Published in final edited form as:

J Neuroimmune Pharmacol. 2016 December ; 11(4): 629–644. doi:10.1007/s11481-016-9683-8.

Mitochondrial Quality Control Proteases in Neuronal Welfare

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Abstract

The functional integrity of mitochondria is a critical determinant of neuronal health and compromised mitochondrial function is a commonly recognized factor that underlies a plethora of neurological and neurodegenerative diseases. Metabolic demands of neural cells require high bioenergetic outputs that are often associated with enhanced production of reactive oxygen species. Unopposed accumulation of these respiratory byproducts over time leads to oxidative damage and imbalanced protein homeostasis within mitochondrial subcompartments, which in turn may result in cellular demise. The post-mitotic nature of neurons and their vulnerability to these stress factors necessitate strict protein homeostatic control to prevent such scenarios. A series of evolutionarily conserved proteases is one of the central elements of mitochondrial quality control. These versatile proteolytic enzymes conduct a multitude of activities to preserve normal mitochondrial function during organelle biogenesis, metabolic remodeling and stress. In this review we discuss neuroprotective aspects of mitochondrial quality control proteases and neuropathological manifestations arising from defective proteolysis within the mitochondrion.

Keywords

Mitochondria; Neurons; Mitochondrial quality control; Neurodegenerative diseases; Mitochondrial proteases; Hereditary neurological diseases

Introduction

Mitochondria are complex multifaceted organelles critical for cellular physiology. Vital mitochondrial functions include energy conversion, synthesis of key cellular cofactors and metabolites, ion homeostasis, signaling and initiation of apoptosis. Among different cell types, the post-mitotic neurons are particularly dependent on properly functioning mitochondria (Lin and Beal 2006; Rugarli and Langer 2012). One central aspect of mitochondrial function is bioenergetics—the organelle's ability to produce energy for the cell, and related functions of regulating redox status and Ca^{2+} concentration (Wallace 2013). Mitochondrial bioenergetics plays a critical role in neuronal development and maturation (Mlody et al. 2016), as well as in neuronal function (Amadoro et al. 2014). The morphology and dimensions of neurons necessitate efficient long-distance transport of mitochondria to

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Compliance with ethical standards **Conflict of interest** The authors declare that they have no conflict of interest.

energy-demanding presynaptic and postsynaptic sites. Significant energy is needed to transport mitochondria to the synapse. Likewise, segregation and removal of malfunctioning mitochondria from synaptic sites requires major expenditure of energy. Hence, when these organelles do not operate efficiently, incur significant damage, and cannot be removed, the cell exhausts the possibilities for normal functioning (Lax et al. 2011). Given such critical importance of the functional integrity of mitochondria for neuronal health, it is not surprising that impaired mitochondrial bioenergetics and related mitochondrial deficits underpin a large number of neurological diseases. Mitochondrial respiration is inevitably linked to generation of reactive oxygen species (ROS), which eventually can overburden antioxidant defense systems and cause oxidative damage, and subsequently impair protein homeostasis in mitochondrial subcompartments (Lin and Beal 2006; Ugarte et al. 2010). These perturbations result in the build-up of underfolded, misfolded or aggregated proteins further leading to altered mitochondrial dynamics, calcium overload, impaired cellular energetics and ultimately cellular damage or death (Fig. 1). This problem is especially pronounced in neurons, whose metabolism is prone to generation of excessive oxidative stress (Rugarli and Langer 2012). Mitochondrial biogenesis is the process of expansion of mitochondrial mass and network which largely involves precisely coordinated import and assembly of multiple mitochondrial proteins encoded by the nuclear and mitochondrial genomes (Diaz and Moraes 2008). Increase in mitochondrial biogenesis is often observed as a compensatory adaptation to mitochondrial deficits, which can provide relatively limited, but sufficient physiological benefit. However, the efficiency of this process critically depends on proper protein folding and homeostasis at each mitochondrial sub-location, which become compromised under prolonged oxidative stress (Kallergi et al. 2014). Thus, maintenance of protein homeostasis is one of the key aspects in preservation of functional integrity of neuronal mitochondria.

Mitochondrial integrity is maintained through a group of interdependent mechanisms known as mitochondrial quality control (MQC) (Cook et al. 2012; Bohovych et al. 2015a). MQC relies on several partially overlapping systems operating at molecular, organellar and cellular levels (reviewed in (Livnat-Levanon and Glickman 2011; Chan and Chan 2011; Jovaisaite et al. 2014; Quirós et al. 2015; Hamon et al. 2015)). A key facet of molecular MQC is represented by a number of evolutionarily conserved proteases localized across mitochondrial compartments. MQC proteases are involved in a variety of proteolytic activities including removal of damaged, misfolded and surplus polypeptides, regulatory processing, maturation cleavage and assembly of proteins. These proteolytic enzymes can be roughly classified into two groups: ATP-dependent and ATP-independent proteases. The former group encompasses the members of the AAA+ (ATPases associated with diverse cellular activities) superfamily; the latter group is more heterogeneous and includes several structurally distinct types of proteases.

There is evolving evidence that neuronal dysfunctions stem not merely from oxidative stress but its combination with bioenergetic deficits and impaired MQC activity. Combined, these factors affect neurons in a variety of settings, manifesting in a spectrum of early- to late-onset neurological disorders (Andrews et al. 2005; Wider and Wszolek 2008; Cook et al. 2012). In this review we will focus on neuroprotective aspects of MQC proteases and

clinical neurological manifestations arising from defective proteolysis within the mitochondrion.

Mitochondrial AAA+ Proteases

AAA+ proteases reside in the mitochondrial matrix and inner mitochondrial membrane and play critical roles in mitochondrial protein homeostasis (Fig. 3). Much of the current insight into the mechanistic aspects of eukaryotic AAA+ proteases was gained from studies in yeast. AAA+ proteases comprise a highly conserved group of mitochondrial enzymes bearing several distinctive features. The key hallmark is the presence of a conserved AAA+ superfamily ATPase domain comprising Walker A and B ATP-binding loops. The second feature is a protease domain, which can contain either a serine (LONP and ClpXP proteases) or a zinc-coordinated labile water molecule (m-AAA and i-AAA proteases) as the nucleophile in the active site. Another important characteristic is oligomerization, which allows the formation of a cooperative ATPase ring and barrel-like proteolytic compartments (Fig. 2). These features provide substrate specificity and highly efficient proteolysis as AAA+ proteases combine their proteolytic activity with translocation and unfolding of their substrates. A significant number of neurological disorders are linked to dysfunctional AAA+ proteases (Table 1).

LON Protease

Mitochondrial matrix peptidase LONP (also known as LON and LONP1) forms a barrel-shaped homo-hexamer (Liu 2004), in which each subunit has three domains, including N-terminal, AAA+ ATPase, and serine protease domains (Lee and Suzuki 2008). In vitro studies indicate that LONP can degrade both unfolded and folded proteins, thereby suggesting a broad repertoire of substrates. However, the list of known LONP substrates remains somewhat short as general rules for structural or sequence features allowing computational prediction are yet to be defined. While specific recognition sequences (degrons) have been identified for prokaryotic Lon proteases, the identity of recognition signals for the eukaryotic enzyme remains obscure. In mammalian cells, the oligomeric LONP is responsible for ATP-dependent degradation of oxidatively damaged proteins (Fig. 3a); among them citric acid cycle enzyme aconitase, COX4I1 subunit of cytochrome *c* oxidase (CcO), mitochondrial thioredoxin 2 (Trx2) and mitochondrial transcription factor A (TFAM) (Watabe et al. 1994; Bota et al. 2002; Stiburek and Zeman 2010).

LONP expression is increased upon various stress conditions including heat, oxidative stress and hypoxia (Fukuda et al. 2007; Bezawork-Geleta et al. 2015; Pomatto et al. 2016). Moreover, LONP overexpression protects mitochondria from damage caused by hypoxia or ER stress (Hori et al. 2002). The protective hypoxic remodeling of the mitochondrial respiratory chain has been attributed to LONP-mediated degradation of the aerobic COX4I1 CcO subunit (Fukuda et al. 2007). Conversely, aging and prolonged oxidative stress were shown to decrease LONP levels, which in turn might be responsible for accumulation of dysfunctional proteins within the mitochondria (Bota et al. 2002). In a way, the proteolytic function of LON in mitochondria resembles that of the 20S proteasome in the cytoplasm (Ngo and Davies 2007; Ngo and Davies 2009). However, LONP preferentially degrades

unfolded polypeptides, rather than aggregated protein structures (Bezawork-Geleta et al. 2015). In addition to its proteolytic function, LONP can also act as a chaperone for the assembly of respiratory complexes (Bota et al. 2002; Ngo and Davies 2007). Additionally, LONP may contribute to the regulation of mtDNA copy number either through direct binding to DNA (Lu et al. 2007; Chen et al. 2007; Xin et al. 2008) or via selective degradation of TFAM (Matsushima and Kaguni 2012) after its dissociation from the mtDNA (Matsushima et al. 2010; Lu et al. 2013). The physiological importance of LONP is further underscored by the fact that *Lonp1* deletion causes embryonic lethality in mice (Quirós et al. 2014).

Several human genetic studies implicate LONP in neural pathologies. A truncated Arg419X mutant in the *cereblon* (*CRBN*) gene, which encodes for the LONP protease, was found in a large kindred with mild mental retardation to be expressed in hippocampus. The mutation preserves the active domain, but decreases LONP functionality likely by disrupting a myristoylation signal and eliminating a phosphorylation site on the C-terminal portion of the enzyme (Higgins et al. 2004). Additional clinical manifestations may be linked to LONP dysfunction through the protease's substrates. For instance, metabolic enzyme aconitase plays an important role in mitochondrial physiology, and its attenuated activity is a contributing factor in progressive supranuclear palsy (PSP) (Park et al. 2001) and influences the progression of Huntington's disease (HD) (Tabrizi et al. 1999). PSP is characterized by progressive neurodegeneration resulting in postural instability and increased risk of falling, parkinsonism and pseudobulbar palsy (Arena et al. 2016). The disease has late onset and commences with the symptoms of motor dysfunction (Boeve 2012). Similarly, the expression of LONP is significantly decreased in another instance of PSP that has been linked to a mutation in mitochondrial chaperone HSP60/HSPD1 (Hansen et al. 2008).

The data highlighting dual role of LONP in oxidative and hypoxic stress tolerance correlate well with the low levels of LONP reported in neurons during such pathological conditions such as dominant autosomal hereditary spastic paraplegia (HSP) and amyotrophic lateral sclerosis (ALS) (Fukada 2004; Hansen et al. 2008). On the contrary, mitochondria from patients with Friedreich's ataxia (FRDA) and myoclonic epilepsy with red ragged fibers (MERRF) exhibit elevated levels of LONP (Venkatesh et al. 2012). Interestingly, LONP dysfunction is also believed to be one of the contributing factors in MERRF, due to its oxidative inactivation by excessive ROS produced under this condition (Wu et al. 2010).

ClpXP Protease

Caseinolytic peptidase XP (ClpXP) is a heteromeric serine protease complex that resides in the mitochondrial matrix and shares some similarities with LONP. However, unlike the former, the proteolytic and AAA+ modules are not a part of the same molecule. CLPXP is formed through interaction between the ATPase CLPX and protease CLPP (Fig. 3a). In this complex two CLPP heptamer barrels and two CLPX hexamer lids are joined together to form a functional unit. The CLPXP complex is capable of ATP-dependent serine protease activity with CLPP hexamer barrels acting as proteolytic chambers (Voos 2009). CLPX lid components of the complex are responsible for recognition, unfolding and spooling of the substrates into the proteolytic chamber formed by CLPP. Unfolding the proteins requires

ATP hydrolysis and can take several cycles (Baker and Sauer 2012). While prokaryotic ClpXP has been extensively studied, the role and function of CLPXP in mitochondria remain less defined.

The substrate's repertoire of eukaryotic CLPXP is largely elusive. Mitochondrial matrix GTPase NOA1 is the only physiological substrate of mammalian CLPXP that has been identified so far (Al-Furoukh et al. 2014). Just like in the case of LONP protease, no information regarding specific recognition signals of eukaryotic CLPXP substrates is currently available. Interestingly, a recent study reported that mitochondrial CLPXP does not seem to participate in unfolded protein degradation when LONP is available, and is thought to engage only when other degradation mechanisms are overburdened (Bezawork-Geleta et al. 2015).

Studies in nematodes implicated the ClpXP as a key component of mitochondrial unfolded protein response (UPR_{mt})—a specific transcriptional pro-survival response activated by mitochondrial dysfunction (reviewed in Haynes et al. 2013). During this response, expression of several mitochondrial heat shock proteins and proteases including CLPP is activated to cope with accumulation of unfolded or misfolded polypeptides within mitochondria (Haynes et al. 2007). Such activation is achieved through enhanced peptide extrusion from mitochondria and transcriptional factor ATFS-1, which—depending on physiological conditions—can be localized to mitochondria or nucleus. ClpXP protease has been shown to contribute towards generation of this signaling peptide flux (Haynes et al. 2010). Of note, LONP protease contributes to the other facet of UPR_{mt} by degrading (and thus inactivating) mitochondria-targeted ATFS-1 (Nargund et al. 2012). UPR_{mt} appears to be conserved in higher eukaryotes. Consistently, it has been shown that the expression of mammalian CLPP is upregulated upon the accumulation of unfolded proteins in the matrix along with enhanced expression of the other canonical UPR_{mt} genes (Aldridge et al. 2007). While it remains to be elucidated if defects in UPR_{mt} signaling may contribute to neural pathologies, mutations in CLPP are reported in several disease-related studies.

In a *Clpp*^{-/-} knockout mouse model, hearing loss, infertility, and motor activity reduction were reported, similar to traits observed in Perrault syndrome patients. At the cellular level *Clpp*^{-/-} cells exhibit reduced respiration and mitochondrial accumulation of ClpX and other UPR_{mt} connected chaperones, as well as accumulation of mtDNA (Gispert et al. 2013). This mtDNA accumulation is putatively linked to mtDNA maintenance function of ClpP through the transcriptional factor TFAM (Kasashima et al. 2012).

In humans most recessive CLPP mutations are linked to Perrault syndrome (Jenkinson et al. 2013) which is an autosomal recessive disease that is characterized by progressive sensorineural hearing loss and ovarian failure (Pallister et al. 1979) and can also include additional symptoms, such as ataxic gait or mild mental retardation (Nishi et al. 1988; Linssen et al. 1994; Gottschalk et al. 1996; Fiumara et al. 2004). One relatively common mutation in patients with Perrault syndrome is a CLPP splice site mutation 270 + 4A >G which results in increasing the probability of retaining intron 2 in the final transcript, and as a result producing a truncated version of CLPP (Jenkinson et al. 2013). Other known pathological substitutions in CLPP are Thr145Pro and Ser147Cys mutations, with the

former having a more pronounced phenotype due to the loss of a hydrogen bond that is critical for catalytic activity. The Ser147Cys mutation is less severe and results in only a mild reduction of CLPP catalytic activity (Jenkinson et al. 2013). The individuals with the Thr145Pro mutation also show progressive spastic paraplegia, similar to that caused by mutations in paraplegin subunit of m-AAA protease (discussed later in this text).

Another set of CLPP point mutations is linked to a different autosomal recessive neurological phenotype. Most patients with Thr268Met, Met411Ile, Thr617Cys, Arg408Gly, Gly646Val, Cys486Arg, Ala591Val, Tyr272Cys, Cys647Lue, and Gly501Lys mutations in CLPP display various degrees of developmental delay and intellectual disability, severe spasticity and epilepsy. Magnetic resonance imaging scans of these patients reveal different degrees of cerebellar atrophy. Additionally, most of these patients exhibit microcephaly. Interestingly, in addition to these neurological symptoms, the patients also present with a pronounced immunological phenotype (neutropenia), which correlates with the severity of neurological symptoms (Saunders et al. 2015; Wortmann et al. 2015).

m-AAA Protease

m-AAA is an inner membrane-anchored oligomeric metallopeptidase with its active site projecting into the matrix (hence the acronym). In humans, m-AAA consists of either homo-oligomers of ATPase family gene 3-like 2 (AFG3L2) or hetero-oligomers of AFG3L2 and spastic paraplegia type 7 (SPG7), also known as paraplegin (Fig. 3b) (Atorino et al. 2003; Martinelli et al. 2009). Both proteins have modular structure and encompass AAA+ ATPase and zinc metallopeptidase domains within the same polypeptide. Expression patterns of AFG3L2 and SPG7 appear to be closely correlated (Banfi et al. 1999). Of note, SPG7 appears to be one of the canonical UPRmt response genes, whose expression is upregulated during proteostatic stress (Haynes et al. 2013). The homo- and hetero-oligomeric isoforms of m-AAA appear to perform largely redundant functions (Martinelli et al. 2009). Nevertheless, AFG3L2 seems to be the rate-limiting factor in m-AAA assembly, as it is required for proteolytic maturation and assembly of SPG7 into functional hetero-oligomers. AFG3L2 is processed in an autocatalytic manner before becoming an active protein (Koppen et al. 2009). Interestingly, rodents express an additional m-AAA subunit Afg3l1, which is represented by a non-functional pseudogene in humans (Koppen et al. 2009). m-AAA is a critical regulator of protein homeostasis in the IM and matrix. It is involved in degradation of damaged and surplus proteins—primarily subunits of the respiratory chain complexes—in these mitochondrial subcompartments. In addition, m-AAA proteolytic activity is central for maturation of several mitochondrial proteins including the components of mitochondrial translation machinery (Nolden et al. 2005; Almajan et al. 2012; Wedding et al. 2014).

The connection of m-AAA to neuronal dysfunctions is well documented. Loss of either SPG7 or AFG3L2, or both proteins in HEK293 cells affects respiratory complex I and impairs oxidative stress tolerance – mimicking pathologic phenotypes described in patients with familial hereditary spastic paraplegia (HSP type 7) (Atorino et al. 2003). Mice deficient in paraplegin demonstrate axonal degeneration, dysfunctional axonal transport and abnormal mitochondria (Ferreirinha et al. 2004). Purkinje cells of mice deficient in Spg7 exhibit swollen mitochondria with abnormal ultrastructure, which accumulate in cells' bodies and

dendrites. Affected mitochondria display a large loss of mtDNA and decreased stability of the respiratory chain complexes. These neurons are characterized by swelling in the synaptic areas and high propensity for cell death. (Martinelli et al. 2009).

As can be deduced from its name, mitochondrial deficits due to mutations in SPG7 are linked to both dominant and recessive forms of HSP in humans (Settasatian et al. 1999; McDermott et al. 2001; Koppen et al. 2007). This progressive disease is characterized by spasms and loss of vibratory sense in the lower extremities (Fink 2006), which are associated with degeneration of corticospinal tract axons and *fasciculus gracilis* fibers (Martinelli et al. 2009). Synapses of HSP patients exhibit swelling due to accumulation of organelles and filaments, which points to deficiencies in anterograde axonal transport mechanisms (Ferreirinha et al. 2004). It is assumed that axonal transport is disrupted due to mitochondrial dysfunction, hence a lack of energy for anterograde transport of the organelles. At the molecular level, these events are associated with significant mitochondrial oxidative damage and impaired stability of the respiratory complexes I and IV (Casari et al. 1998; Atorino et al. 2003; McDermott et al. 2003). Of note, mutagenesis experiments show that loss of proteolytic activity due to the Glu575Gln mutation that disables the catalytic site of the enzyme does not prevent complex I assembly (Atorino et al. 2003), thereby suggesting that other functional impediments in SPG7 may be contributing to the disease phenotypes as well.

About 10 % of HSP cases are attributed to mutations in paraplegin (Martinelli et al. 2009). Known SPG7 mutations related to HSP are deletions of exons 8 and 11 and, point mutations Gly28Ala, Ala510Val, Gly334Asp, Arg398Gln, Arg470Gln and His701Pro (Wilkinson et al. 2004; Warnecke et al. 2007; Schlipf et al. 2011; van Gassen et al. 2012; Pfeffer et al. 2014). The Ala510Val mutation seems to be the most common in HSP patients (Sánchez-Ferrero et al. 2013). This substitution destabilizes the hydrophobic core and active center of the enzyme (Karlberg et al. 2009). Recently, an Arg688Gln mutant was observed to be a constitutively active variant of SPG7, which avoids AFG3L2-mediated regulatory processing. Such deregulation results in higher respiratory rates to produce ATP and generation of excessive amounts of reactive oxygen species—a consequence of reduced proton leak and attenuated ROS buffering capacity (Almontashiri et al. 2014).

AFG3L2 is selectively and highly expressed in Purkinje cells and plays a crucial role in mitochondrial metabolism and axon development (Maltecca et al. 2008) (Di Bella et al. 2010). Rodent models of AFG3L2 deficiency display impaired motor coordination, defective axonal growth with poor myelination, and degeneration of GABAergic neurons in the cerebellum. On a cellular level these animals exhibit excessive mitochondrial fragmentation, lower mitochondrial Ca^{2+} up-take and increased ROS production. (Maltecca et al. 2009; Almajan et al. 2012). These effects were attributed to defective mitochondrial ribosome assembly and impaired protein synthesis within the organelle (Almajan et al. 2012). Additional defects include affected microtubule network, aberrant anterograde transport and tau hyperphosphorylation. These factors were related to the inability of the mitochondria to adapt to oxidative stress, which is confirmed by rescue of cells with antioxidants (Kondadi et al. 2014). On a related note, Maltecca et al. recently showed that synaptic glutamate clearance caused by administration of the antibiotic ceftriaxone to Afg3l2-deficient mice

reduced Ca^{2+} influx and improved ataxia-like symptoms observed in these animals (Maltecca et al. 2015).

Mutations in AFG3L2 are also linked to several neural pathologies in humans. Most of the disease-related alterations were mapped to the region encompassing the peptidase domain (amino acids residues 654 through 700) (Edener et al. 2010; Cagnoli et al. 2010; Löbbe et al. 2014; Smets et al. 2014; Zühlke et al. 2015). A number of heterozygous, substrate recognition-perturbing mutations in AFG3L2 were associated with autosomal dominant spinocerebellar ataxia type 28 (SCA28) (Di Bella et al. 2010). SCA28 is associated with degeneration of Purkinje cell and typically is manifested by progressive spastic gait, lower extremity spasms, and ophthalmoparesis (Mariotti et al. 2012). Clinical manifestations may vary from the typical case depending on which part of the AFG3L2 is affected (Mariotti et al. 2012; Löbbe et al. 2014; Musova et al. 2014). For instance, the Glu700Lys mutation is detected in patients with early onset dominant SCA28 (Maltecca et al. 2008; Edener et al. 2010), while Tyr689Asn and Tyr689His substitutions were detected in the late onset form of the disease (Löbbe et al. 2014; Zühlke et al. 2015).

A recent study identified a novel SCA28-related pathologic substitution. The homozygous Met625Ile mutation, which disables the proteolytic activity of AFG3L2 was reported in patients that succumb to ataxia. This condition, however, is preceded by early onset progressive myoclonus epilepsy (Muona et al. 2014). There are also reports of patients with AFG3L2 haploinsufficiency due to deletions or frame-shift mutations. Clinical manifestations for this condition appear to be similar to those of SCA28 (Musova et al. 2014; Myers et al. 2014).

Finally, mutations in AFG3L2 have also been identified in patients with recessive ataxia. A homozygous Tyr616Cys mutation was recently reported in patients with early onset spastic ataxia-neuropathy syndrome (SPAX5) (Pierson et al. 2011). The disease is associated with cerebellar atrophy and characterized by early onset spastic gait followed by progressive myoclonic epilepsy and motor degeneration. The Tyr616Cys mutation partially affects AFG3L2 assembly into functional hetero-complexes with SPG7, while the activity of the ATPase domain is preserved.

i-AAA Protease

Mitochondrial peptidase Yeast mitochondrial DNA escape 1-like (YME1L), commonly known as i-AAA (where *i* denotes intermembrane space-exposed active site) is a conserved protein that combines ATPase and metalloprotease domains in a single polypeptide (Fig. 3b). YME1L is a IM-anchored protein, which is organized into homo-hexamers that appear to function as a single proteolytic module (Scharfenberg et al. 2015). Dissociation of the i-AAA complex (e.g. due to oxidative stress or low ATP levels) reportedly inactivates the protease and causes degradation of the monomeric subunits (Rainbolt et al. 2015). Studies in yeast indicate that i-AAA can also function as a chaperone for a number of proteins in the intermembrane space (Schreiner et al. 2012). The AAA domain of the protease appears to bind unfolded polypeptides and prevent their aggregation. The N-terminal domain is essential for binding substrates in both the chaperone and proteolytic activity (Graef et al.

2007). It is assumed that mammalian YME1L acts in a similar fashion (Leonhard et al. 1999). Consistent with its multifaceted role in mitochondrial protein homeostasis, YME1L is one of the genes activated in the UPR_{mt} response (Zhao et al. 2002; Aldridge et al. 2007).

The substrate repertoire of i-AAA appears to be diverse and includes subunits of respiratory chain complexes (Stiburek et al. 2012), proteins involved in transport and synthesis of lipids (Potting et al. 2010) and IM dynamics (Song et al. 2007). One YME1L substrate that is receiving a lot of attention recently is optic atrophy 1 (OPA1). This IM-localized dynamin-like GTPase appears to play several central roles in mitochondrial physiology and metabolism, which are linked to balanced mitochondrial dynamics (Griparic et al. 2007; Mishra et al. 2014; Wai et al. 2015), shaping of cristae architecture (Frezza et al. 2007) and apoptotic resistance (Stiburek et al. 2012). The term “mitochondrial dynamics” is used to describe changes in shape and motion of mitochondria, which have profound roles in cellular physiology and pathology (reviewed in Archer 2013; Burté et al. 2015). Mitochondria are able to fuse, forming interconnected networks, which allows mixing of content of the individual organelles, thereby promoting exchange and/or replenishment of mitochondrial DNA, “dilution” of damaged biological molecules, and efficient communication with other organelles such as endoplasmic reticulum. Reciprocally, mitochondrial network can be fragmented, thus permitting mitochondrial inheritance during cell division or segregation and selective removal of irreversibly damaged organelles through the process of mitophagy (detailed later in the text). Mitochondrial dynamics is critical for post-mitotic cells like neurons, wherein balanced mitochondrial fusion and fission ensure proper cellular functioning and tolerance towards various homeostatic insults. Proteolytic processing of OPA1 is one of the central functional points where MQC proteases and mitochondrial dynamics intersect.

YME1L conducts cleavage of the long, membrane-anchored form of OPA1 (L-OPA1) at the so-called S2 site thereby generating a short, soluble variant of OPA1 (S-OPA1), which associates with L-OPA1. The ratio between long and short forms of OPA1 appears to be critical for maintaining balance between mitochondrial fusion and fragmentation. Loss of YME1L in cultured mammalian cells sensitizes them to oxidative stress, causes defects in cell proliferation and triggers mitochondrial fission and altered cristae ultrastructure (Song et al. 2007; Stiburek et al. 2012; Ruan et al. 2013; Anand et al. 2014). At least some of these effects were attributed to stress-activated proteolysis of OPA1 executed by a different protease, OMA1 (discussed below) (Anand et al. 2014).

Even though YME1L1 has not been associated with any known neuropathies in humans, studies in mouse models indicate its critical importance. Whole-body homozygous *Yme1l*^{-/-} mouse embryos display significant developmental delay and die by embryonic day E13.5 (Wai et al. 2015). Depletion of YME1L in mouse heart leads to imbalanced OPA1 processing and a profound mitochondrial deficit that manifests as dilated cardiomyopathy and premature death of the animals (Wai et al. 2015). It is plausible that the loss of *Yme1l* in neural tissues may have a similarly detrimental effect. Mutations in OPA1 were linked to the autosomal dominant optic atrophy (reviewed in (Chao de la Barca et al. 2015)). However, at present it is unclear whether defective processing of OPA1 by YME1L contributes to the development of this disease.

ATP-independent Mitochondrial Proteases

Although the AAA-proteases represent a significant portion of the molecular MQC, the mitochondrion is dually equipped with a multitude of ATP-independent proteases. Just like AAA+ proteases, this heterogeneous group of enzymes maintains mitochondrial health through a variety of critical functions including protein processing, regulation of mitochondrial morphology and apoptosis. An important distinction between these two groups of proteases is that the lack of ATP hydrolyzing capacity necessitates motif recognition-based and/or site-specific cleavage as opposed to unfolding-linked processive proteolysis typical for the AAA+ peptidases. ATP-independent proteases are conserved, structurally diverse proteins playing divergent, but equally important roles in mitochondrial physiology (Fig. 4). With regard to their catalytic mechanism (and similar to AAA+ proteases), these enzymes can be grouped into metalloproteases (OMA1, MPP, IMP, PreP) and serine proteases (PARL, HTRA2). Several mitochondrial ATP-independent peptidases are implicated in neuronal pathologies (Table 1) and will be discussed in the following paragraphs.

OMA1 Protease

The Overlapping activity with m-AAA protease 1 (OMA1) is a conserved zinc metalloprotease. It is integral to the inner mitochondrial membrane and appears to exist as a high molecular mass homo-oligomeric complex (Fig. 4a) (Kaser et al. 2003; Ehses et al. 2009; Head et al. 2009; Baker et al. 2014; Bohovych et al. 2014). Originally identified in yeast, it has been proposed to serve as a back-up partner of the m-AAA protease (Kaser et al. 2003). However, more recent studies in yeast and cultured mammalian cells established that the enzyme exists in a dormant state under normal conditions but is rapidly activated upon various homeostatic insults (Khalimonchuk et al. 2012; Baker et al. 2014; Zhang et al. 2014; Bohovych et al. 2014). Stress activation of yeast Oma1 appears to involve conformational rearrangements within the homo-oligomer without affecting the stability of individual subunits (Baker et al. 2014; Bohovych et al. 2014). OMA1 activation in mammalian mitochondria is slightly different from the yeast protease and is associated with autocatalytic degradation of the enzyme (Baker et al. 2014; Zhang et al. 2014). This activation mode is likely a measure for spatiotemporal control of OMA1 activity. The GTPase OPA1 is now emerging as one of the key substrates of OMA1. Stress activation of the protease leads to a cleavage of OPA1 by OMA1 at the S1 processing site (distinct from the S2 site processed by YME1L peptidase). These events result in rapid elimination of the L-OPA1 form, which in turn leads to massive mitochondrial fragmentation (Ehses et al. 2009; Head et al. 2009; Baker et al. 2014; Zhang et al. 2014). Additional known substrates of OMA1 include the misfolded or unassembled IM translocase Oxa1, the Cox1 subunit of the respiratory complex IV in yeast (Kaser et al. 2003; Khalimonchuk et al. 2012), and the complex III assembly factor UQCC3 in mammals (Desmurs et al. 2015). The mechanisms by which OMA1 is activated and recognizes its substrates remain to be clarified.

Consistent with the key roles of OPA1 in mitochondrial physiology and apoptosis, loss of Oma1 in cultured cells has an anti-apoptotic effect (Quirós et al. 2012; Jiang et al. 2014; Korwitz et al. 2016). OMA1 depletion in rodents protects against ischemic acute kidney

injury (Xiao et al. 2014). Moreover, depletion of OMA1 in YME1L-deficient cultured cells or heart-specific *Yme1l*^{-/-} mice eliminates stress-induced proteolysis of OPA1 thereby suppressing mitochondrial fission and restoring normal cardiac function and metabolism (Anand et al. 2014; Wai et al. 2015). Likewise, loss of OMA1 in forebrain-specific prohibitin 2 (Phb2) knockout mice significantly delays the onset of neurodegeneration and extends the lifespan of these animals (Korwitz et al. 2016). The latter findings indicate that OMA1-mediated stress-processing of OPA1 is an important event in the neuroinflammatory response and point at the protease's key role in neuronal survival. Although seemingly beneficial, loss of OMA1 appears to dampen the ability of cells to cope with dynamic metabolic demands and reduces bioenergetic plasticity of mitochondria. Yeast cells deficient in Oma1 display reduced stability of the respiratory complex III/IV supercomplexes and aging-dependent progressive respiratory decline (Bohovich et al. 2015b). Morpholino-specific depletion of OMA1 in a zebrafish model causes developmental defects including increased density in the brain region and impaired heart and eye development—conditions associated with bioenergetic deficit. Similarly, OMA1-deleted mouse fibroblasts are unable to maximize their respiratory output (Bohovich et al. 2015b). Finally, *Oma1*^{-/-} mice present with signs of metabolic distress—diet-induced obesity and impaired energy expenditure (Quirós et al. 2012).

Mutations in OMA1 are reported in several patients suffering from Amyotrophic Lateral Sclerosis. This devastating progressive motor neuron disease affects the upper and lower motor neurons in several regions of the brain and spinal cord. Neuronal demise is associated with muscular stiffness and twitching and later muscular atrophy (Rowland and Shneider 2001). Daoud et al. performed a sequencing study in a large cohort of patients with familial and sporadic forms of ALS and identified two heterozygous mutations in conserved residues of OMA1 (Daoud et al. 2011). His69Tyr and Glu272Gly substitutions were found in several patients with sporadic ALS whereas they were not in the healthy control group. Given the protease's ability to form homo-oligomers, it is likely that the presence of mutant OMA1 variants in oligomeric complexes may interfere with their stability and/or function.

HTRA2 Protease

The high temperature requirement protein A2 (HTRA2 also known as Omi and PARK13) is a conserved IM-anchored serine protease with its active site facing the mitochondrial intermembrane space (Fig. 4a). HTRA2 is a typical representative of its class with several distinctive features. One hallmark is the presence of a characteristic Postsynaptic density of 95 kDa, Discs large and Zonula occludens 1 (PDZ) structural domain, which follows the catalytic module and contributes to substrate binding, oligomerization and regulation of proteolytic activity. The protease is organized into functional homotrimers in both active and inactive states—monomeric form of HTRA2 appears to be devoid of proteolytic activity (Li et al. 2002). Similar to its bacterial homologs, HTRA2 is likely to be primarily engaged in the degradation of oxidized proteins within the IMS. For more information on the mechanistic aspects of HTRA proteases see the review by Clausen et al. (Clausen et al. 2011).

HTRA2 was initially branded as an apoptosis-related protein and has since been best studied mostly as an important director of cell death. Contradictory reports exist regarding the apoptosis-related activation of HTRA2. One scenario considers the processing of the HTRA2's transmembrane domain by the partner protease PARL (discussed below), thus possibly preventing build-up of pro-apoptotic factors and promoting neuronal survival (Chao et al. 2008). Other reports suggest autocatalytic activation of HTRA2 followed by its dissociation from the IM and release into the cytosol where the protease is involved in processing several anti-apoptotic factors commonly known as inhibitor of apoptosis proteins (IAPs). These events are then followed by further progression of the apoptotic program (Suzuki et al. 2001; Hartkamp et al. 2010). Of note, recent reports also implicate HTRA2 in several other non-apoptotic forms of cell death (Sosna et al. 2013; Yacobi-Sharon et al. 2013). HTRA2's functions are not limited to apoptotic activity as *Htra2*^{-/-} mice exhibit dissipated membrane potential and depletion of cellular ATP levels (Plun-Favreau et al. 2012). Further, *Htra2*-deficient animals show substantial loss of glutamatergic and dopaminergic neuron populations in the striatum, quickly leading to a neurodegenerative condition resembling the clinical phenotypes of Parkinson's disease (PD) (Casadei et al. 2016). These progressive neurological declines also lead to a higher rate of mortality at younger ages in mice lacking proteolytically active HtrA2 (Patterson et al. 2014). Together with recently reported involvement of HtrA2 in cerebral ischemia-associated neostriatum injury (Yoshioka et al. 2013), these data highlight the neuroprotective role of HtrA2. Interestingly, depletion of HtrA2 in the mouse brain is associated with tissue-specific transcriptional stress response. While this response appears to be distinct from UPR_{mt} described above, it is also purposed to prevent accumulation of unfolded proteins (Moisoi et al. 2009).

Missense mutations in HTRA2 were reported in patients with familial cases of hereditary tremor, which is considered a predisposing factor for parkinsonism (Strauss et al. 2005; Ross et al. 2008; Krüger et al. 2011; Unal Gulsuner et al. 2014). The characterized substitutions include Gly399Ser and Ala141Ser with the latter mutation identified as a polymorphism. Both mutations were reported to decrease proteolytic function of HTRA2.

PARL Protease

Presenilins-associated rhomboid-like protein (PARL) is a serine protease integral to the IM. Distinctive hallmarks of this rhomboid-family peptidase include complex topology (7 transmembrane domains) and the ability to perform proteolytic processing within the membrane bilayer (Fig. 4a). The latter feature is made possible by specific positioning of PARL's active site—a catalytic dyad of serine and histidine residues located within helices 4 and 6, respectively. More details on the molecular organization of rhomboid proteases can be found in the following reviews (Ha 2009; Sun et al. 2016). To date, researchers have identified several PARL substrates. Besides the aforementioned HTRA2 protease, the other notable substrates of PARL include PTEN-induced putative kinase 1 (PINK1)—a Ser/Thr ubiquitin kinase—and Ser/Thr phosphatase phosphoglycerate mutase 5 (PGAM5) (Jin et al. 2010; Sekine et al. 2012). Notably, both proteins are shown to play important roles in regulation of selective autophagic removal of mitochondria known as mitophagy (Jin et al. 2010; Chen et al. 2014). This type of macroautophagy involves selective targeting and

autophagosomal sequestration of mitochondria followed by their degradation in lysosomes. This mitochondrial clearance mechanism is commonly recognized as a cellular level-facet of MQC, which plays central roles in stress tolerance and neuroprotection. Conversely, malfunctioning mitophagy is associated with accumulation of damaged organelles and has been linked to several neurological diseases (Yamaguchi et al. 2016). Mitophagy relies on proteins participating in mitochondrial fission and fusion machinery, as well as specific proteins, such as PINK1 and E3 ubiquitin ligase Parkin (Green et al. 2011) (Hamacher-Brady and Brady 2016). Just like in the case of mitochondrial dynamics, regulation of mitophagy is linked to the function of several MQC proteases, particularly PARL. Under normal conditions, PINK1 is imported into mitochondria and released into the IM where it is subsequently processed by PARL (Jin et al. 2010; Meissner et al. 2011). Cleaved PINK1 is metastable and can escape into the cytosol where it is eliminated by the ubiquitin-proteasome system (Narendra et al. 2010; Greene et al. 2012). Mitochondrial stress conditions (e.g. depolarization) arrests PINK1 translocation, thereby allowing it to avoid PARL-mediate processing and lead to accumulation of the functional kinase on the OM—an event that leads to recruitment of the E3 ubiquitin ligase Parkin and subsequent mitophagy (Matsuda et al. 2010; Narendra et al. 2010; Vives-Bauza et al. 2010). It is noteworthy that PARL does not seem to be the only protease able to process PINK1. Other peptidases including mitochondrial processing peptidase (MPP; discussed below), m-AAA and CLPXP appear to be involved in PINK1 degradation within the mitochondria (Greene et al. 2012). Loss of mitochondrial membrane potential also leads to PARL-mediated processing of PGAM5. Interestingly, it has been proposed that the depolarization-triggered PARL-PGAM5 interaction may serve to prevent normal PINK1 processing by PARL and thus promote mitophagy (Sekine et al. 2012). PARL has also been implicated in cell death-related activities. In addition to its possible role in apoptotic activation of HTRA2, PARL may exert anti-apoptotic function by maintaining normal cristae morphology and inhibiting cytochrome *c* release at the commencement of apoptosis (Cipolat et al. 2006). Along these lines, *Parl*-deficient mice are characterized by increased apoptosis, general cachexia and premature death (Cipolat et al. 2006). Similarly to HtrA2, PARL is linked to cerebral ischemia-associated striatal injury (Yoshioka et al. 2013). Moreover, PARL's role in the careful balance of mitophagy is believed to be central to neuronal well-being. Consistent with this idea, a missense Ser77Asn mutation in PARL was identified in a cohort of patients suffering from PD (Shi et al. 2011). This substitution alters proteolytic activity of PARL and prevents Parkin recruitment to stressed mitochondria. However, subsequent studies reported that the Ser77Asn mutation does not appear to be a frequent cause of PD onset (Heinitz et al. 2011; Wüst et al. 2015). A significant number of mutations in PINK1 are linked to an autosomal recessive early-onset form of PD (reviewed in Corti et al. 2011). However at present it is unclear whether any of them might be related to PARL's function.

Other Proteases

Several more mitochondrial peptidases are implicated in neuronal health. These enzymes are primarily involved in maturation processing of polypeptides within the mitochondria rather than quality control (Fig. 4b). For more information on mitochondrial processing enzymes, the reader is referred to several specialized reviews (Gakh et al. 2002; Mossmann et al.

2012; Teixeira and Glaser 2013). At any rate, improper maturation of certain substrates may result in their erroneous assembly or turnover, thereby leading to functional impairment. For instance, PARL cleavage of PINK1 appears to be dependent on PINK1 processing by the mitochondrial processing peptidase MPP. In cells with lowered expression of MPP, PINK1 amasses at the mitochondrial surface. Such accumulation leads to Parkin recruitment—similar to that observed in PARL-deficient mitochondria (Greene et al. 2012). MPP exists in the matrix as a heterodimer encompassing proteolytically active β -subunit (PMPCB) and the recognition/binding partner α -subunit (PMPCA) (Fig. 4b). The ability of the α -subunit to recognize the presequence is critical for proper processing of a substrate protein. Recently, a study identified a homozygous Ala377Thr mutation in PMPCA in patients with non-progressive cerebellar ataxia—a type of static infantile encephalopathy (Jobling et al. 2015). The mutation affects the stability of PMPCA, thereby impairing overall proteolytic activity of the MPP complex.

Mitochondrial inner membrane protease (IMP) is another peptidase involved in maturation and assembly of a subset of proteins localized to the IM. This metallopeptidase complex is anchored in the IM and encompasses two distinct subunits, IMMP1L and IMMP2L (Fig. 4b). One important function of IMP may be related to its role in the maturation of the proapoptotic protein DIABLO/Smac. The latter molecule is released from mitochondria upon the initiation of apoptotic sequence and binds to IAPs to promote apoptosis. IMP-mediated processing of the protein's N-terminal moiety is required to expose its IAP binding domain (Burri et al. 2005). Microdeletions within the IMMP2L-coding sequence were reported in patients with Tourette syndrome (Petek et al. 2007; Bertelsen et al. 2014). Similarly, DNA deletions or duplications disrupting the *IMMP2L* gene were identified in patients with delayed psychomotor development (Gimelli et al. 2014). In addition, several polymorphisms in *IMMP2L* are associated with the incidences of autism spectrum disorder (Casey et al. 2012). Molecular underpinnings of these pathologies remain to be clarified.

Finally, mitochondrial matrix presequence peptidase PITRM1 (also known as PreP or mitochondrial peptidase) is implicated in neural health. This metalloenzyme participates in disposal of oligopeptide sequences generated by MPP during protein import into mitochondria (Fig. 4b). Falkevall et al. identified PITRM1 as a key protease in the clearance of mitochondria-localized amyloid β peptide deposits (Falkevall et al. 2006). Consistent with this finding, a subsequent study demonstrated that proteolytic activity of PTRIM1 is decreased in brain mitochondria of patients suffering from Alzheimer's disease (Alikhani et al. 2011).

Concluding Remarks

The past decade gave rise to impressive research progress pertinent to mitochondrial quality control. Identification of novel substrates and generation of animal models helped to determine novel critical roles of MQC proteases in neural physiology and pathology. Combined with clinical reports and genome-wide association studies, these findings provided major insights into the etiology of neurological and neurodegenerative diseases. Recent developments highlight the increasing potential of MQC proteases as prospective theranostic targets in neural dysfunctions. However, as our understanding of protein quality

control activities in mitochondria continues to expand, its complex, multifaceted nature also becomes evident. Modulation of proteolytic activity of MQC proteases may impact delicate proteostatic balance within the organelle and lead to adverse cytotoxic effects. These possibilities should be carefully considered when designing therapeutic strategies against neuropathological conditions. Clearly, there is more to be learned about proteolytic modules of mitochondria. A vast number of exciting questions at levels ranging from basic biochemistry and cell biology to physiology and molecular medicine remain to be addressed. For instance, better understanding of functional relationships between various proteolytic modules and reciprocal effects of their loss is central for identifying primary targets and “windows” for therapeutic interventions. Systematic identification and cataloging of substrate pools for each MQC protease will be another important advance to comprehend their role in health and disease. Together with structural data, these findings may help to understand molecular aspects of substrate recognition and stress-activation of MQC proteases; likewise, this information will aid in designing specific inhibitors or agonists of mitochondrial proteolytic enzymes. Last but not least, further insight into the interplay between mitochondrial proteases and other branches of MQC is needed. Particularly with regards to facets of MQC that extend outside of mitochondria (e.g. UPRmt and mitophagy), as interfering with these mechanisms may impose limitations to neuroprotective therapies targeting mitochondrial proteases.

Our current knowledge regarding MQC proteases and their role in mitochondrial health and pathology continues to evolve. Recent new discoveries underscore vital roles of mitochondrial peptidases in neuronal welfare and open novel exciting research avenues that will likely help to develop effective clinical applications to prevent or combat neurodegenerative diseases.

Acknowledgements

We apologize to those authors whose work we were unable to cite due to space constraints. We also would like to thank members of the Khalimonchuk lab and Dr. Donald Becker for useful comments. This work was supported, in whole or in part, by the National Institutes of Health grants R01 GM108975 (to O.K.), P30GM103335 (to Nebraska Redox Biology Center). E.M.G is a trainee of the Molecular Mechanisms of Disease predoctoral program supported by the NIH training grant 1T32GM107001-01A1.

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Fig. 1.

Progressive mitochondrial dysfunction and its impact on neuronal health. Spontaneous mutations in the nuclear (nDNA) or mitochondrial (mtDNA) genomes may lead to accumulation of misfolded proteins within the mitochondrion. These polypeptides may in turn affect biogenesis and/or function of the electron transport chain complexes (ETC). Such an impediment may result in the enhanced production of reactive oxygen species (ROS) due to increased incomplete reduction of molecular oxygen by escaping electrons (ROS leakage). Accumulating ROS can further affect mitochondrial protein homeostasis via induction of additional mtDNA mutations and/or direct perturbation of protein folding, thereby sustaining the cycle of the deleterious events. Unopposed gaining of alterations in mitochondrial protein and metabolic homeostasis has a profound impact on cellular physiology and results in progressive decline of neuronal health, which ultimately leads to irreversible cell damage and death

Fig. 2.

General principle of protein degradation by mitochondrial AAA+ proteases. Combination of ATPase and peptidase modules allows repetitive ATP hydrolysis cycles by the hexameric AAA+ ring. These events are associated with conformational changes leading to active unfolding and translocation of the substrate polypeptide into proteolytic chamber and subsequent facile proteolysis of the denatured substrate

Fig. 3.

Distribution and specialization of mitochondrial AAA+ proteases. **a** Two soluble serine AAA+ proteases, Lon-like peptidase LONP and caseinolytic peptidase CLPXP perform quality control activities in the mitochondrial matrix. LONP peptidase is a homo-hexameric complex, each subunit of which combines ATPase and protease domains. This protease mediates facile degradation of unfolded or oxidatively damaged soluble polypeptides as well as some inner mitochondrial membrane (IM)-anchored proteins. The CLPXP heteromeric complex is composed of AAA+ ATPase CLPXP and serine protease CLPP subunits. Together, these components form a functional proteolytic unit, which appears to participate in the degradation of certain soluble proteins in the matrix. **b** The IM-tethered m-AAA and i-AAA proteases survey for misfolded, damaged or unassembled polypeptides within the IM, as well as on the matrix (m-AAA) and intermembrane space interfaces (i-AAA) of the membrane. The i-AAA protease is composed of six copies of YME1L protein, which encompasses IMS-facing ATPase and zinc metalloprotease domains. The enzymatic domains of the m-AAA complex are exposed to the matrix. In human mitochondria, m-AAA complex may exist as a hetero- (composed of SPG7 and AFG3L2 subunits) or homo-hexamer (composed of AFG3L2 subunits only). More details are available in the text. *OM* outer membrane, *IMS* intermembrane space

Fig. 4.

Distribution and specialization of mitochondrial ATP-independent proteases. **a** OMA1, PARL and HTRA2 appear to be the main ATP-independent proteases in the inner membrane (IM). Homo-oligomeric zinc metallopeptidase OMA1 complex processes membrane-bound polypeptides on the intermembrane space side of the IM. OMA1 appears to be dormant under normal conditions but is rapidly activated by various stress stimuli. Presenilins-associated, rhomboid-like protease PARL is another module implicated in protein homeostasis within the IM. This membrane-embedded serine protease mediates intramembrane cleavage of IM-anchored polypeptides. One model posits that PARL may be involved in proteolytic activation of yet another serine protease in the IM, HTRA2. The latter peptidase forms membrane-associated homotrimeric complex, which is believed to be involved in the degradation of oxidized proteins within the intermembrane space. HTRA2 has also been shown to play important roles in the regulation of programmed cell death. **b** Several processing peptidases involved in the maturation of mitochondrial proteins are related to neuroprotective activities. The mitochondrial processing peptidase, MPP, comprises the non-catalytic subunit PMPCA and metallopeptidase PMPCB. The MPP complex resides in the matrix and is involved in recognition and proteolytic removal of positively charged targeting sequences at the N-terminus of cytosolic polypeptides that are being imported into the organelle via the translocase of outer membrane and translocase of inner membrane (TIM23) import channels. The MMP-mediated processing is followed by the engagement of the metallopeptidase PITRM1, which is involved in disposal of cleaved presequences. The mitochondrial intermembrane protease IMMP is formed by two related subunits, IMMP1L and IMMP2L. The active site of this IM-localized metallopeptidase complex is facing the intermembrane space. IMMP is involved in the maturation processing

of several polypeptides in the IM. The substrates of IMMP can be of either nuclear or mitochondrial origin. Additional information about these ATP-independent proteases can be found in the text. *OM* outer membrane, *IMS* intermembrane space, *TOM* translocase of the outer membrane, *TIM* translocase of the inner membrane

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Table 1

Hereditary neurological diseases associated with mutations in mitochondrial proteases

Protease	Gene	Location	Neurological disease	Mutation inheritance	OMIM number
LONP	<i>LONP/CRBN</i>	Matrix	Non-syndromic mental retardation	Autosomal recessive	609262
CLPXP	<i>CLPP</i>	Matrix	Perrault syndrome, type 3	Autosomal recessive	614129
m-AAA	<i>SPG7</i>	IM	Spastic paraplegia, type 7	Autosomal recessive	607259
m-AAA	<i>AFG3L2</i>	IM	Spastic ataxia-neuropathy syndrome, type 5	Autosomal recessive	614487
m-AAA	<i>AFG3L2</i>	IM	Spinocerebellar ataxia, type 28	Autosomal dominant	610246
HTRA2	<i>HTRA2/PARK13</i>	IMS	Parkinson's disease, type13	Autosomal dominant (?)	610297
HTRA2	<i>HTRA2/PARK13</i>	IMS	Hereditary tremor	Autosomal dominant	190300
PARL	<i>PARL</i>	IM	Parkinson's disease	Autosomal recessive	607858, 168600
MPP	<i>PMPCA</i>	Matrix	Spinocerebellar ataxia, type 2	Autosomal recessive	613036, 213200
IMMP	<i>IMMP2L</i>	IM	Tourette syndrome	Autosomal dominant	605977, 137580

Abbreviations: OMIM Online Mendelian Inheritance in Men database, IM inner membrane, IMS intermembrane space