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Focus Quality Control

Quality control of mitochondria: protection against neurodegeneration and ageing

Takashi Tatsuta and Thomas Langer*

Institute for Genetics and Centre for Molecular Medicine (CMMC), University of Cologne, Cologne, Germany

Dysfunction of mitochondria has severe cellular consequences and is linked to ageing and neurodegeneration in human. Several surveillance strategies have evolved that limit mitochondrial damage and ensure cellular integrity. Intraorganellar proteases conduct protein quality control and exert regulatory functions, membrane fusion and fission allow mitochondrial content mixing within a cell, and the autophagic degradation of severely damaged mitochondria protects against apoptosis. Here, we will summarize the current knowledge on these surveillance strategies and their role in human disease.

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Mitochondria: essential and delicate organelles

After the symbiotic engulfment of aerobic α-proteobacteria by pre-eukaryotic cells more than 1.5 billion years ago, mitochondria evolved as specialized organelles with a plethora of cellular functions. They not only house the respiratory chain (RC) and provide cellular energy but are also the site of essential biosynthetic pathways. Mitochondria serve as calcium stores and are integrated in a number of signalling pathways, including cell death cascades, thus controlling cellular homoeostasis in multiple ways (McBride *et al*, 2006). Given the multitude of functions, it does not surprise that mitochondrial dysfunction has severe cellular consequences, and is linked to ageing and neurological disorders in human (Lin and Beal, 2006).

Partly as a consequence of their endosymbiotic origin, the maintenance of mitochondrial activities imposes considerable challenges on eukaryotic cells. While the majority is nuclearly encoded, a few subunits of the RC are encoded by the mitochondrial genome, which requires a coordinated expression of nuclear and mitochondrial genomes. Moreover, mitochondria are highly dynamic organelles. Constant fusion and fission events, mediated by conserved cellular machineries, lead to the formation of a reticulated mitochondrial network (Cerveny *et al*, 2007; Detmer and Chan, 2007; Hoppins *et al*, 2007). This allows adapting mitochondrial activities to different physiological demands, but makes the assembly of functional mitochondria a difficult task to achieve.

Additional challenges for mitochondrial integrity come from reactive oxygen species (ROS), which induces protein modifications, lipid peroxidation and DNA damage and are an inevitable by-product of oxidative phosphorylation. Dysfunctional mitochondria bear the risk of futile ATP hydrolysis and increased oxidative stress. Even more, extensive mitochondrial damage may lead to the dissipation of the membrane potential across the inner membrane and induce cell death by the release of pro-apoptotic proteins (Kroemer *et al*, 2007). It is therefore crucial to tightly control oxidative phosphorylation and monitor the functionality of the RC to maintain mtDNA integrity and limit mitochondrial damage.

Cellular defence against mitochondrial damage

Cells have evolved elaborate systems to cope up with the diverse challenges imposed on mitochondrial integrity (Figure 1). The first line of defence is provided by a highly conserved, intraorganellar proteolytic system that conducts the surveillance of protein quality control (QC) within mitochondria (Koppen and Langer, 2007). Molecular chaperones and energy-dependent proteases monitor the folding and assembly of mitochondrial proteins and selectively remove excess and damaged proteins from the organelle. A second line of defence is provided at the organellar level by the dynamic nature of the mitochondrial population of a cell. The functionality of damaged mitochondria can be restored by fusion with neighbouring, intact mitochondria, assigning an important role for QC to components regulating mitochondrial dynamics (Detmer and Chan, 2007). Severe damage of mitochondria, however, impairs fusion and results in fragmentation of mitochondria, which are then selectively removed by an autophagic process, termed mitophagy (Kim *et al*, 2007). Mitophagy prevents the release of pro-apoptotic proteins from damaged mitochondria. Consistent with a cytoprotective function of autophagy, apoptosis is suppressed upon induction but induced upon inhibition of autophagy (Maiuri *et al*, 2007).

Selective degradation of mitochondrial proteins: the first line of defence

The mitochondrial QC system recognizes and removes non-assembled and misfolded proteins selectively. Key

*Corresponding author. Institut für Genetik, Universität zu Köln, Zülpicher Str. 47, Köln 50674, Germany. Tel.: +49 221 470 4876; Fax: +49 221 470 6749; E-mail: Thomas.Langer@uni-koeln.de

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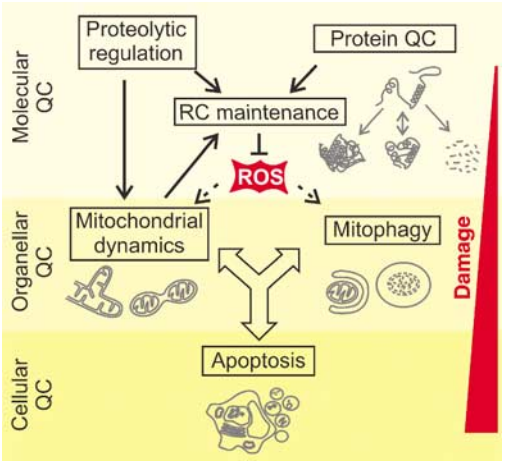

Figure 1 Quality control (QC) surveillance of mitochondria. Intraorganellar proteases exert QC and regulatory functions to maintain respiratory chain (RC) activity. The functionality of damaged mitochondria can be restored by fusion and content mixing within the mitochondrial network. Severely damaged mitochondria fragment and are removed by mitophagy or induce apoptosis by the release of pro-apoptotic proteins.

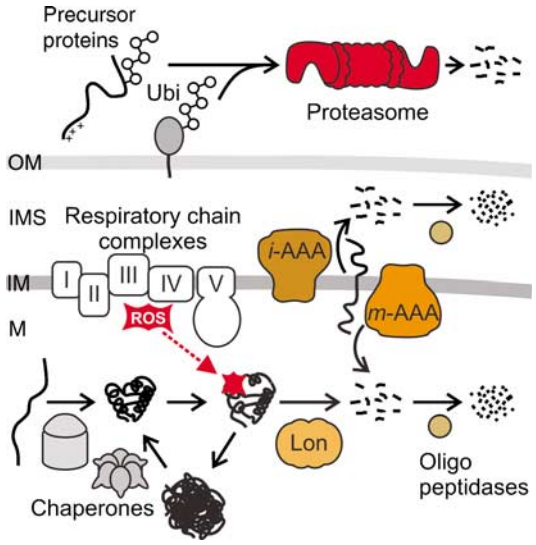

Figure 2 Quality control (QC) of mitochondrial proteins. ATP-dependent proteases present in various subcompartments of mitochondria recognize non-native polypeptides and trigger their proteolysis to peptides that are further degraded by oligopeptidases. At the same time, energy-dependent proteases can act as processing enzymes ensuring assembly and integrity of RC. Recent evidence links the UPS to mitochondrial QC and the regulation of mitochondrial dynamics. OM, outer membrane; IMS, intermembrane space; IM, inner membrane; M, matrix.

components are ATP-dependent proteases, which are derived from bacterial proteases and highly conserved in eukaryotes (Koppen and Langer, 2007). These proteases degrade damaged proteins to peptides, which are subsequently either exported from the organelle or degraded further to amino acids by various oligopeptidases (Figure 2). As many substrates are part of multimeric protein complexes within mitochondria, degradation of damaged proteins may involve substrate extraction from large assemblies and is therefore likely closely linked to assembly and disassembly processes. ATP-dependent proteases sense the folding state of substrates by exerting chaperone-like properties and trigger the proteolysis of non-native proteins. A central role in this process is exerted by conserved ATPase modules, which are characteristic of the AAA^+ family of ATPases and present in all ATP-dependent proteases.

QC of matrix proteins

Up to two ATP-dependent proteases, the Lon and the ClpXP proteases, are present in the mitochondrial matrix in various organisms. While next to nothing is known about the physiological function of ClpXP in mitochondria, Lon protease (also termed PIM1 protease in yeast) has been demonstrated to degrade various misfolded and non-assembled polypeptides in the matrix. These include thermally denatured proteins as well as oxidatively damaged (carbonylated) proteins, such as the matrix-localized iron–sulphur protein aconitase (Bota and Davies, 2002; Major *et al*, 2006). In most cases, impaired folding appears to trigger protein degradation by Lon. Molecular chaperone proteins of the Hsp70 and Hsp100 family stabilize misfolded proteins against aggregation or mediate the dissolution of protein aggregates and thereby ensure proteolysis (Wagner *et al*, 1994; Bateman *et al*, 2002; Röttgers *et al*, 2002).

The limited capacity of the QC system in the matrix is illustrated by the accumulation of oxidatively modified and aggregated aconitase in aged cells or in age-related human disorders and may contribute to the age-related mitochondrial decline (Bota *et al*, 2002). Consistently, Lon-deficient cells accumulate protein aggregates in mitochondria and show deterioration of mitochondrial functions both in human and yeast (Suzuki *et al*, 1994; Bota *et al*, 2005). It is presently unclear whether these cellular defects are simply caused by the accumulation of protein aggregates within mitochondria. Alternatively, protein aggregation could be cytoprotective and may represent dumping of non-native, otherwise deleterious polypeptides. Cellular defects in the absence of Lon, such as the loss of the mitochondrial genome, may instead arise from the impaired proteolysis of specific regulatory proteins. Such a regulatory role could explain the presence of Lon protease in mitochondrial nucleoids and its affinity for DNA (Liu *et al*, 2004; Cheng *et al*, 2005).

QC by AAA proteases in the inner membrane

The mitochondrial inner membrane harbours the RC making inner membrane proteins a prime target of mitochondrial ROS. At least two membrane-integrated ATP-dependent proteolytic complexes, termed AAA proteases, conduct the QC surveillance in the inner membrane and the selective degradation of non-assembled and damaged proteins (Koppen and Langer, 2007). Whereas the *i*-AAA protease is active on the intermembrane side, *m*-AAA proteases expose their catalytic domains to the matrix side of the inner membrane. *m*- and *i*-AAA proteases exert overlapping substrate specificities in yeast and it is apparently solely the membrane topology of substrates that determines which protease is involved in degradation. AAA proteases recognize misfolded, solvent-exposed domains of substrates, loops of multispanning membrane proteins, or short N- or C-terminal tails protruding from the lipid bilayer (Leonhard *et al*, 2000). The presence of at least two AAA proteases inversely inserted

into the inner membrane therefore ensures degradation of membrane proteins irrespective of their topology. AAA proteases mediate the ATP-dependent dislocation of substrate proteins from the membrane allowing their degradation in a hydrophilic environment (Leonhard *et al*, 2000). This is reminiscent of the turnover of ER membrane proteins by 26S proteasomes during ER-associated protein degradation (ERAD) (Meusser *et al*, 2005). Therefore, it appears that AAA proteases represent a distinct proteolytic system in a membrane, which is not accessible for the ubiquitin–proteasome system (UPS) from the cytosol.

Assembly and maintenance of the RC by AAA proteases

In addition to its function in QC, *m*-AAA proteases control directly the biogenesis of the RC. A nuclearly encoded subunit of mitochondrial ribosomes, MrpL32, is matured by yeast and murine *m*-AAA proteases, which act as a processing and not as a QC enzyme in this context (Figure 3A) (Nolden *et al*, 2005). Since MrpL32 processing is a prerequisite for its assembly into ribosomes and activation of mitochondrial translation, the *m*-AAA protease directly regulates the synthesis of RC subunits within mitochondria. Complementation experiments provide direct evidence that the dysfunction of mitochondria in the absence of *m*-AAA protease is mainly caused by an impaired processing of MrpL32 rather than by misfolded polypeptides (Nolden *et al*, 2005). It should be noted, however, that both regulatory and QC functions of the *m*-AAA protease are interdependent and may interfere with each other. For instance, QC substrates accumulating in aged cells or under stress conditions may compete with MrpL32 for binding to the *m*-AAA protease, resulting in an inhibition of translation.

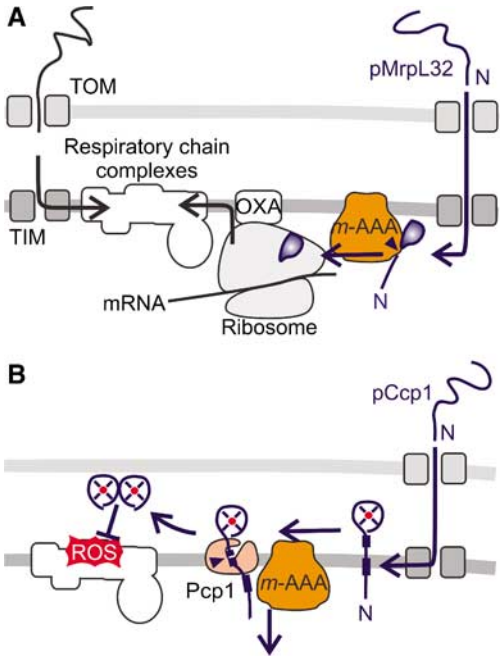

Figure 3 Regulation of RC assembly and maintenance by proteases. (**A**) The assembly of mitochondrial ribosomes and synthesis of mitochondrially encoded RC subunits require maturation of newly imported MrpL32 by the *m*-AAA protease. (**B**) Biogenesis of the ROS scavenger Ccp1 in yeast mitochondria depends on ATP-dependent membrane dislocation of the precursor protein by the *m*-AAA protease and maturation by the rhomboid protease Pcp1.

The *m*-AAA protease also exerts a protective function for assembled RC complexes, strikingly independent of its proteolytic activity. It mediates maturation of the ROS scavenger cytochrome *c* peroxidase (Ccp1) in the intermembrane space of yeast mitochondria and therefore limits ROS damage (Figure 3B). Ccp1 receives two-step processing by the *m*-AAA protease and the rhomboid protease Pcp1, an intramembrane-cleaving peptidase in the inner membrane (Esser *et al*, 2002). Rhomboid cleavage depends on the ATP-dependent membrane dislocation of Ccp1 by the *m*-AAA protease but not on its proteolytic activity (Tatsuta *et al*, 2007). A similar role has been proposed for the *i*-AAA protease assisting the import of mammalian polynucleotide phosphorylase (PNPase) into yeast mitochondria (Rainey *et al*, 2006). It therefore appears that the ATP-dependent membrane dislocation represents a novel function of AAA proteases of general relevance.

QC of mitochondrial proteins by the UPS

Increasing evidence suggests that the cytosolic UPS affects the QC of mitochondrial proteins in two ways (Figure 2): first, in the cytosol prior to import of nuclearly encoded proteins into mitochondria and, second, in the mitochondrial outer membrane. A proteomic survey of ubiquitinated proteins identified many proteins that finally reside in different mitochondrial subcompartments, suggesting that non-imported preproteins are degraded by the cytosolic UPS (Peng *et al*, 2003). Direct evidence for UPS-mediated degradation in the cytosol has been provided for apo-cytochrome *c* or the E2 component of 2-oxoglutarate dehydrogenase complex (Pearce and Sherman, 1997; Habelhah *et al*, 2004). Notably, although imported post-translationally, some preproteins are synthesized from messenger RNAs in close proximity to mitochondria and are found associated with the outer membrane (Zahedi *et al*, 2006). It is therefore conceivable that preproteins are ubiquitinated at the outer membrane and subject to proteolysis by the UPS. This is of interest as a RING-like ubiquitin ligase is present in the outer membrane of mammalian mitochondria that was demonstrated to affect mitochondrial dynamics (Nakamura *et al*, 2006; Yonashiro *et al*, 2006; Karbowski *et al*, 2007). However, it is presently unclear whether this ligase primarily exerts a regulatory or a QC control function. The latter would imply that mitochondrial outer membrane proteins are subject to proteolysis by 26S proteasomes in the cytosol and therefore point to similarities to ERAD.

Mitochondrial dynamics and QC

The dynamic nature of the mitochondrial network provides additional protection against mitochondrial damage. Ongoing cycles of fusion and fission of mitochondrial membranes are mediated by conserved protein machineries (Cerveny *et al*, 2007; Detmer and Chan, 2007; Hoppins *et al*, 2007). At least four dynamin-related GTPases mediate fission and fusion of mitochondrial membranes and determine the shape of the mitochondrial reticulum: mitofusins MFN1 and MFN2 in the outer and OPA1 in the inner membrane control mitochondrial membrane fusion, while DRP1 triggers fission events. Fusion, on the one hand, allows content mixing between intact and

dysfunctional mitochondria. Replacement of damaged material, such as mutant mtDNA, contributes to the integrity and homogeneity of the mitochondrial population in a cell. In agreement with a protective function of mitochondrial fusion, a decline in respiratory activities has been observed in fusion-deficient murine fibroblasts lacking OPA1 or mitofusins (Chen *et al*, 2005, 2007). Fission, on the other hand, allows sequestration of irreversibly damaged, fusion-incompetent mitochondria and their subsequent elimination by autophagy (see below). Fusion and fission are highly regulated and again a central regulatory role of QC enzymes is emerging (Figure 4).

Degradation of yeast mitofusin Fzo1

Fusion of yeast mitochondria depends on the constitutive turnover of the mitofusin Fzo1, an essential component of the fusion machinery in the outer membrane (Figure 4A). Proteolysis of Fzo1 was found to depend on the F-box protein Mdm30 (Neutzner and Youle, 2005; Escobar-Henriques *et al*, 2006). F-box proteins have been identified as components of SCF-E3 ubiquitin ligase complexes mediating proteasomal degradation of specific substrates (Petroski and Deshaies, 2005). Surprisingly, the Mdm30-dependent proteolysis of Fzo1 did not require other SCF complex components or 26S proteasomes, suggesting the existence of a yet unidentified proteolytic system for the turnover of outer membrane proteins (Escobar-Henriques *et al*, 2006). Notably, mating factor-induced cell cycle arrest induces rapid degradation of Fzo1 (Neutzner and Youle, 2005). However, under these conditions, proteolysis does not depend on Mdm30 but requires a functional UPS system. Thus, the level of yeast mitofusin Fzo1 in the mitochondrial outer membrane is controlled by two independent proteolytic pathways. It will be interesting to examine whether mammalian mitofusins are regulated in a similar manner.

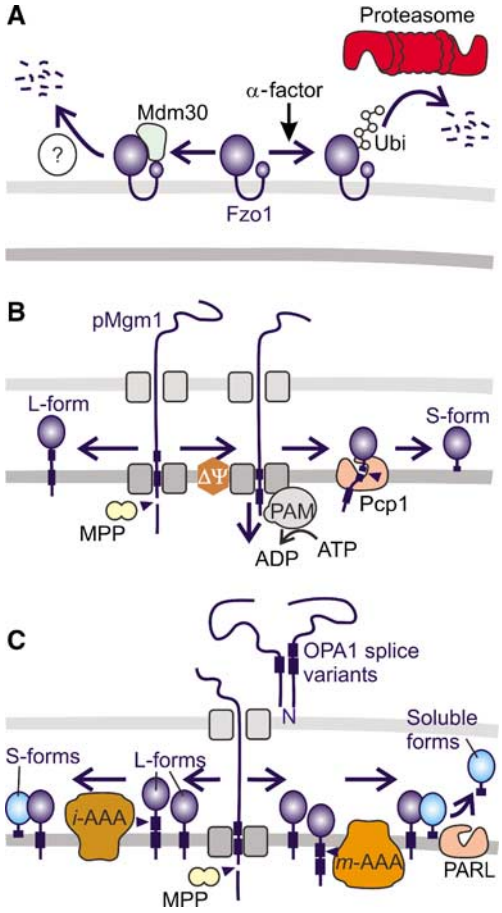

Figure 4 Regulation of mitochondrial dynamics by proteolysis. (**A**) Degradation of the yeast mitofusin Fzo1 can occur along two independent pathways. Constitutive turnover is dependent on the F-box protein Mdm30. Fzo1 turnover induced by cell cycle arrest (in the presence of α-factor) is mediated by UPS but does not require Mdm30. (**B**) Alternative topogenesis of Mgm1. Proteolytic conversion of L-Mgm1 to S-Mgm1 by the rhomboid protease Pcp1 depends on the mitochondrial membrane potential (ΔΨ), matrix ATP, and the mitochondrial import motor (PAM). (**C**) Model for the processing of OPA1 in mammalian mitochondria. After processing of newly imported OPA1 by MPP, long and short isoforms of OPA1 are generated by constitutive (left pathway) and inducible (right pathway) cleavage at sites 2 and site 1, respectively. *m*- and *i*-AAA proteases and PARL have been linked to OPA1 processing, but the exact proteolytic pathways remained speculative.

Processing of the dynamin-like GTPase OPA1

Most compelling evidence for a regulatory role of mitochondrial peptidases exists for inner membrane fusion and cristae morphogenesis mediated by the dynamin-like GTPase OPA1 (Mgm1 in yeast) (Figure 4B and C). Fusion depends on proteolytic processing and the balanced formation of short and long isoform(s) (Herlan *et al*, 2003; Song *et al*, 2007). In yeast, the long isoform of Mgm1 (L-Mgm1) inserts into the mitochondrial inner membrane upon import and is cleaved by the mitochondrial-processing peptidase MPP (Herlan *et al*, 2003). A subset of the long isoforms undergoes a second cleavage event, which is mediated by the rhomboid protease Pcp1 in the inner membrane and results in the formation of the short Mgm1 isoform (S-Mgm1) (Herlan *et al*, 2003; McQuibban *et al*, 2003; Sesaki *et al*, 2003). The efficiency of Pcp1 processing was found to depend on the ATP-dependent protein import motor in the matrix space and thereby on the energy status of mitochondria (Herlan *et al*, 2004). Similarly, the balance between long and short isoforms of OPA1 is determined by matrix ATP also in mammalian mitochondria. However, in contrast to yeast mitochondria, low ATP levels trigger OPA1 cleavage (Duvezin-Caubet *et al*, 2006; Baricault *et al*, 2007). S-OPA1 accumulates and mitochondria fragment in cells harbouring a dysfunctional RC as well as in cells from patients suffering from mitochondrial myopathies (Duvezin-Caubet *et al*, 2006). These observations suggest that mitochondrial functionality and morphology are tightly coupled by the energy-sensitive processing of OPA1.

Eight splice variants of OPA1 are synthesized which are processed at two different sites, sites 1 and 2, resulting in the accumulation of at least five different OPA1 isoforms (Ishihara *et al*, 2006; Duvezin-Caubet *et al*, 2007; Olichon *et al*, 2007). OPA1 processing occurs constitutively at site 2 but cleavage can be induced at site 1 at low membrane potential, by ROS treatment or by apoptotic stimuli (Ishihara *et al*, 2006; Baricault *et al*, 2007; Griparic *et al*, 2007; Song *et al*, 2007). Currently, three peptidases have been linked to the processing of OPA1. Overexpression of S-OPA1 protects cells lacking the rhomboid protease PARL against apoptosis, demonstrating that they act along the same apoptotic pathway (Cipolat *et al*, 2006). However, *Parl*$^{-/-}$ cells do not show an impaired processing of OPA1 or mitochondrial morphology defects (Cipolat *et al*, 2006; Duvezin-Caubet *et al*, 2007). Rather, PARL appears to

facilitate the release of S-OPA1 from the inner membrane (Cipolat *et al*, 2006). Moreover, PARL can substitute for the yeast rhomboid protease Pcp1 in processing Mgm1 but does not process OPA1 when co-expressed in yeast (Duvezin-Caubet *et al*, 2007). The *m*-AAA protease subunit paraplegin has also been proposed to mediate inducible cleavage of OPA1 at site 1 (Ishihara *et al*, 2006). But again, OPA1 processing is not impaired in paraplegin-deficient fibroblasts (Duvezin-Caubet *et al*, 2007). This might be explained by the partial redundancy of various *m*-AAA protease subunits and the formation of homo- and hetero-oligomeric isoenzymes (Koppen *et al*, 2007). Co-expression of OPA1 with *m*-AAA isoenzymes in yeast indeed revealed that OPA1 can be cleaved by mammalian *m*-AAA proteases in the heterologous yeast system (Duvezin-Caubet *et al*, 2007). Knockdown experiments identified the *i*-AAA protease Yme1L as being responsible for the constitutive cleavage of OPA1 at site 2 (Griparic *et al*, 2007; Song *et al*, 2007). The physiological consequence of this cleavage is still unclear as Yme1L affects mitochondrial morphology also in an OPA1-independent manner (Griparic *et al*, 2007). Therefore, it appears that various proteases regulate, directly or indirectly, OPA1 processing and adjust OPA1 activity and mitochondrial morphology to different physiological demands.

Elimination of damaged mitochondria by mitophagy

Mitochondrial dysfunction impairs OPA1 processing and triggers fragmentation sequestering damaged mitochondria from the intact mitochondrial network. Fragmented mitochondria can be removed by mitophagy before apoptosis is induced by the release of pro-apoptotic proteins from mitochondria (Figure 5) (Kim *et al*, 2007). Substantial insight into general mechanisms regulating autophagy came from studies in yeast which identified ~30 proteins (Atg) involved in autophagic processes, many of them conserved in higher eukaryotes (Yorimitsu and Klionsky, 2005). Deletion of yeast *ATG* genes causes mitochondria-related phenotypes, such as growth defects on non-fermentable medium, suggesting a pivotal role of autophagy for QC of mitochondria (Zhang *et al*, 2007). Two mitochondrial proteins, termed Uth1 and Aup1, have been linked specifically to mitophagy and are not part of the general autophagic machinery: Uth1 in the mitochondrial outer membrane and the putative phosphatase Aup1 in the intermembrane space (Kissova *et al*, 2004; Tal *et al*, 2007).

It is currently not understood how damaged mitochondria are specifically selected, but accumulating evidence suggests that mitochondrial dysfunction by itself triggers mitophagy (Kim *et al*, 2007). Mitochondria-derived ROS, at low concentrations, may act as signalling molecules and trigger mitophagy through redox regulation of Atg4, an essential cysteine protease in the autophagic pathway (Scherz-Shouval *et al*, 2007). The formation of autophagosomes depends on Atg4-mediated cleavage of Atg8 and its subsequent conjugation to phosphatidyl ethanolamine at autophagosomal membranes (Yorimitsu and Klionsky, 2005). Antioxidant treatment suppresses lipidation of Atg8 and autophagosome formation, suggesting that lipid conjugation is a direct consequence of ROS production (Scherz-Shouval *et al*, 2007).

Mitophagy was found to depend on mitochondrial fission. In primary neuronal cells, mitophagy can be induced by NO, which results in ROS production and mitochondrial fragmentation (Barsoum *et al*, 2006). Inhibition of mitochondrial fission or induction of mitochondrial fusion inhibits NO-induced mitophagy (Barsoum *et al*, 2006). Similarly, autophagic degradation of yeast mitochondria, induced by depletion of the inner membrane protein Mdm38, depends on fission mediated by the Drp1 homologue Dnm1 (Nowikovsky *et al*, 2007). After mitochondrial fission, progression of mitophagy appears to depend on the mitochondrial permeability transition (MPT) and can be inhibited by MPT inhibitors like cyclosporine A or overexpression of the anti-apoptotic protein Bcl2 (Xue *et al*, 2001; Rodriguez-Enriquez *et al*, 2006). MPT finally leads to the rupture of the outer membrane, the release of pro-apoptotic proteins from the intermembrane space and apoptosis, if mitochondria are not selectively removed by mitophagy. Thus, mitophagy and apoptosis appear to share common steps, although the physiological outcome is strikingly different (Maiuri *et al*, 2007).

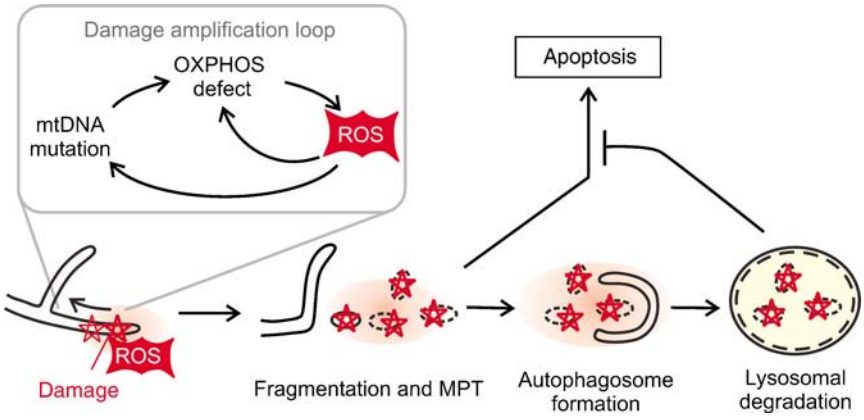

Figure 5 Quality control of mitochondria by mitophagy. ROS produced by damaged mitochondria induces mitochondrial fragmentation and mitochondrial permeability transition (MPT). Damaged mitochondria are engulfed by autophagosomes selectively and eliminated, preventing the release of pro-apoptotic proteins and apoptosis.

Role of mitochondrial QC in disease and ageing

Numerous studies illustrate the relevance of disturbed mitochondrial activities for ageing and degenerative processes (Lin and Beal, 2006). An impaired energy supply or Ca^{2+} buffering, increased ROS production, or control of apoptosis by mitochondria may contribute to the progressive decline of long-lived, postmitotic cells. Mutations in mitochondrial proteins frequently result in neurological symptoms illustrating the susceptibility of neurons for mitochondrial dysfunctions. Given the protective functions of mitochondrial QC, it is not surprising that pathogenic mutations for an increasing number of neurodegenerative disorders are identified in key components of the intraorganellar QC system and the mitochondrial fusion machinery (Table I).

Parkinson's disease

Parkinson's disease (PD), a common age-associated neurodegenerative disorder, is characterized by the preferential loss of dopamine-secreting neurons in the substantia nigra and the accumulation of intraneuronal inclusions (Lewy bodies). Although the aetiology of PD is heterogeneous and pathogenic mutations have been identified in various genes, mitochondrial dysfunction appears to have a prevalent role in the pathogenesis of the disease (Mandemakers *et al*, 2007). A role of protein QC within mitochondria is suggested by the finding of heterozygous missense mutations in *HTRA2/OMI* in sporadic cases of PD. HtrA2, homologous to bacterial Deg proteases, is localized in the mitochondrial intermembrane space and protects against mitochondrial stress. HtrA2 may act as a QC enzyme and degrade misfolded polypeptides in the mitochondrial intermembrane space or, in analogy to bacterial DegS, be part of an adaptive stress signalling cascade. HtrA2 is released from the intermembrane space of mitochondria during apoptosis (Suzuki *et al*, 2001). However, HtrA2-deficient mice show neurodegeneration and Parkinson-like phenotypes, but did not provide evidence for a pro-apoptotic function of HtrA2 (Jones *et al*, 2003; Martins *et al*, 2004).

Intriguingly, HtrA2 associates with PINK1, a serine/threonine kinase, which has been found to be mutated in PD patients (Plun-Favreau *et al*, 2007). Both PINK1 and HtrA2 appear to act along the same stress-protective pathway. PINK1 is required for phosphorylation of HtrA2, which increases its proteolytic activity *in vitro* (Plun-Favreau *et al*, 2007). While it remains to be clarified whether HtrA2 is a PINK1 substrate, PINK1-mediated phosphorylation has been demonstrated for TRAP1, a putative molecular chaperone with significant homology to the HSP90AA1 family (Pridgeon *et al*, 2007). PINK1-mediated phosphorylation of TRAP1 is induced by oxidative stress. Overexpression of PINK1, on the other hand, protects cells from apoptosis induced by oxidative stress, suggesting that PINK1 and TRAP1 are part of an anti-apoptotic signalling cascade (Pridgeon *et al*, 2007). Thus, although neither the physiological role of HtrA2 nor of TRAP1 has been defined, an intimate link among mitochondrial QC, stress signalling and PINK1-associated PD pathogenesis is emerging. Interestingly, Parkin has been genetically linked to PINK and functions downstream as an E3 ubiquitin ligase (Clark *et al*, 2006; Park *et al*, 2006; Exner *et al*, 2007). Therefore, although substrates are largely unknown, Parkin is functionally linked to mitochondria (Dodson and Guo, 2007).

Table I Neurodegenerative diseases linked to mitochondrial QC

Associated disease	Gene	Function
Parkinson's disease (PD)	*HTRA2*	Serine protease in mitochondrial intermembrane space
	PINK1	Serine/threonine kinase in mitochondrial intermembrane space
	PARKIN	E3 ubiquitin ligase
Charcot–Marie–Tooth disease		
Subtype 2A (CMT2A)	*MFN2*	Dynamin-like GTPase in outer membrane mediating fusion
Subtype 4A (CMT4A)	*GDAP1*	Outer membrane protein promoting fission
Autosomal dominant optic atrophy (DOA)	*OPA1*	Dynamin-like GTPase required for inner membrane fusion and cristae morphology
Hereditary spastic paraplegia (HSP)	*Paraplegin*	Subunit of hetero-oligomeric *m*-AAA protease in inner membrane
	HSP60	Matrix-localized chaperonin

Hereditary spastic paraplegia

Mutations in a subunit of the *m*-AAA protease, termed paraplegin, cause an autosomal recessive form of hereditary spastic paraplegia (HSP) (Casari *et al*, 1998). HSP is a genetically heterogeneous group of neurological disorders that is characterized by progressive and cell-specific axononal degeneration of cortical motor neurons starting from their distal extremities (Soderblom and Blackstone, 2006). The pathogenic mechanism is presently not understood but the retrograde mode of degeneration suggests that mitochondria at synaptic endings are affected initially. Paraplegin constitutes a subunit of a hetero-oligomeric *m*-AAA isoenzyme, which can substitute for QC and regulatory functions of the yeast *m*-AAA protease (Atorino *et al*, 2003; Koppen *et al*, 2007). Accordingly, mitochondrial dysfunction and axonal degeneration in the absence of paraplegin may result from the accumulation of non-degraded, misfolded inner membrane proteins or impaired regulatory steps during mitochondrial biogenesis, or both. It is of interest in this context that mutations in the chaperone HSP60, localized in the mitochondrial matrix, have been described to cause an autosomal dominant form of HSP (Hansen *et al*, 2002), suggesting that an impaired mitochondrial QC can indeed cause axonal degeneration.

Insights into the pathogenesis of HSP came from a paraplegin-deficient murine model that recapitulates main clinical features of HSP (Ferreirinha *et al*, 2004). While RC deficiencies were not apparent, enlarged and structurally abnormal mitochondria accumulated in synaptic terminals of motor neurons at early stages, correlating with the onset of motor impairment. These abnormalities become more prominent with age and involve also proximal regions. It is conceivable that the accumulation of aberrant mitochondria leads to clogging and subsequent swelling of axons, as fission and

dynamic changes in mitochondrial morphology affect axonal transport (Rugarli and Langer, 2006). Moreover, an impaired axonal transport of enlarged mitochondria may also affect their autophagic degradation, which requires retrograde transport because of the paucity of lysosomes at synaptic endings (Hollenbeck, 1993).

While these findings could explain the accumulation of dysfunctional mitochondria at synaptic endings, they are difficult to reconcile with the proposed role of paraplegin for OPA1 processing. Impaired OPA1 cleavage in the absence of the paraplegin-containing *m*-AAA protease would impair fusion and cause fragmentation of mitochondria. This could facilitate their retrograde transport and autophagic degradation. Although functional mitochondria would be depleted at synapses, the presence of enlarged mitochondrial structures in the absence of paraplegin remains to be explained. Therefore, it appears that additional effects of paraplegin on mitochondrial fission or mitophagy must be envisioned.

Mitochondrial dynamics and neurodegeneration

The importance of mitochondrial dynamics for neuronal survival is illustrated directly by the identification of pathogenic mutations in components regulating mitochondrial dynamics. Mutations in OPA1 lead to dominant optic atrophy (Alexander *et al*, 2000; Delettre *et al*, 2000), the most commonly inherited optic neuropathy, characterized by the specific loss of retinal ganglion cells (Delettre *et al*, 2002). Moreover, Charcot–Marie–Tooth disease, a frequent peripheral neuropathy affecting both motor and sensory nerves (Züchner and Vance, 2005), is caused by mutations in MFN2 and GDAP1 (ganglioside-induced differentiation protein 1) localized in the mitochondrial outer membrane (Züchner *et al*, 2004; Niemann *et al*, 2005). While mutations of MFN2 inhibit fusion, inactivation of GDAP1 promotes fission of mitochondria.

A conditional mouse model allowing inactivation of MFN2 in the cerebellum of adult mice revealed that Purkinje cells are highly susceptible to mitochondrial fusion defects (Chen *et al*, 2007). In the absence of MFN2 and mitochondrial fusion, they accumulate fragmented mitochondria with an impaired respiratory activity, likely caused by the loss of mtDNA nucleoids. Interestingly, aberrant mitochondria are predominantly detectable in neuronal terminals of MFN2-deficient murine Purkinje cells (Chen *et al*, 2007). This is reminiscent of observations in paraplegin-deficient neurons and consistent with axonal transport deficiencies in the absence of MFN2 (Baloh *et al*, 2007).

The problem of tissue specificity

Although we are beginning to understand the selective vulnerability of neurons for mitochondrial damage, the striking tissue-specific consequences of pathogenic mutations in QC components including the mitochondrial fusion machinery remain difficult to explain. Multiple mechanisms can be envisioned that are not mutually exclusive. It is conceivable that certain cell types are more dependent on mitochondrial activities than others and therefore more susceptible to mitochondrial damage. This may include neurons with a high demand for mitochondrial transport, such as corticospinal motor neurons with long axons or Purkinje cells with highly branched dendrites. Others are inherently more exposed to stress and mitochondrial damage, such as dopamine-producing neurons affected in PD (Mandemakers *et al*, 2007). Moreover, functional redundancy and tissue-specific expression of different mitochondrial QC components could affect QC surveillance and the resistance of mitochondria against damage. For instance, the mitofusins MFN1 and MFN2 were detected in functionally redundant, homo- and hetero-oligomeric complexes (Chen *et al*, 2003). In murine Purkinje cells, MFN2 is more highly expressed than MFN1, providing an explanation for their selective vulnerability in CMT2A (Chen *et al*, 2007). Similarly, overlapping substrate specificity was demonstrated for homo- and hetero-oligomeric isoenzymes of the *m*-AAA protease in murine and human mitochondria (Koppen *et al*, 2007). AFG3L2, homologous to paraplegin, assembles with paraplegin into a hetero-oligomeric *m*-AAA protease, but can also form homo-oligomeric, proteolytically active complexes. Accordingly, homo-oligomeric AFG3L2 complexes may lessen phenotypic consequences of the loss of paraplegin in HSP. At the same time, differences in the relative abundance of AFG3L2 and paraplegin in different tissues, as observed in mouse, may relate to the tissue-specific defects observed in HSP patients (Koppen *et al*, 2007). If differences in the enzymatic properties or substrate specificity of homo- and hetero-oligomeric *m*-AAA isoenzymes exist, the situation may become even more complex. Thus, although recent years have seen considerable progress in our understanding of QC surveillance mechanisms, much remains to be learned before a molecular understanding of pathogenic consequences of mitochondrial damage can be reached.

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