which are assembled into large complexes, often embedded into the mitochondrial inner and outer membrane, respectively. Given that 99% of mitochondrial proteins are nuclear-encoded, most of the organellar proteome needs to be post-translationally imported into the respective sub-compartment within mitochondria (Harbauer *et al.*, 2014).

Import of mitochondrial proteins

Proteins targeted to mitochondria are mainly imported via two channels spanning both mitochondrial membranes, called TOM (translocase of the outer membrane) and TIMs (translocases of the inner membrane) (Wasilewski *et al.*, 2017; Wiedemann and Pfanner, 2017; Pfanner *et al.*, 2019). Together with their interaction partners, these two channels allow directing each protein to their final subcellular destination. Once inside mitochondria, imported proteins must assemble with those encoded by the mitochondrial DNA, which in humans are 13. Interestingly, translation of nuclear-encoded and mitochondrial-encoded OXPHOS components coordinately adapt to metabolic conditions stimulating respiratory growth (Couvillion *et al.*, 2016). Moreover, this response was shown to be unidirectionally controlled by cytosolic translation components.

Mitochondrial protein quality control

Owing to the dimensions of the protein transport pores, proteins cross the membranes in an unfolded state. In addition, the unfolded import-competent state must be protected from non-native interactions. Therefore, chaperones – ensuring proper folding and assembly into active proteins – but also proteases -allowing to eliminate faulty polypeptides- are of extreme importance for mitochondrial protein quality control (Rugarli and Langer, 2012; Voos, 2013; Voos *et al.*, 2016) (Fig. 12.3). In addition, once proteins have reached their destination, compartment-specific mechanisms of protein quality control locally protect the proteome. Upon mitochondrial stress, several proteostasis networks have been nicely shown to operate at damaged mitochondria, for example to protect cells from death signals (D'Amico *et al.*, 2017; Priesnitz and Becker, 2018).

Chaperones guiding mitochondrial proteins

To support efficient import of proteins targeted to mitochondria, these are kept in the cytoplasm in an unfolded state by the cytosolic Hsp70 and Hsp90 machineries (Deshaies *et al.*, 1988; Young *et al.*, 2003; Craig, 2018). In turn, mitochondrial Hsp70 (mtHsp70) – residing at the matrix site of the TIM complex – is crucial for the import and subsequent folding of proteins into mitochondria (Kang *et al.*, 1990; Liu *et al.*, 2001; Schulz *et al.*, 2015). Protein folding inside the mitochondrial matrix largely also depends on the Hsp60-Hsp10 chaperonin, a member of the GroEL family of bacterial chaperones (Cheng *et al.*, 1989; Reading *et al.*, 1989). Importantly, the Hsp70 and Hsp60 systems are not only required for the folding of newly imported proteins, but also support refolding and prevent aggregation of unfolded proteins, which might occur upon proteotoxic stress (Kubo *et al.*, 1999; Bender *et al.*, 2011). Under conditions of severe protein folding stress, as for example acute heat stress, the abundance of unfolded proteins exceeds the capacity of mitochondrial chaperones to maintain these in a soluble state, resulting in protein aggregation. Such aggregates can be resolved

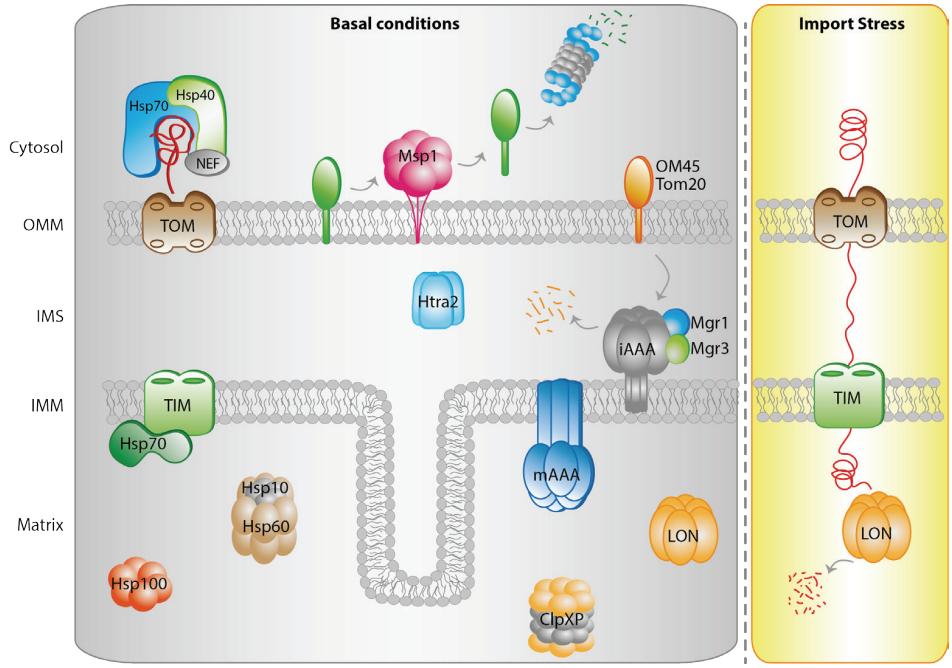

Figure 12.3 Mitochondrial quality control systems. For each individual compartment, mitochondria possess their own quality control proteins required for maintenance of the mitochondrial proteome. Within the matrix, mitochondrial Hsp70 and its cofactors (Hsp10 Hsp60, Hsp100) maintain proper protein folding after import. Turnover of misfolded proteins is regulated via the proteases LON and ClpXP. Proteolytic turnover of inner membrane proteins is mediated by m-AAA at the matrix side and i-AAA on the intermembrane side. The i-AAA protease maintains also proteolytic control of outer membrane proteins like Om45 and Tom20. The protease Htra2 maintains proteolysis of misfolded and damaged proteins in the intermembrane space. At the outer membrane, the AAA-ATPase Msp1 mediates segregation of outer membrane proteins to the cytosol, which results in subsequent proteasomal turnover. Polypeptides are imported via the TOM (translocase of the outer membrane) and TIM (translocase of the inner membrane) channels. On import stress, polypeptides reaching the matrix are degraded by the Lon protease.

by mitochondrial Hsp100 chaperones, which are required for efficient recovery from acute heat stress (Schmitt *et al.*, 1996) (Fig. 12.3).

Mitochondrial proteolytic machinery

Incorrect complex assembly or mis-targeting of membrane spanning proteins is especially prone to result in the accumulation of non-native proteins, which can undergo non-specific interactions, potentially detrimental for the cell. To remove misfolded proteins, mitochondria are equipped with a diversified set of proteases (Hamon *et al.*, 2015; Quirós *et al.*, 2015) (Fig. 12.3). The substrates targeted by mitochondrial proteases mainly depend on their sub-mitochondrial localization and structural properties. In general, mitochondria have ATP-dependent and independent proteases in all its sub-compartments. The first belong to the AAA+ superfamily, characterized by their oligomerization into a beta-barrel structure, enclosing a chamber with ATP-dependent pulling activity. In addition to the complete turnover of their substrates, mitochondrial proteases are also required to process and thus mature pre-proteins, for example by removing the mitochondrial targeting sequence.

The main protease degrading misfolded proteins in the mitochondrial matrix is Pim1/LON (Wagner *et al.*, 1994). In addition, the matrix protease ClpXP has been implicated in degradation of misfolded proteins, but its function is not yet well understood (Haynes *et al.*, 2007). Interestingly, a role of LonP1 and ClpP in regulating heteroplasmy was suggested (Latorre-Pellicer *et*

al., 2016). Indeed, human mitochondrial DNA shows extensive sequence variability, suggested to impact on mitochondrial proteostasis, dependent on LonP1 and ClpP. These findings have clinical implications, in what regards mitochondrial replacement therapies, which should be considered when choosing the mitochondrial DNA donor (Latorre-Pellicer *et al.*, 2016).

The mitochondrial inner membrane harbours two AAA proteases, which are anchored to the inner membrane by transmembrane domains (Gerdes *et al.*, 2012; Rugarli and Langer, 2012; Levytskyy *et al.*, 2017; Patron *et al.*, 2018). The m-AAA protease exposes its catalytic domain to the matrix, while the catalytic domain of the i-AAA protease faces the intermembrane space. These proteases degrade misfolded proteins of the inner membrane, being their substrate specificity mainly depending on the topology of the respective substrates (Leonhard *et al.*, 2000; Almajan *et al.*, 2012; Stiburek *et al.*, 2012; Anand *et al.*, 2014; Kondadi *et al.*, 2014; König *et al.*, 2016; Wai *et al.*, 2016; Wang *et al.*, 2016; Pareek *et al.*, 2018; Sprenger *et al.*, 2019). In the inner mitochondrial membrane space, misfolded and damaged proteins are degraded by the proteases Omi/HtrA2 and Atp23 (Osman *et al.*, 2007; Clausen *et al.*, 2011). At the outer membrane, proteins are surveilled by mitochondrial and cytoplasmic quality control machineries in parallel, as discussed later.

The unfolded protein response – UPR

Mitochondrial stress inhibits mitochondrial translation, but also impacts on nuclear expression. This was termed unfolded protein response (UPR) and depends on the transcription factor ATFS-1 (Nargund *et al.*, 2012; Jovaisaite and Auwerx, 2015; Münch and Harper, 2016; Higuchi-Sanabria *et al.*, 2018; Shpilka and Haynes, 2018). In intact mitochondria, ATFS-1 is imported into the matrix and degraded by the protease LON. However, upon import inhibition, ATFS-1 is diverted from the mitochondria to the nucleus. There, it up-regulates critical detoxifying genes, encoding proteins ensuring proper translation, folding and turnover at mitochondria, thus restoring mitochondrial homeostasis.

Mitochondrial roles in quality control of cytosolic components

Non-native proteins are a general threat for the cellular proteome and their spatial sequestration – into aggregates, inclusions or organelles – is a common strategy to limit such effects (Sontag *et al.*, 2017). Moreover, misfolded proteins are transported between organelles, as it has been shown for terminally misfolded cytosolic proteins, which are transported into the nucleus for degradation (Park *et al.*, 2013). Interestingly, novel roles of mitochondria in coping with cytosolic or cytosolic-exposed proteins have recently emerged.

Mitochondrial contributions to mitigate aggregation toxicity

Asymmetric inheritance of protein aggregates

It is known that protein aggregates that cannot be efficiently cleared by proteolytic systems are asymmetrically inherited during cell divisions, thereby ensuring that one cell deriving from such a division is free of damaged aggregated proteins (Aguilaniu *et al.*, 2003; Shcheprova *et al.*, 2008; Clay *et al.*, 2014; Coelho *et al.*, 2014; Hill *et al.*, 2017; Saarikangas *et al.*, 2017). Interestingly, an active role of mitochondria in restricting the mobility and thus inheritance of protein aggregates residing in the cytosol has been identified (Zhou *et al.*, 2014). In addition, proteins aggregated inside the matrix were sequestered into specific deposits that were also retained in the mother cell (Bruderek *et al.*, 2018). Finally, asymmetric inheritance depended on mitochondrial size and actively engaged the motor components involved in mitochondrial transport (Böckler *et al.*, 2017). Consistent with a cellular mechanism providing for rejuvenated daughter cells, a filtering process that prevented feeble mitochondria from being inherited had equally been shown (Higuchi *et al.*, 2013; Nyström, 2013). In contrast, however, under conditions of mild heat stress these damage-retention quality control mechanisms were inhibited. Instead, inheritance of toxic components to the daughter cell was promoted, which consequently increased longevity of the mother cell (Baldi *et al.*, 2017).

Import and turnover of cytosolic proteins in mitochondria

Recently, it has been observed that mitochondria can also function as a place to dispose misfolded cytosolic proteins under stress conditions. As previously mentioned, initially it had been observed that cytosolic protein aggregates are tethered to mitochondria, which facilitates asymmetric inheritance, keeping daughter cells free of the damaged proteins (Zhou *et al.*, 2014). Consistently, purification of such aggregates revealed a physical interaction with the mitochondrial import pore (Ruan *et al.*, 2017). Moreover, it was observed that the clearance of cytosolic aggregates was supported by import of cytosolic proteins into the mitochondrial matrix, where these proteins were handled by the Lon mitochondrial protease Pim1 (Ruan *et al.*, 2017). The mitochondrial import and clearance of cytosolic proteins was in particular observed on acute heat shock and inhibition of cytosolic Hsp70, suggesting that this pathway functions to buffer extensive cytosolic proteotoxic stress (Ruan *et al.*, 2017). The presence of ubiquitinated proteins inside mitochondria was suggested, which could perhaps result from similar surveillances principles (Lehmann *et al.*, 2016). However, this does not necessarily imply a functional role of ubiquitin inside mitochondria. Import of cytosolic aggregated proteins required the disaggregase Hsp104, probably through generating soluble proteins for mitochondrial import. Surprisingly, this process was independent of cytosolic Hsp70, which usually cooperates with Hsp104 (Ruan *et al.*, 2017). In conclusion, borrowing mitochondrial proteolytic capacity seems to have beneficial effects for stress-release of cytosolic protein load. Nevertheless, it remains an open question to which quantitative extend mitochondrial import of cytosolic proteins contributes to cytosolic proteostasis. In addition, it is still unclear if and how the import of aberrant proteins affects mitochondrial proteostasis and which mechanisms might protect mitochondria. For example, acute heat stress will also affect mitochondrial proteins and the additional uptake of non-native cytosolic proteins can be expected to pose a major challenge for the mitochondrial proteostasis network.

Turnover of cytosolic-exposed proteins by mitochondrial proteases

Transmembrane proteins residing at the outer membrane of mitochondria can, in principle, be degraded by outer membrane-embedded proteases, or be subject to membrane extraction to the cytoplasm or to mitochondria, for turnover. Interestingly, recent studies revealed a role of the i-AAA protease, or Yme1, for turnover of two outer membrane anchored proteins. Indeed, proteolysis of Tom22 and Om45 was independent of the proteasome pathway but instead depended on Yme1 (Wu *et al.*, 2018). In addition, proteolysis required substrate dislocation by Yme1, after recognition of their inner-membrane-space domains by the Mgr1/Mgr3 complex. Mgr1/Mgr3 interact with Yme1, thus enhancing its catalytic activity (Dunn *et al.*, 2008). These findings show a cross-membrane mechanism for proteolytic control at mitochondria.

Quality control of mitochondrial proteins in the cytoplasm

Defects in mitochondrial targeting and import of proteins result in mis-localization of mitochondrial precursor proteins to the cytosol. Multiple concerted responses operating at the mitochondrial surface are now shown, allowing these proteins to be degraded in the cytosol by the UPS.

Rescue of mitochondrial import overload by cytosolic machineries

The import machinery at the outer membrane has an upfront role in determining mitochondrial biogenesis. Indeed, it is now clear that the import process is highly regulated, both under physiological and pathophysiological conditions (Harbauer *et al.*, 2014). Moreover, it plays critical roles in surveilling translocation quality and in signalling import stress (Wasilewski *et al.*, 2017).

Pre-import chaperones

The classical cytosolic Hsp70 and Hsp90 chaperones, their co-factors Sti1 and Ydj1, and ubiquilins (chaperone-like factors), associate with mitochondrial pre-proteins and also physically interact with the outer membrane components of the mitochondrial import channel (Deshaies *et al.*, 1988; Young

et al., 2003; Hoseini *et al.*, 2016; Zanphorlin *et al.*, 2016; Jores *et al.*, 2018; Opaliński *et al.*, 2018). Supporting these physical interaction evidences, a genetic synthetic growth defect was observed between *TOM* and *STI1*, which encode protein forming an important scaffold, by simultaneously binding to Hsp70 and Hsp90 (Hoseini *et al.*, 2016). Moreover, among the TOM components, a prominent role of Tom70 in import control has been suggested (Backes *et al.*, 2018; Hansen *et al.*, 2018; Opaliński *et al.*, 2018). In fact, Tom70 has a tetratricopeptide repeat, a domain known to bind to Hsp90 (Zanphorlin *et al.*, 2016). Consistently, chemical inhibition of the Hsp70/90 interaction with Tom70 reduced the mitochondrial association of protein aggregates (Pavlov *et al.*, 2018).

Dual role of ubiquilins in control of protein import

Ubiquilins were proposed to be involved at the earlier steps of mitochondrial protein biogenesis (Itakura *et al.*, 2016; Whiteley *et al.*, 2017). Ubiquilins are substrate receptors for proteasomal degradation, typically harbouring a ubiquitin-binding (UBA) domain for recognition of ubiquitinated cargo and a ubiquitin like domain, which is required for proteasomal targeting (Buchberger, 2002; Funakoshi *et al.*, 2002). In line with their canonical function, they have been implicated in targeting mislocalized mitochondrial precursor proteins to the 26S proteasome for degradation (Itakura *et al.*, 2016; Whiteley *et al.*, 2017). In addition, by binding to mitochondria-targeted membrane proteins, ubiquilins prevent their aggregation, thus exerting a chaperone like function. At this step ubiquilin binding still allows correct targeting of the bound protein. However, prolonged binding will result in ubiquitination of the bound protein by ubiquitin-ligases recruited by the UBA domain, and subsequent targeting for degradation (Itakura *et al.*, 2016). Thus, ubiquilins not only function in targeting already ubiquitinated substrates for turnover but themselves exert an important role in triage of mitochondrial proteins (Fig. 12.4).

Recognition of J-proteins by mitochondrial receptors

Recent findings shed additional light on the early steps of mitochondrial protein biogenesis, by identifying how – once translated – mitochondrial proteins are targeted intracellularly to the surface of the organelle (Hansen *et al.*, 2018; Opaliński *et al.*, 2018). Djp1, a J-protein that localizes to the surface of the endoplasmic reticulum, was found to contribute to mitochondrial protein import, in cooperation with pre-protein receptors. Therefore,

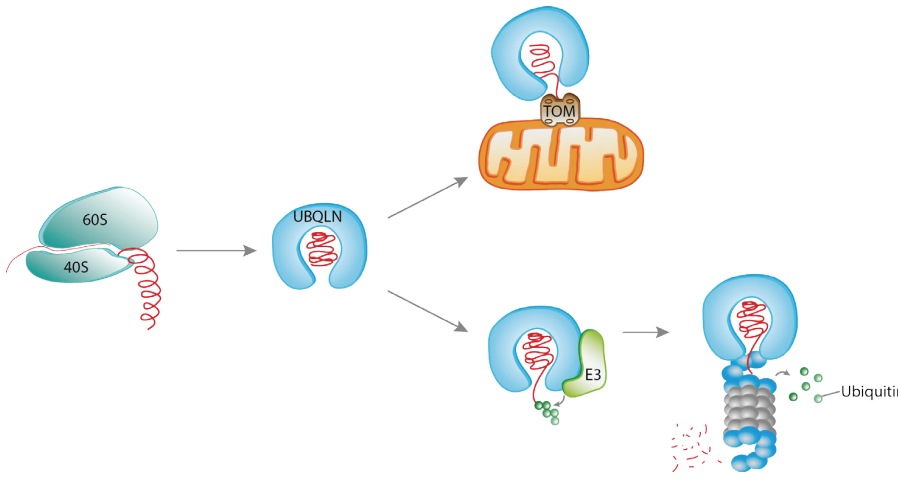

Figure 12.4 Dual role of ubiquilins in control of mitochondrial pre-proteins. The ubiquitine-proteasome receptors Ubiquilin 1 and 2 (UBQLN) guide mitochondrial membrane proteins after translation to the import channel. However, on prolonged binding, UBQLNs recruit E1, E2 and E3 ligase enzymes, required for their ubiquitination, allowing subsequent turnover by the proteasome.

a pathway termed ER-Surf has been proposed, in which the endoplasmic reticulum provide a surface to capture mitochondrial preproteins (Fig. 12.5). In addition, the recognition by J-proteins of mitochondrial translocase components seems to be broad but specific, because although Djp1 cooperates with Tom70, Tom22 recruits the J-protein Xdj1 (Opaliński *et al.*, 2018). Consistent with an important role of J-proteins, Ydj1 and Sis1 were found to mediate import of beta-barrel proteins to the mitochondrial outer membrane (Jores *et al.*, 2018).

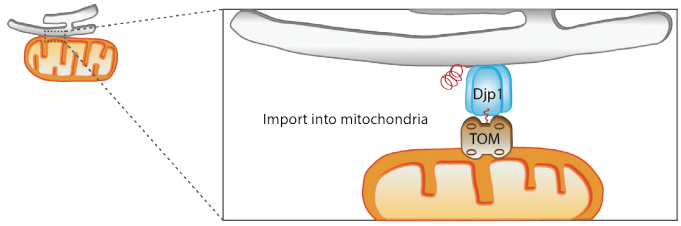

Figure 12.5 ER-associated J-Proteins promote mitochondrial import. The J-protein Djp1, which localizes at the ER surface, contributes with the TOM channel at the mitochondrial outer membrane to import mitochondrial pre-proteins.

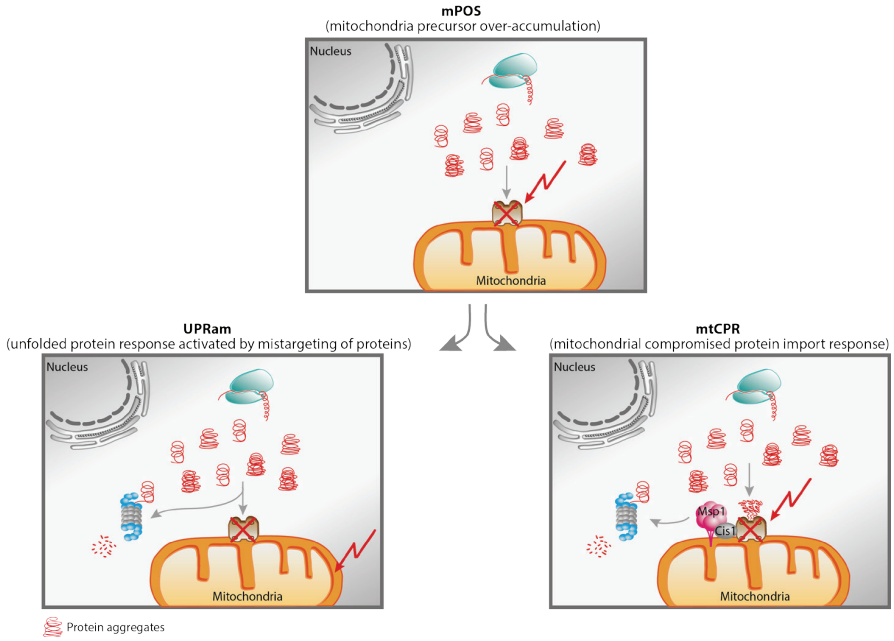

Figure 12.6 Cellular responses to mitochondrial import stress. Different quality control pathways are described maintaining cellular homeostasis on import overload at the mitochondrial surface. Protein import can be reduced due to intra- or extracellular stresses, causing the cytosolic accumulation of protein aggregates destined for the mitochondria (mPOS, mitochondrial precursor over-accumulation). The accumulation of un-imported mitochondrial proteins can activate the UPRam (unfolded protein response activated by mis-targeting of proteins), which mediates the turnover of these proteins via the 26S proteasome. Similarly, import clogging at the TOM channel activates the MitoCPR pathway (mitochondrial compromised protein import response). Thereby, the AAA-ATPase Msp1 interacts with the TOM channel via Cis1 and mediates the extraction of clogging proteins to the cytosol, where they are targeted for proteasomal turnover.

UPRam – unfolded protein response activated by mistargeting of proteins

The global cellular responses caused by accumulation of mitochondrial precursor proteins in the cytosol were recently addressed, by provoking a defective protein import in the intermembrane space (Wrobel *et al.*, 2015). Interestingly, mis-targeted mitochondrial proteins activated a concerted proteostatic response in the cytosol, whereby protein synthesis was inhibited and the proteasome was activated. Importantly, these responses were key in alleviated systemic pathology of the organelle and organismal death. In conclusion, UPRam allows buffering the consequences of physiological slowdown in mitochondrial protein import, thus promoting cellular survival under stress (Fig. 12.6).

Consistently, it was previously shown that reduced mitochondrial-membrane potential induced aggregation in the cytosol (Erjavec *et al.*, 2013). A role of faulty protein import and accumulation of unprocessed mitochondrial proteins in the cytosol was equally proposed. Such defects generated by mitochondrial dysfunctions could be compensated for by a boost in cytosolic protein quality control, thus maintaining viability despite chronic failures in mitochondrial function (Erjavec *et al.*, 2013). Another study suggested that defects in protein import lead to increased levels of reactive oxygen species, which in turn affect protein synthesis by modification of cytosolic ribosomes (Livnat-Levanon *et al.*, 2014). Interestingly, reducing cytosolic synthesis of mitochondrial proteins has even been shown to reduce mitochondrial degeneration, emphasizing its impact on mitochondrial integrity (Wang *et al.*, 2008).

mPOS – mitochondrial precursor over-accumulation stress

Simultaneously to UPRam, a study addressed the global consequences of aberrant accumulation of mitochondrial precursors in the cytosol, triggered either by impairing protein import or by clinically relevant mitochondrial damage (Wang and Chen, 2015). Consistent with UPRam, a cytosolic proteostatic network could be observed (Fig. 12.6). In particular, ribosomal biogenesis was modulated, where cap-dependent and thus major translation was down-regulated, to suppress protein synthesis. Moreover, cap-independent translation was up-regulated for a particular set of proteins that prevent ribosome assembly, thus reinforcing inhibition of general translation. Finally, this cytosolic network also suppressed cell death, confirming the physiological relevance of mPOS.

MitoCPR – mitochondrial compromised protein import response

An artificial precursor protein leading to clog of the protein import machineries revealed a role of the dislocase Msp1(yeast)/ATAD1(mammals) (Weidberg and Amon, 2018) (Fig. 12.6). Msp1 is a AAA-ATPase inserted at the outer membrane and facing the cytosol, assembling into a hexameric ring (Wohlever *et al.*, 2017). An analysis of the genes transcriptionally correlated with import clogging allowed the identification of Cis1, which physically interacts with Msp1 but also with the Tom70 component of the outer membrane translocase. Clearance of the precursor proteins, which depended on Cis1, Tom70 and Msp1, also required the proteasome to degrade the non-imported proteins.

IPTP – Interplay between mitochondrial translation and cytosolic responses

Apart from the responses just described, primarily induced at the surface of mitochondria, a proteostasis retrograde mechanism initiated in the matrix was also reported. The unfolded protein response (UPR) had already revealed that mitochondrial stress can inhibit translation at the mitochondria. Now, a crosstalk mechanism of mitochondrial translation accuracy impacting on cytoplasmic proteostasis was also proposed (Suhm *et al.*, 2018). Mitochondrial translation is signalled by a novel interorganellar proteostasis transcription program (IPTP), impacting chronological lifespan. Hyperaccurate mitochondrial translation stimulated Hsp104-mediated refolding and proteolytic capacity of a proteasomal model substrate. This infers that decreased accuracy of mitochondrial translation impaired management of cytosolic protein aggregates, eliciting a general transcription stress response. It also shows that cytosolic proteostasis, nuclear stress signalling and mitochondrial translation are closely coordinated in determining cellular homeostasis and lifespan (Suhm *et al.*, 2018).

Turnover of mitochondrial proteins by the UPS

In contrast to the nucleus, proteasomes are not present in mitochondria. Nevertheless, the UPS was shown to degrade some mitochondrial proteins after their insertion in the outer membrane, independently of the import surveillance mechanism described below. In the endoplasmic reticulum, ubiquitinated proteins are extracted to the cytoplasm by Cdc48/p97 to be degraded by the proteasome (Rape *et al.*, 2001; Franz *et al.*, 2014). This process is called ERAD (endoplasmic reticulum associated degradation). A similar mechanism was identified in mitochondria and named OMAD, for outer membrane associated degradation, in analogy to ERAD (Neutzner *et al.*, 2007; Braun and Westermann, 2017).

OMAD – extraction of cytosolic-exposed proteins by the cytosolic dislocase Cdc48

As previously mentioned, Cdc48 assembles with a myriad of partners that assist the AAA protein in extracting proteins from complexes or membranes (Fig. 12.7). Cdc48 was suggested to extract the yeast mitofusin Fzo1 from mitochondria under oxidative stress conditions (Heo *et al.*, 2010; Esaki and Ogura, 2012). In absence of external stress, Cdc48 formed a complex with Doa1, Ufd1 and Npl4 to retrogradely translocate ubiquitinated membrane-anchored proteins to the cytoplasm, including tagged versions of Msp1 and Tom70 (Wu *et al.*, 2016). Although tagged Fzo1 was also a MAD substrate (Wu *et al.*, 2016), the endogenous protein is instead stabilized by endogenous Cdc48 (Simões *et al.*, 2018), demonstrating limitations of working with tagged proteins, and thus accessing the real Cdc48 substrates. Nevertheless, the work from Wu *et al.* (2016) clearly highlights the importance of Cdc48 in quality control mechanisms at the outer membrane. In mammals, p97 was also required for the extraction and proteasomal turnover of outer membrane proteins, under damaging conditions, including mitofusins and Mcl-1 (Neutzner *et al.*, 2007; Tanaka *et al.*, 2010; Xu *et al.*, 2011).

Interestingly, Cdc48 performs opposing roles to MAD, by instead increasing the stability of the Fzo1 protein (Fig. 12.7). In fact, mitochondria form a dynamic network that is continuously remodelled by fusion and fission events. Fzo1, present at the

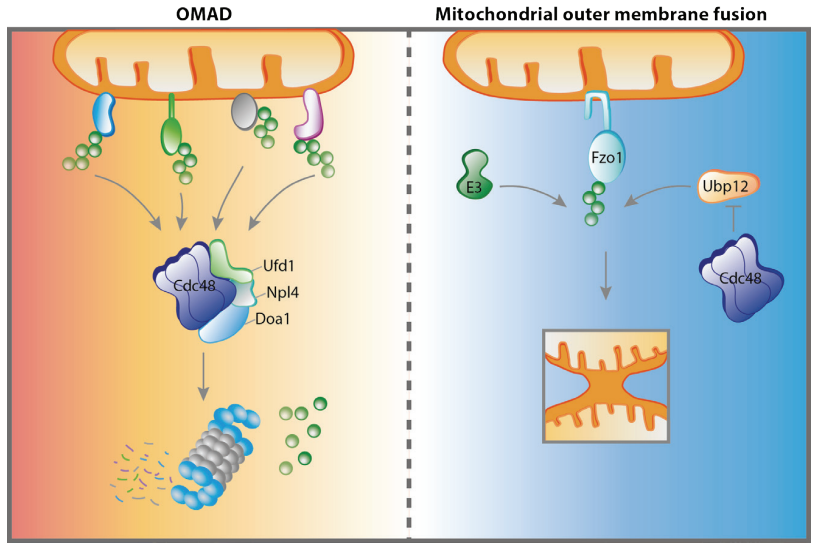

Figure 12.7 Dual roles of Cdc48 at the mitochondrial surface. The ubiquitin-specific AAA-ATPase Cdc48 maintains protein quality control at the mitochondrial outer membrane and regulates mitochondrial dynamics. On the one hand, Cdc48 acts as a cytosolic dislocase, which segregates ubiquitinated outer membrane proteins, allowing their degradation by the 26S proteasome. On the other hand, Cdc48 protects ubiquitination on Fzo1, and controls a cascade of deubiquitinases, like Ubp12, thus promoting the mitochondrial outer membrane fusion process.

mitochondrial outer membrane, is required for mitochondrial fusion. In contrast to MAD, Fzo1 was shown to be protected from the UPS by Cdc48 (Simões *et al.*, 2018). Instead, Cdc48 was required for the turnover of a deubiquitinating enzyme which inhibits Fzo1. In addition, Cdc48 served as a binding platform, allowing crosstalk regulation between deubiquitinases and thus promoting membrane merging and mitochondrial fusion.

Extraction of cytosolic-exposed proteins by the mitochondrial dislocase Msp1

Similar to Cdc48/p97, Msp1/ATAD1 is a AAA-ATPase at the mitochondrial outer membrane that assembles into an hexameric ring, as previously mentioned (Schnell and Hebert, 2003). Therefore, Msp1/ATAD1 constitutes an alternative machinery to segregate substrates from the mitochondria. Indeed, recent findings showed that Msp1/ATAD1 participates in a local organelle surveillance pathway, to deal with proteins inappropriately inserted into mitochondria (Hegde, 2014; Okreglak and Walter, 2014; Opaliński *et al.*, 2014). Correct targeting of proteins to their respective organelles is a general challenge, given that the vast majority of organellar proteins are synthesized as precursors on cytosolic ribosomes and have to be transported to their intracellular destinations (Schnell and Hebert, 2003). This challenge is even bigger for tail-anchored proteins, i.e. with a single transmembrane segment at the C-terminus. Msp1 was shown to extract tail-anchored proteins mis-targeted from peroxisomes to mitochondria (Chen *et al.*, 2014; Okreglak and Walter, 2014). Consistently, purified Msp1 drove ATP-dependent extraction of tail-anchored proteins from the lipid bilayer (Wohlever *et al.*, 2017). It is highly likely that the proteasome will degrade these mis-localized proteins, once extracted by Msp1, as it is the case in mitoCPR.

In conclusion, the Msp1/ATAD1 protease ensures the fidelity of organelle specific-localization of tail anchor proteins. Moreover, as previously described, it also functions in pre-protein clearance during mitochondrial import stress. This highlights critical functions of an outer membrane dislocase in maintaining mitochondrial integrity.

IMS proteins

The proteasome was also shown to degrade proteins present at the inner-membrane space, after their retrograde translocation back to the cytosol, mediated by Tom40 (Bragoszewski *et al.*, 2015) This is consistent with a previously observed accumulation of mitochondrial inner membrane proteins, upon chemical inhibition of the proteasomal activity (Radke *et al.*, 2008). Interestingly, under those conditions, the inner membrane space protease Omi/HtrA2 could degrade inner membrane proteins. However, it should be noted that mitochondrial proteases can also be sensitive to proteasomal inhibitors. Collectively, it is possible that faulty folding of inner membrane space proteins during import could lead to ubiquitination of their cytosolic exposed parts, providing access to the UPS. This is consistent with the observation that a fraction of newly synthesized intermembrane space precursors are degraded in the cytosol before reaching this subcompartment, event in the absence of stress-inducing conditions (Bragoszewski *et al.*, 2013; Kowalski *et al.*, 2018). In conclusion, the UPS plays a constitutive role for the biogenesis of intermembrane space proteins.

Mitochondrial damage overload

In addition to the previously described removal of individual proteins for proteasomal degradation, excessive damage can be repaired by eliminating whole mitochondrial fragments, by mitophagy, thus protecting the healthy mitochondria (Harper *et al.*, 2018; Pickles *et al.*, 2018). In addition, selected mitochondrial components can be eliminated from the mitochondrial network by mitochondria-derived vesicles or mitochondrial derived compartments (Sugiura *et al.*, 2014; Hughes *et al.*, 2016) (Fig. 12.8).

Mitophagy

The panoply of different mechanisms described, e.g. UPRmt, UPRam, POS, mitoCPR, allow coping with transient stress, still repairable at the level of the mal-functioning proteins. However, prolonged or acute stress conditions can no longer be reversed by these pathways, and mitochondria need to be eliminated by mitophagy, in order to restore cellular homeostasis. A prominent role of mitochondrial fission in allowing the detachment of damaged pieces from the entire network was recently proposed (Burman *et al.*, 2017).

One of the early steps and hallmarks in mitophagy is the general ubiquitination of outer

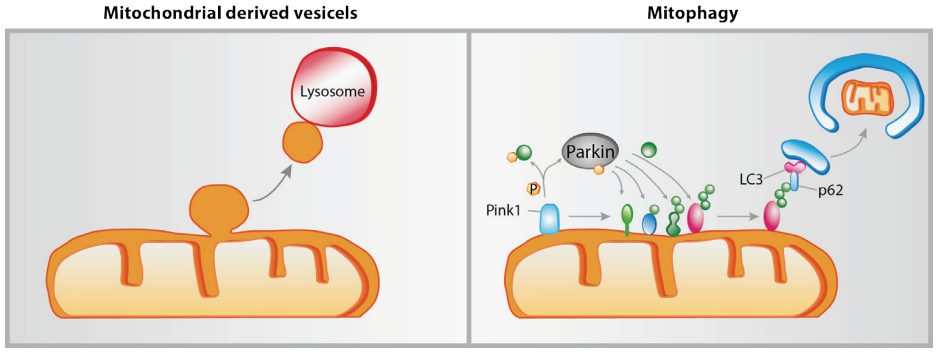

Figure 12.8 Elimination of damaged mitochondria. Local mitochondrial defects cause the budding of mitochondrial-derived vesicles (MDVs), which can consist only of the outer membrane, or of outer and inner membrane. Such vesicles, forming on oxidative stress, segregate from the mitochondrial network and fuse subsequently with lysosomes, where final degradation takes place. However, acute mitochondrial defects initiate the clearance of mitochondria via mitophagy. Loss of the mitochondrial membrane potential causes the accumulation of the kinase Pink1 at the outer membrane. Pink1 recruits and phosphorylates Parkin, which activates the E3 ligase. In addition, Pink1 phosphorylates ubiquitin, which is required for Parkin activation as well. Activated Parkin ubiquitylates several mitochondrial outer membrane proteins, which serve as a platform for the recruitment of the ubiquitin-like modifier LC3 via different adaptor autophagic receptors. LC3 lipidation with PE (Phosphatidylethanolamine) allows autophagosome expansion, which engulfs the whole damaged mitochondria. Subsequent mitochondrial degradation takes place within the lysosome on fusion of autophagosomes with lysosomes.

membrane proteins. This is believed to recruit several autophagy receptors and therefore mark mitochondria that should be eliminated. The most famous components performing this task are the E3 ligase Parkin and the mitochondrial kinase Pink1 (Pickles *et al.*, 2018). In healthy mitochondria, Pink1 is imported into mitochondria, processed during import and then released to the cytosol and degraded by the N-end rule. In contrast, upon loss of membrane potential, the kinase is arrested due to import failure, thus integrating in the outer membrane, and exposing the catalytic domain to the cytosol (Matsuda *et al.*, 2010; Vives-Bauza *et al.*, 2010). There, it phosphorylates Parkin at its ubiquitin-like domain, which changes its conformation and leads to enzymatic activation. Moreover, Pink1 phosphorylates serine 65 of ubiquitin chains assembled by Parkin at the outer membrane, further increasing Parkin recruitment to the mitochondria and activation, by feedforward loop mechanisms. These chains bind several receptors like Optineurin, NDP52 and p62, which then engage the autophagic machinery and culminates by releasing mitochondria into the lysosome for destruction (McWilliams and Muqit, 2017) (Geisler *et al.*, 2010; Narendra *et al.*, 2010; Lazarou *et al.*, 2015; Khaminets *et al.*, 2016). In addition to Parkin, other E3 ligases stimulate mitophagy (Covill-Cooke *et al.*, 2018). For example, the outer membrane RING ligase March5 induced mitophagy on hypoxic conditions, together with the autophagic receptor FUNDC1 (Chen *et al.*, 2017). In addition, March5 has many additional physiological functions (Covill-Cooke *et al.*, 2018). Finally, ubiquitin-independent mitophagy pathways have also been described as for example the role of the ATG8 receptor NIX in hypoxia (Khaminets *et al.*, 2016).

Mitochondrial-derived vesicles

In contrast to mitophagy, where the whole organelle is degraded, mitochondria can also dispose content in the form of vesicles, called MDVs (mitochondria-derived vesicles), which transport proteins and lipids to other cellular organelles (Sugiura *et al.*, 2014). Mitochondria can form different types of vesicles, with different cargoes and also with different cellular destinations, thus facilitating intracellular communication. In yeast, vesicles allowing selective degradation of a protein subset were also found, suggesting that budding of mitochondria could be a conserved mechanism (Hughes *et al.*, 2016). Pink1 and Parkin are also involved in the formation of MDVs containing oxidized cargo and formed after oxidative stress.

Consistent with their quality control roles, these MDVs were destined to the lysosomes, suggesting a similar role to mitophagy of Pink1 and Parkin, just more confined and not so extreme. Interestingly, however, a role of Pink1 and Parkin in repressing vesicle formation was also recently reported (Matheoud *et al.*, 2016). Indeed, MDVs containing antigens were negatively regulated by Pink1 and Parkin. These MDVs were targeted to the cellular surface, to present the cargo on major histocompatibility (MHC) class I molecules, triggering an immune response. Consistently, Pink1 and Parkin also prevented the activation of an inflammatory response caused by excessive mutations in the mitochondrial DNA (Sliter *et al.*, 2018). Finally, other roles of Parkin have been proposed, as for example in mitochondrial trafficking along neurons (Scarffe *et al.*, 2014).

Role of ribosomal quality control (RQC) in mitochondria

Fidelity of protein synthesis is essential for mitochondrial function, since the vast majority of mitochondrial proteins are synthesized by cytosolic ribosomes. However, protein synthesis at ribosomes can go wrong, resulting in aberrant translation products, which are subjected to ribosomal quality control (RQC) (Brandman and Hegde, 2016) (Fig. 12.9). Failure in RQC of mitochondrial proteins results in defective proteins, which are still imported into mitochondria and interfere with mitochondrial proteostasis (Izawa *et al.*, 2017). RQC monitors ribosomal activity and is activated on stalling of translation. Potential causes for stalling are damaged or truncated mRNAs, particular mRNA sequences including poly(A)-tails, excessive mRNA secondary structures and insufficient amounts of amino acids or tRNAs (Brandman and Hegde, 2016). As a consequence of stalling, ribosomes are disassembled and the potentially defective mRNA is degraded, as well as the nascent polypeptide chain.

RQC

The first step of RQC is the splitting of the ribosome, resulting in a 60S ribosomal subunit bound to the nascent polypeptide chain. This complex is then recognized by the RQC complex, which consists of Rqc1, Rqc2, the E3 ubiquitin ligase Ltn1 (Listerin in mammals) and Cdc48 with its co-factors Npl4 and Ufd1 (Brandman *et al.*, 2012). The function of Ltn1 is to ubiquitylate the stalled polypeptide at the ribosomal exit site, resulting in proteasomal degradation (Bengtson and Joazeiro, 2010). This process requires the action of Cdc48 to extract the ubiquitinated nascent chain from the 60S ribosome (Defenouillère *et al.*, 2013). In addition to ubiquitination, the stalled polypeptide can be further modified by addition of multiple alanine and threonine residues, at the C-terminal. The synthesis of this amino acid extension -termed CAT-tail (c-terminal Alanine Threonine tail) occurs independently of mRNA or 40S subunits. Instead, it depends on the recruitment of charged t-RNAs by Rqc2 (Shen *et al.*, 2015). CAT-tails increase the probability of exposing lysines present

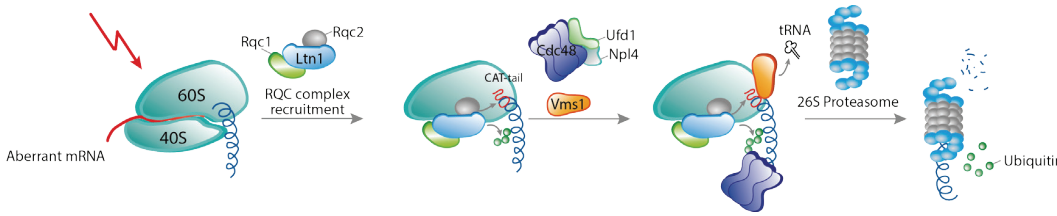

Figure 12.9 Ribosomal quality control. Translation of defective proteins requires the ribosomal quality control (RQC) machinery. On stalling of translation, ribosomes disassemble resulting in the 60S subunit bound to the nascent polypeptide chain. This recruits the RQC complex consisting of Rqc1, Rqc2 and Ltn1. Rqc1 recruits Cdc48 for the segregation of the polypeptide chain from the 60S subunit, which is subsequently degraded via the 26S proteasome. Rqc2 recruits charged tRNAs for the assembly of a CAT tail at the C-terminus of the nascent poly-peptide. The CAT tail increases exposure of lysine residues, which are ubiquitinated by the E3 ligase Ltn1, thus addressing the stalled polypeptide for proteasomal degradation. Additionally, the peptidyl-tRNA hydrolase Vms1 mediates the removal of the bound tRNA from the nascent chain, facilitating release from the ribosome.

on the stalled chain to Ltn1. In turn, Rqc1 recruits Cdc48 to ribosomal subunits. When ubiquitination and degradation are compromised, the nascent polypeptides are still released from the ribosome and form CAT-tail dependent aggregates in the cytosol (Choe *et al.*, 2016; Defenouillère *et al.*, 2016; Yonashiro *et al.*, 2016).

Role of Vms1/ANKZF1 in RQC

Another factor recently implicated in RQC is Vms1. Initially, Vms1 had been identified as a co-factor recruiting Cdc48 to mitochondria on stress (Heo *et al.*, 2010). Despite being a cytosolic soluble protein, Vms1 translocates into mitochondria on oxidative stress, dependent on ergosterol peroxide, suggesting the presence of an oxidized sterol receptor at the outer membrane (Nielson *et al.*, 2017). Further studies revealed that Vms1 binds to ribosomes and the RQC complex, suggesting a role of RQC in mitochondrial quality control and a general function of Vms1 in RQC (Izawa *et al.*, 2017). Indeed, Vms1 and its human homologue ANKZF1 have then been identified as peptidyl-tRNA hydrolase, required to remove the bound tRNA from the nascent chain, thereby facilitating release from the ribosome (Zurita Rendón *et al.*, 2018; Verma *et al.*, 2018).

Mitochondrial functions of Vms1/ANKZF1

Vms1, despite being a general factor in RQC, appears to particularly affect mitochondrial proteostasis (Izawa *et al.*, 2017). Indeed, a combined deletion of the genes encoding the proteins Vms1 and Ltn1 resulted in severe growth inhibition under respiratory conditions. This growth defect was entirely dependent on CAT-tail formation by Rqc2. Consistently, aggregation and sequestration were observed, mainly of mitochondrial proteins, highlighting the importance of RQC for mitochondrial integrity (Izawa *et al.*, 2017). Conversely, overexpression of Vms1 reduced the Rqc2-dependent aggregation, by inhibiting Rqc2 binding to ribosomes. The strong impact of Vms1 on mitochondrial proteostasis might be explained by differences in the fate of cytosolic and mitochondrial proteins. Nascent mitochondrial proteins might still be partially imported, thanks on their N-terminal mitochondrial targeting sequence. This might reduce the efficiency of Ltn1-dependent ubiquitination on ribosome stalling. Consequently, an increase in the requirement of Vms1 in RQC, to clear nascent mitochondrial polypeptides and prevent clogging of the import channel, is not surprising. Though initially identified as a Cdc48-interacting protein (Heo *et al.*, 2010), the function of Vms1 in RQC was reported to be independent of Cdc48 (Verma *et al.*, 2018). In contrast, the general role of Vms1 in RQC was shown to depend on Cdc48 interaction (Izawa *et al.*, 2017).

Concluding remarks

Increased proteotoxic burden is a hallmark of neurodegeneration. The main cellular strategies to cope with proteotoxic stress are to increase the levels of chaperones, activate proteolytic pathways and reduce protein synthesis. Recent events highlighted that these main strategies come in different flavours and involve crosstalk between different cellular compartments. Knowing that mitochondria joined this team considerably broadens our knowledge of the ubiquitin dependent and independent cross-functional cellular stress response mechanisms. Hopefully these findings will get us closer to therapies for the myriad of diseases caused by insufficient handling of protein damage.

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