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Protein quality control at the mitochondrion

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Mitochondria are essential constituents of a eukaryotic cell by supplying ATP and contributing to many mayor metabolic processes. As endosymbiotic organelles, they represent a cellular subcompartment exhibiting many autonomous functions, most importantly containing a complete endogenous machinery responsible for protein expression, folding and degradation. This article summarizes the biochemical processes and the enzymatic components that are responsible for maintaining mitochondrial protein homoeostasis. As mitochondria lack a large part of the required genetic information, most proteins are synthesized in the cytosol and imported into the organelle. After reaching their destination, polypeptides must fold and assemble into active proteins. Under pathological conditions, mitochondrial proteins become misfolded or damaged and need to be repaired with the help of molecular chaperones or eventually removed by specific proteases. Failure of these protein quality control mechanisms results in loss of mitochondrial function and structural integrity. Recently, novel mechanisms have been identified that support mitochondrial quality on the organellar level. A mitochondrial unfolded protein response allows the adaptation of chaperone and protease activities. Terminally damaged mitochondria may be removed by a variation of autophagy, termed mitophagy. An understanding of the role of protein quality control in mitochondria is highly relevant for many human pathologies, in particular neurodegenerative diseases.

Introduction

Living cells exist in a state of dynamic equilibrium where its components are permanently synthesized and eliminated again. This applies also to most, if not all, proteins of a cell or organism. Cells have evolved intricate mechanisms responsible for protein biosynthesis, folding and polypeptide degradation. The entirety of the processes contributing to the maintenance of a functional state of proteins in the cell is summarized under the designation 'protein homeostasis' or, in short, 'proteostasis'. In order to reach this state of homoeostasis, apart from the typical biosynthesis and turnover processes, cells also contain dedicated enzymatic systems that deal with conditions leading to protein dysfunction, either by repair (refolding) or removal of damaged polypeptides. This PQC (protein quality control) system consists of different types of molecular chaperones, also called Hsps (heat-shock proteins), that co-operate closely with dedicated PQC-related ATP-dependent proteolytic enzymes [1]. An imbalance of proteostasis, either due to external or internal influences, will result in defective protein function and accumulation of damaged polypeptides. This progressive pathological process is now widely accepted as a cause or a contributing factor to important human diseases, including aging, cancer or neurodegeneration [2].

As organelles of endosymbiotic origin, mitochondria represent, in principle, autonomous entities inside a eukaryotic cell, being able to perform endogenous replication, protein expression and various metabolic reactions. However, as mitochondrial and cellular metabolism are closely linked, defects in mitochondrial functions will have serious effects on the cell as a whole. Mitochondria contain their own system of molecular chaperones and proteases that is distinct and independent of the cytosolic set of PQC components. On the basis of their close relationship with prokaryotic cells, mitochondria usually contain a full set of molecular chaperones that are closely homologous to their bacterial relatives (Table 1). The main chaperone families are distinguished by their approximate relative molecular mass: Hsp100/Clp, Hsp90, Hsp70, Hsp60 and sHsp (small Hsp). In particular, the roles of Hsp70s and Hsp60s have been extensively

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Table 1. List of PQC-related chaperones and proteases

The first column for each species gives protein names and the most common alias names in parentheses; the second column for each species gives UniProt entry numbers.

Class	*Escherichia coli*		*Saccharomyces cerevisiae*		*Homo sapiens*	
Hsp60	GroEL (Cpn60)	P0A6F5	Hsp60 (Mif4)	P19882	HSPD1 (Hsp60)	P10809
	GroES (Cpn10)	P0A6F9	Hsp10	P38910	HSPE1 (Hsp10)	P61604
Hsp70	DnaK	P0A6Y8	Ssc1	P12398	HSPA9 (mortalin, Grp75)	P38646
	hscA (Hsc66)	P0A6Z1	Ssq1	Q05931	–	
	–		Ecm10 (Ssc3)	P39987	–	
	DnaJ	P08622	Mdj1	P35191	DNAJA3 (Tid-1)	Q96EY1
	GrpE	P09372	Mge1	P38523	GRPEL1 GRPEL2	Q9HAV7 Q8TAA5
Hsp100/Clp	ClpB	P63284	Hsp78	P33416	–	
	ClpX	P0A6H1	Mcx1	P38323	CLPX	O76031
Proteases	ClpP	P0A6G7	–		CLPP	Q16740
	Lon	P0A9M0	Pim1 (Lon)	P36775	LON	P36776
	DegP	P0C0V0	–		HtrA2 (OMI)	O43464
	FtsH	P0AAI3	Afg3 (Yta10)	P39925	AFG3L2	Q9Y4W6
	–		Rca1 (Yta12)	P40341	SPG7 (paraplegin)	Q9UQ90
	–		Yme1	P32795	YME1L1 (FTSH1)	Q96TA2

characterized in mitochondria as well as in bacteria, where they are responsible for the 'classical' folding catalysis of newly synthesized polypeptides or the refolding of misfolded proteins under stress conditions. Interestingly, the mitochondrial Hsp70 (mtHsp70) has acquired an additional role in providing the driving force for the translocation of mitochondrial preproteins through the inner membrane, a mechanism that is not necessary in bacteria. Not much functional information is available about the mitochondrial Hsp90, called Trap1, but in analogy to the role of its cytosolic relative, it may be involved in as yet unknown signal transduction processes. Chaperones from the Hsp100/Clp family represent a special case as most of its members co-operate closely with the ATP-dependent protease of this family, named ClpP (caseinolytic protease, P-subunit). In mitochondria, these protease cofactors belong to the ClpX family. In contrast, the members of the ClpB chaperone family have an independent role and are involved in the protection against protein aggregation. In addition, mitochondria contain several other ATP-dependent protease systems, a soluble protease of the Lon family in the matrix and two protein complexes integrated into the inner membrane, the AAA (ATPase associated with various cellular activities) proteases. All Hsp100/Clp chaperones as well as the ATP-dependent proteases belong to the widespread but structurally conserved AAA+ protein family [3].

On the basis of the special relationship of mitochondria with the eukaryotic 'host' cell, there are two levels of quality control in case of an accumulation of damaged polypeptides. First, the internal mitochondrial PQC system will deal with the problem by trying to repair the damage on the molecular level. If the endogenous system is overwhelmed, there are essentially three forms of reactions on a cellular level that affect the organelle as a whole, all of them requiring specialized signalling reactions between the organelle and the cell. (i) Via a specific up-regulation of the protein expression of mitochondrial PQC components, termed mitochondrial unfolded protein response (UPR^{mt}), the cells will try to increase the polypeptide repair capacity. (ii) If the damage is too severe and dysfunctional mitochondria accumulate, the cell will specifically remove these organelles by a dedicated form of autophagy, called mitophagy. (iii)

In case the cellular damage caused by mitochondria is too severe, the cell might elicit its own removal by programmed cell death or apoptosis.

PQC mechanisms in mitochondria can be largely generalized and are principally conserved throughout the eukaryotic kingdom. However, there are some species-dependent differences in the set of PQC components present, which may reflect so far unknown functional requirements [3]. Fungal mitochondria do not possess an Hsp90 family member and have lost the ATP-dependent protease ClpP, but contain multiple copies of the Hsp70-type chaperone. Metazoan mitochondria on the other hand do not contain a ClpB family member. UPR^{mt} has been characterized in detail only in the nematode *Caenorhabditis elegans*, whereas its role in fungi or mammals remains unclear [4]. In contrast, a specific removal of mitochondria by mitophagy as a damage-control pathway has been mainly established for mammalian cells [5].

Protein biogenesis

The process of mitochondrial protein biogenesis already presents unique challenges to the PQC system due to the dual origin of mitochondrial proteins. Mitochondria have lost the majority of their genome during evolution either by transferring genetic information to the host nucleus or by deletion of genes no longer needed for the endosymbiotic lifestyle. Hence mitochondria themselves encode and express only a few polypeptides, 13 in humans, whereas the vast majority, probably more than 1000 different proteins, is encoded in the nucleus. The nuclear-encoded components are synthesized at cytosolic ribosomes and have to be imported mainly post-translationally to their mitochondrial localization [6]. As some enzymes, in particular the respiratory complexes of the inner membrane, contain both nuclear and mitochondria-encoded subunits, the biosynthesis has to be co-ordinated between the different encoding organelles, nucleus and mitochondria. As a detailed description of the mitochondrial protein import system would go beyond the scope of this article, we focus on two import-related phenomena that affect the mitochondrial PQC system directly. First, mitochondrial proteins imported from the cytosol have to be folded inside the organelle to their native conformation. Secondly, in many cases, the newly imported polypeptides have to be assembled into active oligomeric enzyme complexes, in part together with internally expressed subunits. Both cases represent a challenge to the endogenous molecular chaperone system.

Owing to the dimensions of the protein transport pores in the inner membrane, mitochondrial polypeptides destined for the matrix compartment cross the membranes in a completely unfolded conformation. In this context, the matrix chaperone mtHsp70 interacts physically with the incoming polypeptide chain and provides an inward-directed translocation force in an ATP-dependent reaction [7]. Typically for Hsp70 chaperone systems, with the bacterial DnaK as the most well-characterized member, mtHsp70 is supported in this function by a co-operation with a nucleotide-exchange factor of the GrpE protein family, Mge1, and a specialized member of the DnaJ protein family, Pam18, that is integrated into the inner membrane and a component of the membrane translocase complex [8]. Similar to other proteins of the DnaJ family, Pam18 stimulates the substrate interaction of mtHsp70 and also activates its ATPase activity. In order to exert its function as a translocation motor, mtHsp70 is anchored at the inner face of the inner membrane by a specific interaction with the translocase component Tim44, a unique property of the mitochondrial Hsp70 chaperone system [9]. As the first chaperone to come into contact with the incoming polypeptide chain, mtHsp70 is also the first member of the PQC machinery that initiates further folding and assembly reactions.

In order to express mitochondria-encoded genes, the organelle needs its own machinery for DNA replication, transcription and translation. Most of the components needed are encoded in the nucleus and represent nearly one-third of the mitochondrial proteome [10]. However, mitochondria still contain their own set of genes encoding rRNAs, tRNAs and mRNAs, expressing hydrophobic membrane subunits of the respiratory chain complexes. Synthesizing those proteins inside mitochondria may be an advantage as hydrophobic proteins are difficult to be imported into the organelle from the cytosol. Interestingly, mitochondria exhibit an altered genetic code different from the universal code. A particular property of the mitochondrial translation system is the co-translational insertion of newly synthesized nascent chains into the mitochondrial inner membrane. During their passage through the ribosome exit tunnel, which exhibits hydrophobic residues creating an environment similar to the mitochondrial membrane, polypeptides can start to fold. The co-translational mechanism of the mitochondria-encoded membrane proteins prevents aggregation of the newly synthesized highly hydrophobic polypeptides during transport. The insertion into the inner membrane is mediated by the protein Oxa1 (oxidase assembly 1), a protein that interacts with the mitochondrial ribosomal exit tunnel [11]. Although the co-translational insertion mechanism minimizes the potential interactions of nascent mitochondrial polypeptides, there might be a requirement to prevent irregular reactions directly at the ribosome surface. Indeed, the mammalian mitochondrial elongation factor Tu, which delivers the aminoacyl-tRNA

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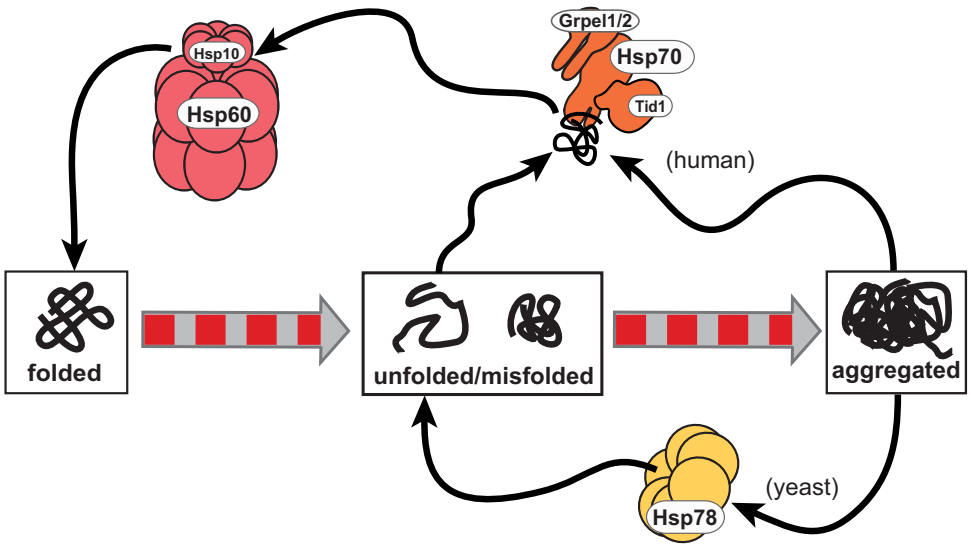

Figure 1. Schematic depiction of the chaperone network in human and yeast mitochondria

Red arrows indicate pathological processes, and black arrows symbolize chaperone-mediated PQC reactions. The yeast chaperone Hsp78, a member of the Hsp100 family, is able to disaggregate protein aggregates to an unfolded state in order to refold proteins into their functional conformation with the support of the Hsp70 and the Hsp60 system. In human cells Hsp70 in co-operation with the J-domain protein Tid1 and the nucleotide-exchange factor GrpEl1/2 directly acts on protein aggregates also mediating proteins for refolding by the Hsp60 system.

to the ribosome during translation, is supposed to interact with nascent proteins and to possess also a chaperone-like activity by preventing thermal aggregation of proteins and enhancing protein refolding, which has been shown *in vitro* in mammalian mitochondria [12].

Protein folding and assembly

After completion of translocation into the matrix, the newly imported polypeptides have to fold to their native conformation in the mitochondrial matrix. It is likely that a certain amount of these polypeptides do not complete the folding reaction and acquire a misfolded conformation. The amount of unfolded or misfolded proteins would be increased in the presence of external stress stimuli such as heat or toxic reagents that can cause protein damage. Hence the mitochondrial PQC system directly supports protein-folding reactions and stabilization of newly imported unfolded polypeptides [3].

Owing to its involvement in the translocation reaction, mtHsp70 provides the primary contact point for newly imported unfolded polypeptides (Figure 1). Molecular chaperones of the Hsp70 family stabilize unfolded or misfolded proteins by a direct interaction with exposed hydrophobic patches. This interaction assists refolding by avoiding kinetic traps on the folding pathway and is also able to prevent aggregation processes [13]. In the yeast *Saccharomyces cerevisiae*, there are three different mitochondrial homologues of Hsp70, which have widely different molecular functions, illustrating the diversity of these chaperones. The most abundant and essential family member is mtHsp70, in yeast encoded by the gene *SSC1* that drives the import of preproteins into the mitochondrial matrix and is also fundamental for protein folding. The second member in yeast is the non-essential Ssq1 that plays a specialized role in the assembly of iron–sulfur clusters. The third member Ssc3 has a very high sequence similarity to Ssc1, but its expression level is very low and its cellular function is still not fully understood [14]. Hsp70 proteins generally contain an N-terminal ATPase domain and a C-terminal substrate-binding domain. The binding affinity for unfolded peptides is regulated by an allosteric mechanism based on the nucleotide state of the ATPase domain, with a low affinity in the ATP-bound state to a high affinity in the ADP-bound state [13]. A high variability in the cellular function of Hsp70 is achieved by a co-operation with different proteins from the DnaJ family that can support substrate interaction and also stimulate ATPase activity. In yeast mitochondria, the soluble J-protein Mdj1 promotes the ATP-dependent binding of unfolded substrate polypeptides to Ssc1 and is a major cofactor in protein-folding reactions [14]. Moreover, Mdj1 itself can directly prevent heat-induced aggregation of substrate proteins even in the absence of Ssc1. As J-proteins in

general exhibit substrate interaction abilities themselves, they are also categorized as heat-shock proteins (Hsp40s). Also during protein-folding reactions, nucleotide exchange in Ssc1 is facilitated by the exchange factor protein Mge1, thus reducing substrate affinity and promoting substrate protein release [15].

Hsp70 chaperones also supply the Hsp60 chaperone system, also called chaperonins, with substrate polypeptides for additional folding catalysis (Figure 1). The co-operation between mtHsp70 and Hsp60 is essential for the folding of newly imported proteins that have more stringent folding requirements [16]. Hsp60 is formed by two stacked rings composed of seven subunits each and provides a hydrophobic surface in its inner cavity where unfolded polypeptides up to a size of 50 kDa can bind. The cavity of the Hsp60 complex is covered by a cofactor named Hsp10 that influences substrate binding and release. After ATP hydrolysis the polypeptide is released by the opening of the Hsp10 lid and can exit the protein complex, ideally having acquired its functional conformation [17]. Yeast cells that are lacking Hsp60 are not viable, whereas cells carrying a conditional Hsp60 mutation accumulate misfolded proteins in the matrix under non-permissive conditions, demonstrating directly its importance for mitochondrial folding reactions [18]. Similarly, when cells are exposed to stress stimuli such as elevated temperatures, increased amounts of misfolded polypeptides accumulate resulting in protein aggregation processes. Hsp60 mutant mitochondria tend to accumulate more aggregated polypeptides, indicating the importance of the chaperonin system for protection against protein aggregation [19].

In humans, three of the four complexes of the mitochondrial respiratory chain consist of subunits of different genetic origin, the nuclear and mitochondrial genome. Due to their different translation sites, their assembly in the inner mitochondrial membrane causes specific problems. This highly co-ordinated process is mediated by assembly factors, which stabilize complex assembly intermediates and facilitate enzyme maturation [20]. Mutations in assembly factors have been shown to cause severe mitochondrial disorders, e.g. a mutation in the gene of the assembly factor SURF1 (surfeit locus protein 1), which is required for COX (cytochrome *c* oxidase) assembly, result in COX deficiency in Leigh syndrome patients [21]. As the complexes of the respiratory chain are essential for the metabolism of the whole cell, a precise co-ordination of the synthesis of the complex subunits, which are performed by two distinct translation systems, one in the cytosol and the other in the mitochondria, is necessary to avoid the accumulation of assembly intermediates which could impair the mitochondrial function. To ensure the correct co-ordination of mitochondrial and nuclear gene expression, nuclear-encoded translational activators can regulate the translation of the mitochondria-encoded mRNA. These feedback loops are well described so far for the cytochrome bc_1 complex as well as cytochrome *c* oxidase. For example, expression of the mitochondria-encoded subunit Cox1 in humans is regulated by the COX assembly intermediate, which contains assembly factors such as C12ORF62 and MITRAC12 that bind to newly synthesized Cox1 and stimulate its translation [11].

Protein disaggregation

Protein aggregation may occur after the capacity of the PQC system is overwhelmed, e.g. after extended exposure to stress conditions or by the accumulation of folding-deficient polypeptides [22]. Cells have therefore developed mechanisms to dissolve protein aggregates to provide further possibilities to repair or degrade damaged proteins. Chaperones from two families, Hsp70 and Hsp100/Clp, have been described to exhibit such disaggregation activity (Figure 1). Hsp100 proteins belong to the AAA + protein group, exhibiting an intrinsic ATPase activity and being able to form ring-shaped protein complexes typically composed of six identical subunits with a central pore. Their duty is to remodel polypeptides by disasembly and unfolding, aimed at their refolding or degradation - thereby exhibiting a significant protective role under proteotoxic conditions.

Whereas most members of the Hsp100/Clp chaperone family act as unfoldases during protein degradation reactions, proteins of the ClpB subfamily function exclusively in polypeptide disaggregation [23]. ClpB proteins consist of an N-terminal substrate-binding domain and two AAA domains connected by a middle domain that enables interaction with the Hsp70 system. Hsp78, the mitochondrial homologue in yeast cells, represents a shorter form lacking the typical N-terminal domain being replaced by an import-targeting sequence [24]. ClpB proteins are able to resolubilize aggregated proteins by pulling the polypeptide chain through the central pore of their homohexameric complex. Experiments demonstrated that Hsp78 has no protective role on aggregate formation as such, but is able to disaggregate proteins for subsequent refolding in close co-operation with the Hsp70 system or degradation by the LON protease system [25]. As this disaggregation activity becomes important after the respective stress conditions have subsided, the role of ClpB chaperones is mainly to prevent further proteotoxic damage. In this context, ClpB proteins are required for 'acquired thermotolerance', where a short sub-lethal heat treatment enables cells to survive otherwise lethal heat stress conditions. Hsp78 has also a positive influence on mitochondrial thermotolerance by supporting

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the chaperone function of Ssc1 [25], mitochondrial DNA replication [26] and protein translation [27], all representing essential processes for mitochondrial protein homoeostasis. Interestingly, in contrast with other members of the ClpB family, yeast Hsp78 has been shown to directly support degradation of proteins in the mitochondrial matrix by the soluble protease Pim1 (proteolysis in mitochondria 1) [28]. However, the natural substrate spectrum of Hsp78 in mitochondria has not yet been established. Whereas ClpB proteins have been identified in bacteria, fungi and plants, they are not present in metazoans. This species specificity and the fact that ClpB proteins show a heat-induced expression pattern indicates their functional role in achieving thermotolerance in organisms that are obligatorily exposed to external heat stress conditions. Despite a reduced danger of encountering temperature stress conditions, it was shown that human Hsp70 (mortalin) in co-operation with its J-protein Tid1 is able to take over the disaggregation function of the missing ClpB-type chaperones in human mitochondria [29]. It has to be noted that protein databases list a "mitochondrial ClpB" also in metazoan species. Although this protein contains an AAA domain, its overall homology with genuine ClpB-type chaperones is only superficial. As neither its mitochondrial localization nor any chaperone-like activity has yet been demonstrated, its classification as ClpB family member remains questionable.

Protein degradation

Degradation of damaged proteins becomes a major aspect of PQC in cases where the amount of affected polypeptides surpasses the refolding capacity of the chaperone system or when proteins fail to be refolded to the native conformation, for example due to covalent modifications. Mitochondria contain several PQC-related proteolytic systems, their activity and substrate spectrum depending largely on the intramitochondrial location of the protease and the individual properties of the substrate protein [3]. The main PQC protease for soluble polypeptides in the matrix compartment is the LON protease, in yeast called Pim1, a homologue of the bacterial protease Lon. There is another soluble protease system in the matrix with homology with the bacterial Clp protease; however, its role in PQC reactions is less well defined. Damaged inner membrane proteins, in particular non-assembled subunits of the respiratory chain complexes, are degraded by either of two membrane-integrated protease systems, the AAA proteases. All of these proteases are members of the ATP-dependent AAA + enzyme family (Figure 2).

The matrix protease Pim1/LON is arguably the most important PQC-related proteolytic system in mitochondria. It has been directly or indirectly implicated in a multitude of human pathologies that are affected by mitochondrial dysfunction [30]. As a member of the AAA + protein family, Pim1/LON forms ring-shaped protein complexes with the proteolytic sites protected inside the barrel-shaped enzyme. On the basis of a correlation with the bacterial homologue, it is likely that the complex is formed by six identical subunits. The analysis of the primary structure of Lon-like proteases revealed a roughly two-domain composition, with one domain involved in substrate recognition and the other domain containing the ATP-dependent proteolytic activity. Interestingly, mutants without proteolytic activity still exhibit chaperone-like functions [31]. Pim1 mutants in yeast have a severe phenotype losing respiratory competence and accumulating aggregate structures in the matrix compartment [32]. A direct involvement of Pim1/LON in the degradation of misfolded polypeptides was shown using imported artificial reporter proteins [33]. A proteomic screen for endogenous substrate proteins revealed that polypeptides exhibiting a low conformational stability are preferentially removed by Pim1/LON-mediated proteolysis. In its PQC-related function for soluble proteins, Pim1/LON closely co-operates with the Hsp70 and Hsp60 chaperones in the matrix [33]. The substrate specificity of Pim1/LON for unfolded or misfolded polypeptides is an intrinsic property of the enzyme complex that results from the combination of two characteristic features: (i) on the basis of the dimensions of the enzyme complex, Pim1 requires an unordered peptide segment of approximately 50–60 amino acids to initiate its progressive degradation reaction, and (ii) as it exhibits a low or even absent intrinsic protein unfolding capacity, only unstructured or destabilized polypeptides will be degraded completely [34]. This characterization correlates well with the observation that Pim1/LON is a main protective factor under oxidative stress conditions. An increased concentration of ROS (reactive oxygen species) results in the covalent modification of proteins, resulting in inactivation and misfolding, in particular if ROS-sensitive cofactors such as iron–sulfur complexes are part of the enzyme structure. These damaged polypeptides cannot be refolded and need to be removed by proteolysis. Both in yeast as well as in mammalian mitochondria, Pim1/LON has been shown to degrade oxidatively modified proteins [35,36]. Consequently, an overexpression of Pim1/LON in fungal model systems showed reduced ROS-dependent protein modifications, a decreased accumulation of protein aggregates under stress conditions and, most interestingly, increased lifespan [19,37]. It has to be noted that LON exhibits binding affinity for mitochondrial DNA and may also affect DNA damage control under oxidative stress [38].

Mitochondria also contain a proteolytic system that is dedicated to membrane protein degradation [39]. Again, there are significant homologies with a bacterial enzyme, in this case the FtsH protease. Proteases of this class are also

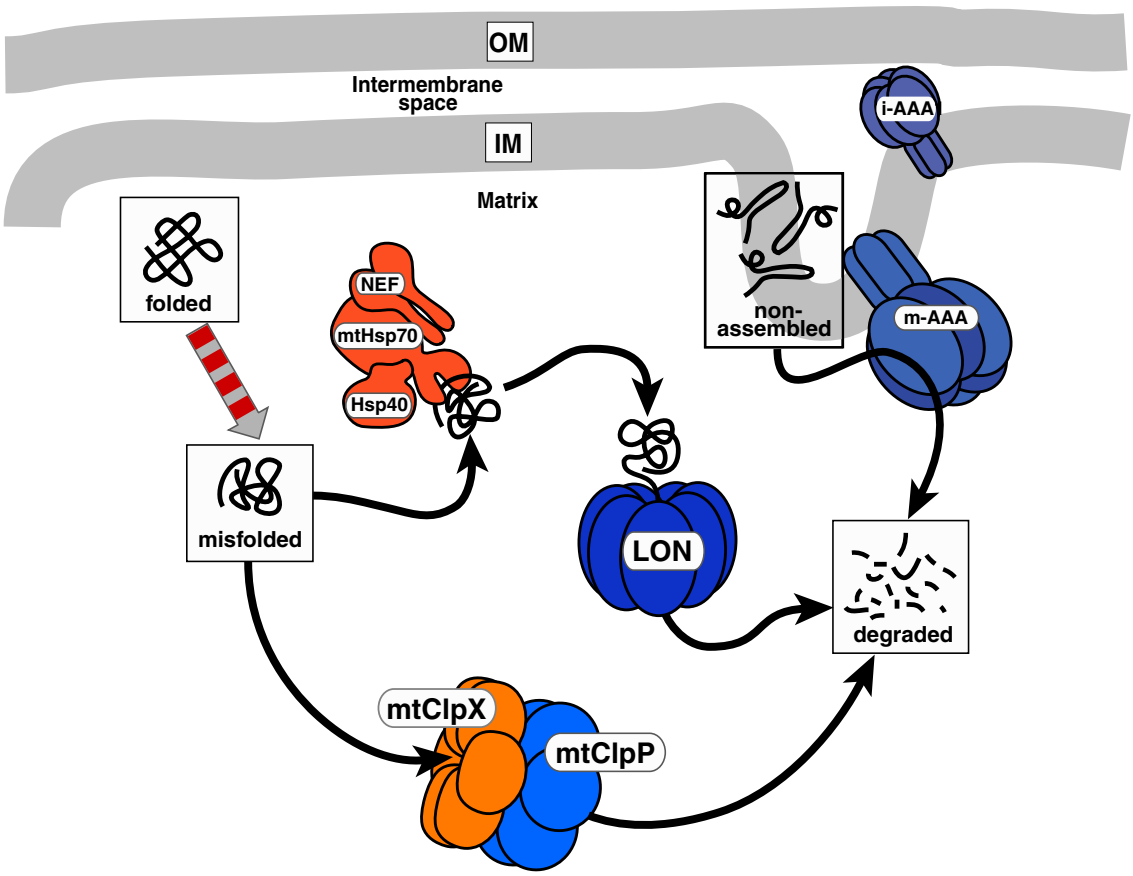

Figure 2. Schematic depiction of the chaperone-protease network in mitochondria

Red arrows indicate off-pathway reactions of folded to misfolded proteins due to protein damage and mutations. Misfolded proteins within the mitochondrial matrix can undergo degradation either by the AAA+ protease Lon/Pim1 in co-operation with the Hsp70 chaperone or by the ClpX/ClpP system. In contrast, non-assembled membrane proteins of the inner membrane (IM) are degraded by the m-AAA protease facing the mitochondrial matrix or become degraded by the i-AAA protease, facing the intermembrane space. OM, mitochondrial outer membrane; NEF, nucleotide-exchange factor.

members of the AAA+ family and form hexameric complexes. In contrast to LON, they contain an additional transmembrane domain that anchors the protease in the inner mitochondrial membrane. In mitochondria, there are two types of this protease, the i-AAA protease, in yeast composed of the single subunit Yme1, that exposes its proteolytic domain to the intermembrane space, and the m-AAA protease, usually composed of two homologous components, that faces the matrix compartment (Figure 2). Both AAA proteases are able to extract and degrade membrane proteins from the inner membrane in an ATP-dependent reaction [40]. Which protease becomes active depends on the topology of the respective substrate protein. The impact of the membrane AAA protease on mitochondrial PQC has not been characterized in detail, but it is assumed that they are important to avoid the accumulation of non-assembled membrane components of respiratory chain complexes [41]. Apart from a genuine role in general PQC processes, both AAA proteases are involved in specific proteolytic processing reactions that are important for mitochondrial function. On the basis of their localization, the AAA proteases are particularly important for the respiratory activity of mitochondria. In addition, the m-AAA protease contributes to the maturation of a ribosomal protein during import in mitochondria that eventually controls ribosome assembly [42]. The i-AAA protease affects the cleavage of the inner membrane dynamin homologue (Opa1) that is important for mitochondrial dynamics in the cell [43]. Typical for proteins with such diverse roles, loss-of-function mutants of either protease have a very pleiotrophic phenotype affecting many mitochondrial activities. In humans, defects of the AAA proteases have been directly associated with a neurodegenerative disease, spastic paraplegia [44].

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Although the role of the above-described proteases within the PQC system is already well understood, the function of the second mitochondrial matrix protease ClpP (mtClpP) remains largely elusive. ClpP is present in many branches of eukaryotes, except in fungi. To date, most information on mtClpP has been obtained by the analogy to its bacterial homologue [45]. ClpP is a protease of the AAA + family and consists of a barrel-like structure formed by a two-stack heptameric complex, creating a shielded chamber for proteolytic activity. Degradation by ClpP requires previous unfolding of the substrate protein that is achieved by the action of an additional unfoldase component. In mitochondria, the second part of this two-component proteolytic system is the hexameric ring-shaped complex ClpX, a member of the Hsp100 chaperone family. In analogy to the bacterial system, it is thought that ClpX complexes cover the openings of the ring-shaped ClpP complex and feed unfolded polypeptides into the interior of the protease where the proteolytic sites are located. As the chaperone component initially interacts with the substrate polypeptides, it is likely to be responsible for the substrate specificity of the protease. Interestingly, mitochondria from the model organism *Saccharomyces cerevisiae* lack a ClpP protease, while the chaperone component ClpX still exists [46]. Owing to their close relation to its bacterial counterpart, the ClpXP protease system in mitochondria is assumed to be involved in PQC in the matrix compartment [47], even if the substrate specificity has not yet been investigated in detail. Although the molecular function of ClpXP in mitochondria is not well understood, there is a correlation of ClpP defects with mitochondrial dysfunction and human diseases. A knockout model in mice revealed a function of ClpP during organismal development and growth [48]. Additionally, patients suffering from hereditary spastic paraplegia or Perrault's syndrome showed decreased expression or mutations of mitochondrial ClpP, indicating severe dysfunction of mitochondria with impaired activity of ClpXP [49,50].

Cellular level of mitochondrial quality control

Severe defects in mitochondrial functions could endanger the survival of the whole cell. Apart from a reduction or complete loss of essential nutrients, dysfunctional mitochondria typically produce toxic chemicals such as ROS that directly affect the functional integrity of cellular processes. In addition to mitochondrial PQC processes that act on the individual protein molecule as described above, we need to consider organellar quality control processes, affecting the quality of the mitochondria on a cellular level (Figure 3).

Apart from protein degradation reactions, experiments indicated an involvement of ClpXP in the up-regulation of mitochondrial proteins in response to mitochondrial dysfunction. Initially discovered in mammalian cell lines, most studies were carried out in the nematode *Caenorhabditis elegans* in which over 400 genes were described to be regulated by the UPR^{mt} [51]. In *C. elegans*, the main actor of the UPR^{mt} is ATFS-1 (activating transcription factor associated with stress 1), containing a mitochondria-targeting sequence at the N-terminus as well as an NLS (nuclear localization signal) at the C-terminus. In healthy mitochondria, ATFS-1 is imported into the mitochondrial matrix and degraded directly by the protease LON. As a consequence of mitochondrial dysfunction, damaged polypeptides start to accumulate in the mitochondrial matrix and become degraded to small peptides by the ClpP/ClpX system. Subsequently, they are exported into the mitochondrial intermembrane space via the inner-membrane peptide transporter Haf1. It was proposed that Haf1 interferes under these conditions with the mitochondrial protein import activity, which in turn leads to an accumulation of ATFS-1 in the cytosol. ATFS-1 then migrates to the nucleus and induces the transcription of UPR^{mt} genes [52]. In mammalian cells, the signalling pathway of UPR^{mt} established for *C. elegans* does not seem to be conserved, since homologues of Haf1 as well as ATFS-1 could not yet be identified. Although a transcriptional activation of protein expression similar to UPR^{mt} has been described in mammalian mitochondria [53], the details of the process remain to be clarified. Interestingly, a recent proteome study described that the up-regulation of ClpX resulted in the up-regulation of mitochondrial PQC components in myoblasts [54]. It seems likely that a reaction similar to UPR^{mt} in mammalian cells is induced by multiple signal transduction pathways, depending on the mitochondrial compartment and the type of stress condition.

A selective elimination of defective mitochondria as a whole is an alternative mechanism to maintain the functional quality within the mitochondrial population of a cell [55]. In general, the process of degrading cellular constituents, such as organelles, cytosol or protein aggregates is called autophagy. Cellular components are enclosed in a double-membraned structure, the autophagosome, which fuses with a lysosome to degrade and recycle the cargo. In contrast with non-selective autophagy, which is caused by nutrient deprivation and helps to adjust to metabolic requirements and recycle intracellular components, the mitochondria-specific autophagy, called mitophagy, removes superfluous or damaged mitochondria by a dedicated biochemical mechanism [56]. Studies in yeast have shown that the autophagy protein Atg32 plays an important role in mitophagy. This outer mitochondrial membrane protein interacts with Atg11 and Atg8 in mediating the recruitment of damaged mitochondria to the autophagosome [56]. So far no

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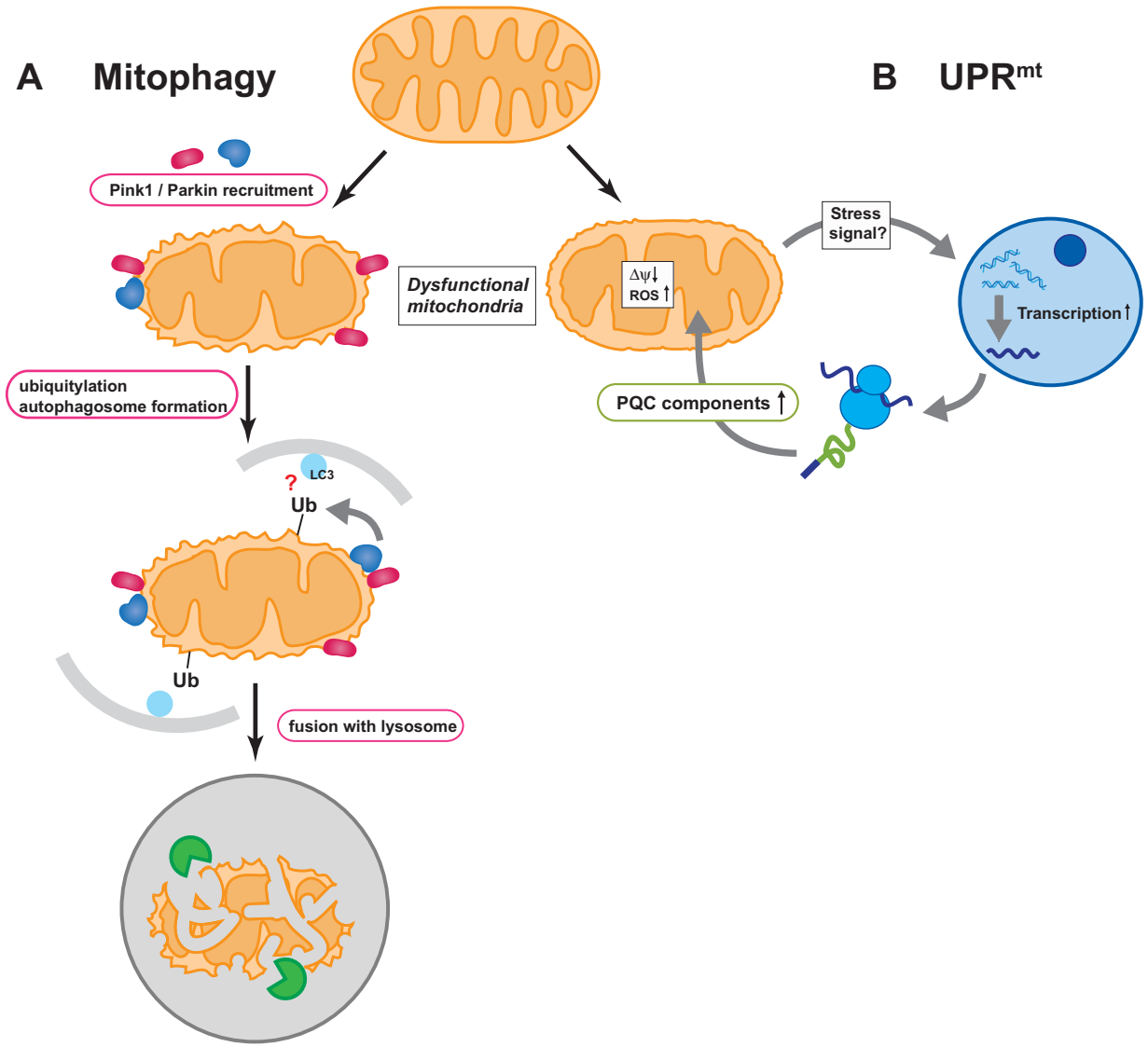

Figure 3. Principles of mitophagy and UPR^{mt}

(**A**) Damaged mitochondria are eliminated by mitophagy, a mitochondria-specific autophagy process. In this mechanism, the kinase PINK1 accumulates at the surface of dysfunctional mitochondria and recruits parkin, an E3 ubiquitin ligase. Parkin marks proteins of the outer mitochondrial membrane for proteasomal degradation by ubiquitylation and also mediates the recruitment of autophagosomal membranes for the engulfment of the damaged mitochondria. The autophagosome fuses with the lysosome to degrade and recycle the cargo. (**B**) Damaged mitochondria are able to mediate the transcriptional up-regulation of components of mitochondrial PQC via a stress signal. This mechanism, the UPR^{mt}, ensures the increase of the repair capacity of the organelle under stress conditions such as elevated levels of ROS.

mammalian homologue of Atg32 is known. However, the outer-membrane protein NIX (NIP3-like protein X) is, like Atg32, able to bind LC3 (light chain 3), the mammalian Atg8 orthologue, as well as the GABA (γ-aminobutyric acid) receptor-associated protein, which result in the recruitment of isolation membranes to the mitochondria. Defects in mitophagy, which are implicated in the failure to eliminate damaged mitochondria and therefore the accumulation of toxic material in the cell, are supposed to contribute to several neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases [57]. An impaired mitophagy due to mutations in the kinase PINK1 [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase protein 1] and the E3 ubiquitin ligase parkin have been shown to cause familiar autosomal recessive Parkinson's disease [56]. The pathway regulating mitophagy mediated by PINK1 and parkin is present in many metazoans [55]. In this mechanism, PINK1

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marks damaged mitochondria by an accumulation at the outer mitochondrial membrane and recruits the cytosolic E3 ubiquitin ligase parkin, which mediates the selective elimination of these mitochondria (Figure 3). Pink1 is targeted to the mitochondrial outer membrane by a variation of the standard import pathway of mitochondrial preproteins. It contains an N-terminal mitochondria-targeting sequence that is cleaved off after insertion into the inner membrane. Additional C-terminal targeting sequences retain the polypeptide in the outer membrane so that the kinase domain is exposed to the cytosol [58]. In healthy mitochondria, the cleaved PINK1 may even be released to the cytosol where it interferes with parkin activation [59]. It is not fully established how PINK1 is involved in the recognition of damaged mitochondria. The prevalent model is based on the loss of an inner membrane potential ($\Delta\Psi$) as the event that would lead to an impaired import and degradation of PINK1 [60]. However, PINK1 accumulation has also been observed under other conditions independently of the $\Delta\Psi$ [61], so that also alternative mechanisms might exist. Irrespective of the mechanistic details, PINK1 at the outer mitochondrial membrane is able to phosphorylate parkin, which is important for its activation and mitochondrial localization. In addition PINK1 also activates parkin indirectly via phosphorylation of ubiquitin. As a consequence of the binding of these two phosphorylated proteins, the ubiquitin ligase parkin becomes fully active. Ubiquitin chains, phosphorylated by PINK1, also constitute receptors for parkin on the mitochondrial surface. This process ensures that parkin recruitment and mitophagy is limited to impaired mitochondria. Following translocation and activation, parkin targets several proteins of the mitochondrial outer membrane for proteasomal degradation by ubiquitylation and recruits further receptor proteins to the mitochondria, which subsequently activate autophagosomes for engulfment of the damaged mitochondria [62].

Conclusion

It is expected that in modern societies the number of patients with aging-related pathologies like neurodegeneration, diabetes or cancer will substantially rise in the next decades and will put an increasing financial burden on healthcare systems. On the basis of the prominent role of mitochondrial defects in these human pathologies, an improved understanding of the biochemical mechanisms of mitochondrial protein homoeostasis will be a critical requirement for the development of cause-related therapies and treatment options. On the basis of modern methods of genome analysis, many disease-related genetic alterations have been identified that have a direct or indirect link to mitochondrial functions [63]. Research efforts in recent years have made substantial progress in the functional characterization of molecular processes that involve these disease-related proteins as well as the biochemical mechanisms of mitochondrial PQC in general. However, many open questions remain to be solved until a mitochondria-based therapeutic approach will be feasible in the future. On the basic research level, the wealth of information that has been acquired using mostly cellular model systems has to be translated to the situation in a whole organism, ideally to the human patient. Although many specific genetic defects correlating with human diseases have been identified, a particular vexing problem is that the vast majority of disease cases seem to be sporadic without any obvious cause. In this context, it should be noted that degenerative diseases are usually progressive and may not be associated with a specific definable defect or dysfunction. It is likely that even small defects in the efficiency of PQC might disturb the balance between positive and negative effects on cellular and, in particular, mitochondrial health, thereby causing or at least contributing to the progress of the disease. A second major problem in addressing the health effects of PQC-related problems is the lack of technical options for a direct manipulation of PQC component activity. Despite a quite detailed understanding of the biochemistry of the respective enzymes, the development of pharmacological compounds affecting the activities of the responsible molecular chaperones and proteases is still in its infancy. In particular, most potential treatments in the context of PQC will require an increase in the activity of the PQC enzymes, a feat that is difficult to achieve by a pharmacological approach. However, concerning the pathological role of mitochondrial PQC, the main strategy will be to avoid any type of cellular stress condition that may tip the fine-tuned equilibrium underlying protein homoeostasis.

Summary

- Mitochondria are largely autonomous organelles that contain their own machineries for protein expression, folding and degradation.

- Owing to a restricted amount of genetic information, most mitochondrial polypeptides have to be imported after synthesis in the cytosol.
- Protein import and subsequent folding reactions are driven by the activity of molecular chaperones of the Hsp70 and Hsp60 classes, which are closely related to their bacterial relatives.
- Together with specific ATP-dependent proteases, these molecular chaperones form an internal protein quality control network that is responsible for refolding or removal of damaged polypeptides.
- The amounts of protein quality control components under conditions of proteotoxic stress can be up-regulated by an intracellular signalling process called UPR^{mt}.
- Dysfunctional or damaged mitochondria can be removed by a specific form of autophagy, termed mitophagy.
- Failures in protein homoeostasis result in mitochondrial dysfunction that has been implicated in different neurodegenerative diseases.

Abbreviations

AAA, ATPase associated with various cellular activities; ATFS-1, activating transcription factor associated with stress 1; ClpP, caseinolytic protease, P-subunit; COX, cytochrome *c* oxidase; Hsp, heat-shock protein; mtHsp70, mitochondrial Hsp70; Pim1, proteolysis in mitochondria 1; PINK1, PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase protein 1; PQC, protein quality control; ROS, reactive oxygen species; sHsp, small Hsp; UPR^{mt}, mitochondrial unfolded protein response.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Hartl, F.U., Bracher, A. and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature **475**, 324–332 CrossRef PubMed
2 Balch, W.E., Morimoto, R.I., Dillin, A. and Kelly, J.W. (2008) Adapting proteostasis for disease intervention. Science **319**, 916–919 CrossRef PubMed
3 Voos, W. (2013) Chaperone–protease networks in mitochondrial protein homeostasis. Biochim. Biophys. Acta **1833**, 388–399 CrossRef PubMed
4 Arnould, T., Michel, S. and Renard, P. (2015) Mitochondria retrograde signaling and the UPR^{mt}: where are we in mammals? Int. J. Mol. Sci. **16**, 18224–18551 CrossRef
5 Wang, K. and Klionsky, D.J. (2011) Mitochondria removal by autophagy. Autophagy **7**, 297–300 CrossRef PubMed
6 Chacinska, A., Koehler, C.M., Milenkovic, D., Lithgow, T. and Pfanner, N. (2009) Importing mitochondrial proteins: machineries and mechanisms. Cell **138**, 628–644 CrossRef PubMed
7 Strub, A., Lim, J.H., Pfanner, N. and Voos, W. (2000) The mitochondrial protein import motor. Biol. Chem. **381**, 943–949 CrossRef PubMed
8 Truscott, K.N., Voos, W., Frazier, A.E., Lind, M., Li, Y., Geissler, A. et al. (2003) A J-protein is an essential subunit of the presequence translocase associated protein import motor of mitochondria. J. Cell Biol. **163**, 707–713 CrossRef PubMed
9 Voos, W., von Ahsen, O., Müller, H., Guiard, B., Rassow, J. and Pfanner, N. (1996) Differential requirement for the mitochondrial Hsp70–Tim44 complex in unfolding and translocation of preproteins. EMBO J. **15**, 2668–2677 PubMed
10 Mick, D.U., Fox, T.D. and Rehling, P. (2011) Inventory control: cytochrome c oxidase assembly regulates mitochondrial translation. Nat. Rev. Mol. Cell Biol. **12**, 14–20 CrossRef PubMed
11 Ott, M., Amunts, A. and Brown, A. (2016) Organization and regulation of mitochondrial protein synthesis. Annu. Rev. Biochem. **85**, 77–101 CrossRef PubMed
12 Suzuki, H., Ueda, T., Taguchi, H. and Takeuchi, N. (2007) Chaperone properties of mammalian mitochondrial translation elongation factor Tu. J. Biol. Chem. **282**, 4076–4084 CrossRef PubMed
13 Mayer, M.P. (2013) Hsp70 chaperone dynamics and molecular mechanism. Trends Biochem. Sci. **38**, 507–514 CrossRef PubMed
14 Voos, W. and Röttgers, K. (2002) Molecular chaperones as essential mediators of mitochondrial biogenesis. Biochim. Biophys. Acta **1592**, 51–62 CrossRef PubMed
15 Prip-Buus, C., Westermann, B., Schmitt, M., Langer, T., Neupert, W. and Schwarz, E. (1996) Role of the mitochondrial DnaJ homologue, Mdj1p, in the prevention of heat-induced protein aggregation. FEBS Lett. **380**, 142–146 CrossRef PubMed

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DOI: 10.1042/EBC20160009

16 Deocaris, C.C., Kaul, S.C. and Wadhwa, R. (2006) On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. Cell Stress Chaperones **11**, 116–128 CrossRef PubMed
17 Hayer-Hartl, M., Bracher, A. and Hartl, F.U. (2016) The GroEL–GroES chaperonin machine: a nano-cage for protein folding. Trends Biochem. Sci. **41**, 62–76 CrossRef PubMed
18 Cheng, M.Y., Hartl, F.U., Martin, J., Pollock, R.A., Kalusek, F., Neupert, W. et al. (1989) Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature **337**, 620–625 CrossRef PubMed
19 Bender, T., Lewrenz, I., Franken, S., Baitzel, C. and Voos, W. (2011) Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol. Biol. Cell **22**, 541–554 CrossRef PubMed
20 Dennerlein, S. and Rehling, P. (2015) Human mitochondrial COX1 assembly into cytochrome *c* oxidase at a glance. J. Cell Sci. **128**, 833–837 CrossRef PubMed
21 Richter-Dennerlein, R., Dennerlein, S. and Rehling, P. (2015) Integrating mitochondrial translation into the cellular context. Nat. Rev. Mol. Cell Biol. **16**, 586–592 CrossRef PubMed
22 Tyedmers, J., Mogk, A. and Bukau, B. (2010) Cellular strategies for controlling protein aggregation. Nat. Rev. Mol. Cell Biol. **11**, 777–788 CrossRef PubMed
23 Sauer, R.T. and Baker, T.A. (2011) AAA+ proteases: ATP-fueled machines of protein destruction. Annu. Rev. Biochem. **80**, 587–612 CrossRef PubMed
24 Leidhold, C., Janowsky, B.V., Becker, D., Bender, T. and Voos, W. (2006) Structure and function of Hsp78, the mitochondrial ClpB homolog. J. Struct. Biol. **156**, 149–164 CrossRef PubMed
25 von Janowsky, B., Major, T., Knapp, K. and Voos, W. (2006) The disaggregation activity of the mitochondrial ClpB homolog Hsp78 maintains Hsp70 function during heat stress. J. Mol. Biol. **357**, 793–807 CrossRef PubMed
26 Germaniuk, A., Liberek, K. and Marszalek, J. (2002) A bichaperone (Hsp70–Hsp78) system restores mitochondrial DNA synthesis following thermal inactivation of Mip1p polymerase. J. Biol. Chem. **277**, 27801–27808 CrossRef PubMed
27 Schmitt, M., Neupert, W. and Langer, T. (1996) The molecular chaperone Hsp78 confers compartment-specific thermotolerance to mitochondria. J. Cell Biol. **134**, 1375–1386 CrossRef PubMed
28 Röttgers, K., Zufall, N., Guiard, B. and Voos, W. (2002) The ClpB homolog Hsp78 is required for the efficient degradation of proteins in the mitochondrial matrix. J. Biol. Chem. **277**, 45829–45837 CrossRef PubMed
29 Iosefson, O., Sharon, S., Goloubinoff, P. and Azem, A. (2012) Reactivation of protein aggregates by mortalin and Tid1: the human mitochondrial Hsp70 chaperone system. Cell Stress Chaperones **17**, 57–66 CrossRef PubMed
30 Pinti, M., Gibellini, L., Liu, Y., Xu, S., Lu, B. and Cossarizza, A. (2015) Mitochondrial Lon protease at the crossroads of oxidative stress, ageing and cancer. Cell. Mol. Life Sci. **72**, 4807–4824 CrossRef PubMed
31 Rep, M., van Dijl, J.M., Suda, K., Schatz, G., Grivell, L.A. and Suzuki, C.K. (1996) Promotion of mitochondrial membrane complex assembly by a proteolytically inactive yeast Lon. Science **274**, 103–106 CrossRef PubMed
32 Suzuki, C.K., Suda, K., Wang, N. and Schatz, G. (1994) Requirement for the yeast gene *LON* in intramitochondrial proteolysis and maintenance of respiration. Science **264**, 273–276 CrossRef PubMed
33 Wagner, I., Arlt, H., van Dyck, L., Langer, T. and Neupert, W. (1994) Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. EMBO J. **13**, 5135–5145 PubMed
34 von Janowsky, B., Knapp, K., Major, T., Krayl, M., Guiard, B. and Voos, W. (2005) Structural properties of substrate proteins determine their proteolysis by the mitochondrial AAA+ protease Pim1. Biol. Chem. **386**, 1307–1317 CrossRef PubMed
35 Bender, T., Leidhold, C., Ruppert, T., Franken, S. and Voos, W. (2010) The role of protein quality control in mitochondrial protein homeostasis under oxidative stress. Proteomics **10**, 1426–1443 CrossRef PubMed
36 Bayot, A., Gareil, M., Rogowska-Wrzesinska, A., Roepstorff, P., Friguet, B. and Bulteau, A.L. (2010) Identification of novel oxidized protein substrates and physiological partners of the mitochondrial ATP-dependent Lon-like protease Pim1. J. Biol. Chem. **285**, 11445–114457 CrossRef PubMed
37 Luce, K. and Osiewacz, H.D. (2009) Increasing organismal healthspan by enhancing mitochondrial protein quality control. Nat. Cell Biol. **11**, 852–858 CrossRef PubMed
38 Lu, B., Yadav, S., Shah, P.G., Liu, T., Tian, B., Pukszta, S. et al. (2007) Roles for the human ATP-dependent Lon protease in mitochondrial DNA maintenance. J. Biol. Chem. **282**, 17363–17374 CrossRef PubMed
39 Gerdes, F., Tatsuta, T. and Langer, T. (2012) Mitochondrial AAA proteases: towards a molecular understanding of membrane-bound proteolytic machines. Biochim. Biophys. Acta **1823**, 49–55 CrossRef PubMed
40 Leonhard, K., Guiard, B., Pellecchia, G., Tzagoloff, A., Neupert, W. and Langer, T. (2000) Membrane protein degradation by AAA proteases in mitochondria: extraction of substrates from either membrane surface. Mol. Cell **5**, 629–638 CrossRef PubMed
41 Stiburek, L., Cesnekova, J., Kostkova, O., Fornuskova, D., Vinsova, K., Wenchich, L. et al. (2012) YME1L controls the accumulation of respiratory chain subunits and is required for apoptotic resistance, cristae morphogenesis, and cell proliferation. Mol. Biol. Cell **23**, 1010–1023 CrossRef PubMed
42 Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E.I. and Langer, T. (2005) The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. Cell. **123**, 277–289 CrossRef PubMed
43 Anand, R., Wai, T., Baker, M.J., Kladt, N., Schauss, A.C., Rugarli, E. et al. (2014) The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J. Cell Biol. **204**, 919–929 CrossRef PubMed
44 Atorino, L., Silvestri, L., Koppen, M., Cassina, L., Ballabio, A., Marconi, R. et al. (2003) Loss of m-AAA protease in mitochondria causes complex I deficiency and increased sensitivity to oxidative stress in hereditary spastic paraplegia. J. Cell Biol. **163**, 777–787 CrossRef PubMed
45 Baker, T.A. and Sauer, R.T. (2012) ClpXP, an ATP-powered unfolding and protein-degradation machine. Biochim. Biophys. Acta **1823**, 15–28 CrossRef PubMed

46 van Dyck, L., Dembowski, M., Neupert, W. and Langer, T. (1998) Mcx1p, a ClpX homologue in mitochondria of *Saccharomyces cerevisiae*. FEBS Lett. **438**, 250–254 CrossRef PubMed
47 Lowth, B.R., Kirstein-Miles, J., Saiyed, T., Brotz-Oesterhelt, H., Morimoto, R.I., Truscott, K.N. and Dougan, D.A. (2012) Substrate recognition and processing by a Walker B mutant of the human mitochondrial AAA+ protein CLPX. J. Struct. Biol. **179**, 193–201 CrossRef PubMed
48 Gispert, S., Parganlija, D., Klinkenberg, M., Drose, S., Wittig, I., Mittelbronn, M. et al. (2013) Loss of mitochondrial peptidase ClpP leads to infertility, hearing loss plus growth retardation via accumulation of CLPX, mtDNA and inflammatory factors. Hum. Mol. Genet. **22**, 4871–4887 CrossRef PubMed
49 Hansen, J., Corydon, T.J., Palmfeldt, J., Durr, A., Fontaine, B., Nielsen, M.N., Christensen, J.H., Gregersen, N. and Bross, P. (2008) Decreased expression of the mitochondrial matrix proteases Lon and ClpP in cells from a patient with hereditary spastic paraplegia (SPG13). Neuroscience **153**, 474–482 CrossRef PubMed
50 Jenkinson, E.M., Rehman, A.U., Walsh, T., Clayton-Smith, J., Lee, K., Morell, R.J. et al. (2013) Perrault syndrome is caused by recessive mutations in CLPP, encoding a mitochondrial ATP-dependent chambered protease. Am. J. Hum. Genet. **92**, 605–613 CrossRef PubMed
51 Haynes, C.M. and Ron, D. (2010) The mitochondrial UPR: protecting organelle protein homeostasis. J. Cell Sci. **123**, 3849–3855 CrossRef PubMed
52 Schulz, A.M. and Haynes, C.M. (2015) UPR^{mt}-mediated cytoprotection and organismal aging. Biochim. Biophys. Acta **1847**, 1448–1456 CrossRef PubMed
53 Zhao, Q., Wang, J., Levichkin, I.V., Stasinopoulos, S., Ryan, M.T. and Hoogenraad, N.J. (2002) A mitochondrial specific stress response in mammalian cells. EMBO J. **21**, 4411–4419 CrossRef PubMed
54 Al-Furoukh, N., Ianni, A., Nolte, H., Holper, S., Kruger, M., Wanrooij, S. and Braun, T. (2015) ClpX stimulates the mitochondrial unfolded protein response (UPR^{mt}) in mammalian cells. Biochim. Biophys. Acta **1853**, 2580–2591 CrossRef PubMed
55 Eiyama, A. and Okamoto, K. (2015) PINK1/Parkin-mediated mitophagy in mammalian cells. Curr. Opin. Cell Biol. **33C**, 95–101 CrossRef
56 Youle, R.J. and Narendra, D.P. (2011) Mechanisms of mitophagy. Nat. Rev. Mol. Cell Biol. **12**, 9–14 CrossRef PubMed
57 Song, Y., Ding, W., Xiao, Y. and Lu, K.J. (2015) The progress of mitophagy and related pathogenic mechanisms of the neurodegenerative diseases and tumor. Neurosci. J. **2015**, 543758 CrossRef PubMed
58 Becker, D., Richter, J., Tocilescu, M.A., Przedborski, S. and Voos, W. (2012) Pink1 kinase and its membrane potential ($\Delta\psi$)-dependent cleavage product both localize to outer mitochondrial membrane by unique targeting mode. J. Biol. Chem. **287**, 22969–22987 CrossRef PubMed
59 Fedorowicz, M.A., de Vries-Schneider, R.L.A., Rub, C., Becker, D., Huang, Y., Zhou, C. et al. (2014) Cytosolic cleaved PINK1 represses Parkin translocation to mitochondria and mitophagy. EMBO Rep. **15**, 86–93 CrossRef PubMed
60 Pickrell, A.M. and Youle, R.J. (2015) The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron **85**, 257–273 CrossRef PubMed
61 Jin, S.M. and Youle, R.J. (2013) The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. Autophagy **9**, 1750–1757 CrossRef PubMed
62 Ni, H.M., Williams, J.A. and Ding, W.X. (2015) Mitochondrial dynamics and mitochondrial quality control. Redox Biol. **4**, 6–13 CrossRef PubMed
63 Schapira, A.H. (2006) Mitochondrial disease. Lancet **368**, 70–82 CrossRef PubMed