Biochimica et Biophysica Acta 1797 (2010) 1–10

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Emerging roles of mitochondrial proteases in neurodegeneration

Paola Martinelli [a], Elena I. Rugarli [a,b,*]

[a] Laboratory of Genetic and Molecular Pathology, Istituto Neurologico "C. Besta", Milan, Italy
[b] Department of Neuroscience and Medical Biotechnologies, University of Milano-Bicocca, Milan, Italy

ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form 28 July 2009
Accepted 28 July 2009
Available online 5 August 2009

Keywords:
Mitochondrial proteases
Paraplegin
AFG3L2
Neuronal and axonal degeneration
Hereditary spastic paraplegia
Spinocerebellar ataxia

ABSTRACT

Fine tuning of integrated mitochondrial functions is essential in neurons and rationalizes why mitochondrial dysfunction plays an important pathogenic role in neurodegeneration. Mitochondria can contribute to neuronal cell death and axonal dysfunction through a plethora of mechanisms, including low ATP levels, increased reactive oxygen species, defective calcium regulation, and impairment of dynamics and transport. Recently, mitochondrial proteases in the inner mitochondrial membrane have emerged as culprits in several human neurodegenerative diseases. Mitochondrial proteases degrade misfolded and non-assembled polypeptides, thus performing quality control surveillance in the organelle. Moreover, they regulate the activity of specific substrates by mediating essential processing steps. Mitochondrial proteases may be directly involved in neurodegenerative diseases, as recently shown for the *m*-AAA protease, or may regulate crucial mitochondrial molecules, such as OPA1, which in turn is implicated in human disease. The mitochondrial proteases HTRA2 and PARL increase the susceptibility of neurons to apoptotic cell death. Here we review our current knowledge on how disturbances of the mitochondrial proteolytic system affect neuronal maintenance and axonal function.

1. Introduction

Mitochondria are interconnected dynamic organelles that serve pivotal functions in neurons, which depend entirely on aerobic metabolism [1,2]. ATP production is required to maintain ionic gradients and sustain neuronal excitability, and is essential for neurotransmission. Mitochondrial derived reactive species may function as signals in synaptic plasticity, learning and memory. Furthermore, mitochondria regulate Ca^{++} homeostasis and dynamics and are key players in apoptotic and necrotic cell death programs. In highly polarized cells as neurons, mitochondrial function is strictly coupled to efficient transport mechanisms, which ensure delivery of the organelles to dendritic spines and growth cones, sites of high energetic need. Mitochondrial transport and distribution in neurons is a complex and crucial process, modulated by the ability of mitochondria to undergo fusion and fission events, by synaptic activity, and still unknown intracellular signals [3,4]. Given this functional complexity, not surprisingly mitochondrial dysfunction plays a central role in several neurodegenerative diseases. Mutations in mt-DNA are frequently associated with neurological symptoms such as ataxia, external ophthalmoplegia, and peripheral neuropathy. Moreover, mutations in nuclear-encoded mitochondrial proteins often lead to progressive neuronal death [5,6].

Two independent genomes, the mitochondrial and the nuclear genome, are required for mitochondrial biogenesis. Communication mechanisms coordinate the transcriptional activity of the two genomes in response to metabolic demands [7]. To overcome a possible unbalance between nuclear-encoded and mitochondrial-encoded polypeptides, mitochondria have developed efficient quality control systems of newly imported mitochondrial proteins [8,9]. On one hand, chaperones assist the folding of imported polypeptides and the assembly of protein complexes, on the other hand proteases degrade misfolded polypeptides and get rid of excess protein components lacking assembly partners. Proteases serve also as processing enzymes, thus increasing the complexity of the mitochondrial proteome.

Mitochondrial proteases have therefore all the characteristics for being crucial regulators of mitochondrial function. This is illustrated by the emerging role of disturbances of mitochondrial proteolysis in neurodegenerative conditions. We review here the current evidences that mitochondrial proteases can directly or indirectly cause degenerations of axons and neurons.

2. Neurodegenerative diseases due to defects of the *m*-AAA protease

2.1. The m-AAA protease: from protein quality control to regulatory functions

The *m*-AAA protease is a key component of a quality control system that mitochondria employ to conduct the surveillance of proteins of the inner membrane and to degrade in a selective manner non-assembled

* Corresponding author. Laboratory of Genetic and Molecular Pathology, Istituto Neurologico "C. Besta", via Temolo 4 20126, Milano, Italy. Tel.: +39 0223942614; fax: +39 0223942619.
E-mail addresses: rugarli@istituto-besta.it, elena.rugarli@unimib.it (E.I. Rugarli).

0005-2728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2009.07.013

and damaged proteins. The mitochondrial *m*-AAA protease has been first described and studied in *Saccharomyces cerevisiae*. In this organism the *m*-AAA protease is a hetero-oligomeric complex composed by highly homologous subunits (Yta10p and Yta12p) [10]. The human *m*-AAA protease has a native molecular mass of approximately 900 kDa and is composed of paraplegin and AFG3L2 [11,12] (Fig. 1B). Yeast and mammalian subunits of the *m*-AAA protease contain a mitochondrial targeting sequence, two transmembrane domains, and a AAA (triple-A, ATPase Associated with various cellular Activities) domain followed by a metal-dependent proteolytic domain (Fig. 1A). The AAA domain defines the superfamily of AAA+ proteins, a group of ATPases with common ancestry, characterized by a conserved region of 220–250 amino acids, the AAA cassette [13]. The protease owes its name to the presence of this domain, and to the topology of the proteolytic domain that is exposed towards the matrix space of mitochondria. Yeast and mammalian mitochondria also contain an *i*-AAA protease composed of the homo-oligomeric Yme1 containing complex, with an opposite orientation in mitochondria (*i* stands for intermembrane space) (Fig. 1). These proteases actively extract transmembrane segments and pull solvent-exposed domains across the membrane, before exerting proteolytic activity [14,15]. The topology of substrate proteins determines which protease is involved in degradation, although overlapping substrate specificity has been described in yeast [14,16]. Misfolded or damaged proteins are degraded to peptides, which are then either exported from the organelle or degraded further to amino acids by various oligopeptidases. Inactivation of the *m*-AAA protease in yeast leads to the inability to grow on non-fermentable carbon sources, reflecting an important role during mitochondrial biogenesis [10,17]. Known substrate polypeptides of the yeast *m*-AAA protease include respiratory complex components, and F1Fo ATPase subunits [10,17], while the physiological substrates of the mammalian enzyme are still largely unknown.

A more recent role attributed to the yeast *m*-AAA protease involves its ability to mediate proteolytic maturation of specific proteins. The *m*-AAA protease processes the mitochondrial ribosomal components MrpL32 [18]. This maturation is a prerequisite for MrpL32 incorporation into preassembled mitochondrial ribosomes [18]. In absence of MrpL32 maturation, severe defects of mitochondrial protein synthesis occur, explaining respiratory deficiencies of yeast cells lacking Yta10p or Yta12p [18]. This finding opened up a complete new perspective, demonstrating a regulatory role for the protease and suggesting that identification of specific substrates is a key step to understand consequences of the protease dysfunction.

In contrast to yeast, human cells possess two *m*-AAA isoenzymes, a hetero-oligomeric complex made up of paraplegin and the AFG3L2 protein and a homo-oligomeric complex formed by AFG3L2 alone [19] (Fig. 1B). Both the hetero-oligomeric paraplegin-AFG3L2 complex and the homo-oligomeric AFG3L2 complex are able to process the ribosomal subunit MrpL32, and to mediate degradation of non-native membrane proteins in a reconstituted yeast system, demonstrating functional complementation [18,19]. Moreover, these experiments highlight the functional redundancy between the homo-oligomeric and hetero-oligomeric mammalian *m*-AAA proteases.

2.2. m-AAA protease subunits, paraplegin and AFG3L2, are involved in different neurodegenerative diseases

Paraplegin was the first subunit of the *m*-AAA protease associated to a human neurodegenerative disease [12]. The *SPG7* gene, mapping

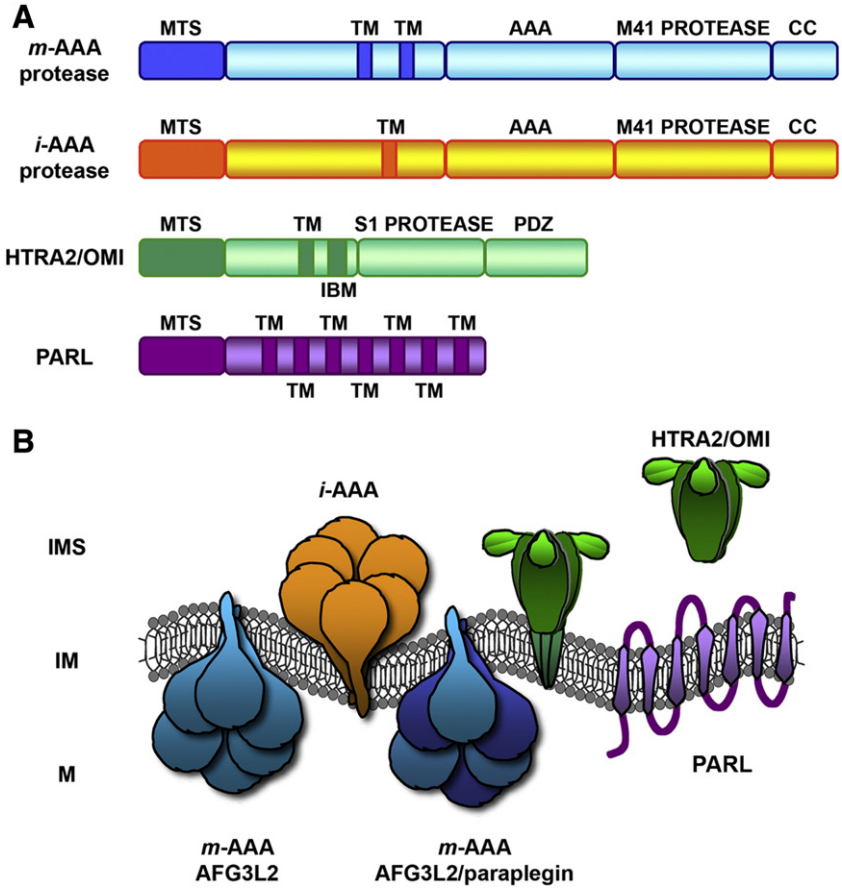

Fig. 1. Structure and topology of the proteases described in this review. A. Schematic representation of domains of the proteases mentioned in this review. Abbreviations: MTS, mitochondrial targeting sequence; TM, transmembrane domain; AAA, triple-A domain (ATPase Associated with various cellular Activities); M41 protease, metal binding proteolytic domain; CC, coiled coil, IBM, inhibitor of apoptosis (IAP)-binding motif; S1 protease, trypsin-like protease domain, PDZ, PDZ domain. B. Illustration of localization and topology of human proteases in mitochondria. Abbreviations: IMS, inter membrane space; IM, inner membrane; M, matrix.

on chromosome 16q24.3 and encoding for paraplegin, was found mutated in a family affected by an autosomal recessive form of hereditary spastic paraplegia (HSP) [12]. This disease is characterized by progressive spasticity and weakness of the lower limbs due to the relentless retrograde degeneration of cortical motor neuron axons [20] (Fig. 2). HSP is genetically heterogeneous, and *SPG7* accounts for 1.5 to 4% of recessive familial cases [21,22]. However, a recent study in 98 Dutch patients with sporadic adult-onset upper motor neuron syndromes revealed a high frequency of mutations in the *SPG7* gene, responsible for about 11% of these cases [23].

SPG7 mutated alleles are clearly loss-of-function, and the pathogenesis of the disease can therefore be ascribed to the lack of the hetero-oligomeric paraplegin–AFG3L2 complex [12,22,24–28] (Fig. 2). Interestingly, in different HSP families analyzed for *SPG7*, the mutation was found in only one allele [22,24]. It is still unclear whether the second mutation escaped identification, or whether a dominant effect of certain mutations or digenic inheritance of the disease could occur in some cases. One study excluded mutations in *AFG3L2* [24].

Recently, the *AFG3L2* gene has been linked to a form of spinocerebellar ataxia (Franco Taroni, personal communication and [29,30]) (Fig. 2). Spinocerebellar ataxias are a heterogeneous group of neurological disorders clinically characterized by loss of balance, progressive gait and limb ataxia, and dysarthria and caused by degeneration of the cerebellum and its afferent and efferent connections [31]. A novel SCA locus (SCA28) was mapped on chromosome 18p11.22–q11.2 in a four-generation Italian family with an autosomal dominant form of cerebellar ataxia (ADCA), defining a critical region that included the *AFG3L2* gene [32]. Ten different missense mutations were found in familial and sporadic ADCA patients [29,30]. Strikingly, mutations hit conserved residues in the peptidase domain, suggesting a possible gain-of-function or dominant-negative effect.

In contrast to paraplegin mutations that affect only the hetero-oligomeric complex, mutations in *AFG3L2* impact both *m*-AAA isoenzymes (Fig. 2). This also rationalizes the fact that recessive mutations of paraplegin cause a rather mild phenotype, while only heterozygous missense mutations of *AFG3L2* are tolerated. Remarkably, from a clinical point of view, phenotypes of patients carrying mutations in one or the other subunit have some points of overlap. Patients with mutations in *SPG7* often show a complicated form of HSP associated with cerebellar atrophy and ataxic signs, and careful examination of HSP patients for signs of cerebellar dysfunction can help identifying patients with *SPG7* involvement [22–24,26,27]. Similarly, SCA28 patients frequently present lower limb hyperreflexia [33]. It will be of interest to explore the possibility that digenic inheritance of mutations in *AFG3L2* and *SPG7* may underlie some forms of cerebellar ataxia, spastic paraparesis, or other neurological phenotypes.

2.3. Pleiotropic roles of m-AAA proteases in neurodevelopment and neurodegeneration

The involvement of *m*-AAA proteases in degeneration of spinal and peripheral axons and in cerebellar dysfunction is nicely recapitulated in mouse models (Fig. 2). Moreover, mouse genetics has clearly indicated that these enzymes are required for development of axonal and dendritic processes in several brain areas (Fig. 2).

Spg7$^{-/-}$ mice were generated by deleting the first two exons of the gene by homologous recombination [34]. They showed motor impairment at the rotarod test at 4.5 months and developed a pronounced scoliosis and abnormal movements of the hindlimbs with increasing age. Neuropathologic analysis revealed a late-onset retrograde axonal degeneration in long descending motor spinal tracts, long ascending sensory spinal tracts, peripheral and optic nerves, resembling the HSP phenotype [34].

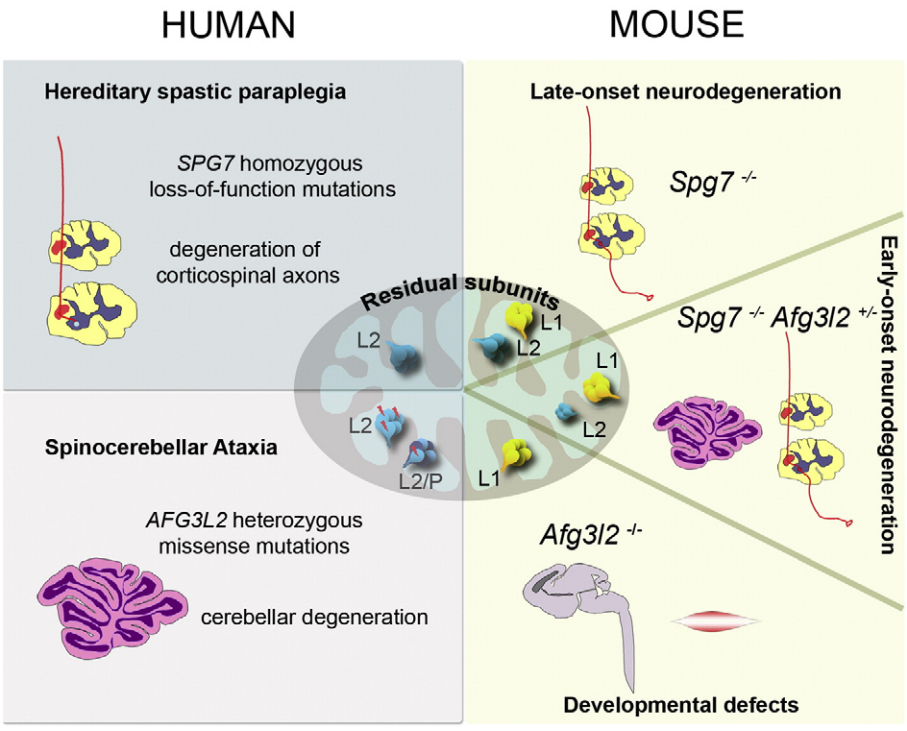

Fig. 2. Phenotypes associated to mutations in *m*-AAA protease subunits in human and mouse. In humans (on the left), mutations on both alleles of *SPG7* lead to degeneration of corticospinal axons, while heterozygous missense mutations in *AFG3L2* cause spinocerebellar ataxia. In the mouse (right), the severity of the phenotype increases along with progressive reduction of the levels of *m*-AAA proteases subunits, and ranges from late-onset neurodegeneration of spinal and peripheral axons, to early-onset neurodegeneration involving also the cerebellum, up to severe developmental defects in brain, spinal cord, and muscle. Residual subunits of *m*-AAA proteases in each condition are depicted in the central mitochondrion. Missense mutations are indicated by the lightning symbol. For the sake of simplicity only homo-oligomeric AFG3L1 and AFG3L2 complexes are shown. Abbreviations, L1, AFG3L1; L2, AFG3L2; P, paraplegin.

Differently from *Spg7* knock-out mice, mice without functional AFG3L2 protein are affected by a severe early-onset neurological phenotype [35]. Two different mouse models are currently available. The first one is the spontaneous mouse mutant *paralysé*, first observed at the Pasteur Institute and recently found to carry a C to G transversion causing the substitution of an arginine by a glycine (R389G) in the conserved AAA domain of AFG3L2 (*Afg3l2*^{par}) [35,36]. A null *Afg3l2* mouse model originated at the Jackson laboratories after ecotropic murine leukemia proviral reinsertion in the MEV/2TyJ strain in intron 14 of the gene (*Afg3l2*^{Emv66}). The reinsertion causes a premature stop codon insertion and the following instability of the protein product [35]. Both mouse models are indistinguishable from control littermates at birth, but they fail to grow and do not survive over 16 days of age, when they are completely paralyzed [35–37]. The phenotype is due to severe defects in neural development, with a retardation of myelination and the absence of large calibre axons in sciatic nerve and spinal cord [35,37]. Interestingly, Purkinje cells were normal in number but failed to develop a branched dendritic tree [35,38]. *Afg3l2* deficient mice have still residual *m*-AAA protease activity, since the murine protease is composed by three different subunits: paraplegin, AFG3L2 and AFG3L1. The last subunit is a pseudogene in humans [39]. Coimmunoprecipitation experiments demonstrated that all the subunits are able to interact with each other and that AFG3L1 can form a homo-oligomeric complex that rescues all the phenotypes of yeast cells deficient of Yta10 or Yta12, similarly to *Afg3l2* [19]. Although *Afg3l1* is poorly expressed in the adult brain [19,38], it is conceivable that the complete absence of *m*-AAA proteases might be embryonic lethal.

The homo-oligomeric AFG3L2 complex plays also an important role in preventing neurodegeneration in the adult brain [38]. Heterozygous *Afg3l2* mice appear normal at birth and do not show a remarkable phenotype up to 3 months of age [38]. However, future studies are required to exclude the appearance of degenerative phenotypes in the spinal axons and/or in the cerebellum at late ages. Mice carrying only one functional allele of *Afg3l2* in a null background of *Spg7* showed instead a severe neurological phenotype from 6 weeks of age, characterized by reduced cage activity, loss of balance, and frank uncoordinated gait, a phenotype highly reminiscent of cerebellar ataxia. Later, they lost significant weight, became immobile, and finally died within the 4th month of life [38]. These animals displayed a remarkable acceleration of the axonal phenotype of *Spg7* knock-out mice in the spinal cord and sciatic nerve, revealing a significant redundancy of the homo-oligomeric and hetero-oligomeric *m*-AAA isoenzymes in adult neurons in vivo. However, at variance with the paraplegin-deficient mice, they also manifested an important loss of Purkinje cells and their afferent and efferent connections, together with prominent reactive astrogliosis, accounting for the ataxic phenotype [38]. Moreover, pyramidal hippocampal neurons specifically in the CA3 field degenerated, stressing the concept that different neurons respond to reduced levels of the *m*-AAA subunits with different thresholds [38].

Afg3l2 and *Spg7* are expressed at different levels in the brain and, while the *Afg3l2* transcript is ubiquitous and highly expressed, *Spg7* is enriched in large neurons, such as pyramidal cortical neurons and Purkinje cells [19,38]. Thus some neurons may be more dependent than others on the action of the hetero-oligomeric protease. Moreover, the different biochemical properties of paraplegin and AFG3L2 may in part explain the various severity and spatio-temporal pattern of neuronal degeneration in case of mutations in one or the other subunit. Crucial questions that need to be addressed are whether neurons are differently affected purely based on their supply of individual subunits, whether neuronal-specific substrates exist driving a certain pattern of degeneration, and finally whether hetero- and homo-oligomeric complexes have also specialized non-redundant functions.

All together, genetic findings in human and mice underline the requirement of an efficient surveillance system by the *m*-AAA protease in neuronal mitochondria to prevent neuronal and axonal degeneration, and to allow normal neural development (Fig. 2).

2.4. Understanding the pathogenesis of m-AAA protease-linked diseases

Pathogenic pathways potentially involved in conditions associated to dysfunction of the *m*-AAA proteases are summarized in Fig. 3.

Axonal and neuronal degeneration in mice deficient for *m*-AAA protease subunits clearly associated with the appearance of structurally abnormal mitochondria. Hypertrophic, altered mitochondria with disrupted cristae were detected in synaptic terminal of spinal axons of paraplegin-deficient mice already at 4.5 months, exactly at the time of onset of motor impairment, and then accumulated in distal affected axons [34]. Similar ultrastructural abnormal mitochondria were observed in cell bodies of motoneurons and Purkinje cells of *Afg3l2* mutants, and in Purkinje cells of *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice [35,38]. Remarkably, the restoration of paraplegin expression in sciatic nerve mitochondria, using AAV vectors expressing the *Spg7* cDNA delivered intramuscularly, rescued the mitochondrial morphology, suggesting that these alterations are in principle reversible [40]. The re-expression of paraplegin also improved neuropathological changes and motor performance of *Spg7*$^{-/-}$ mice [40].

Which are the downstream effects of these mitochondrial alterations? Paraplegin-deficient mice are characterized by a pure axonopathy without cell death. In these mice the most striking phenotype is the appearance of axonal swellings that are loaded with neurofilaments and organelles, particularly the abnormal mitochondria [34]. Impairment of retrograde transport was also evident in old *Spg7*$^{-/-}$ mice [34]. Degeneration of parallel fibers with terminal swellings was observed in the cerebellum of *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice [38]. All together this points to impairment of axonal transport as a common pathogenic bottleneck, leading first to axonal degeneration and then to cell death. The two most affected neuronal types in *m*-AAA protease diseases are characterized either by the longest axons (cortical motor neurons) or by the most elaborate dendritic tree (Purkinje cells), and in both cases depend heavily on proper and regulated mitochondrial transport and distribution. This idea is also supported by the involvement of trafficking genes in several other forms of HSP [41].

Transport of mitochondria along axons and dendrites may be first affected because of mechanical clogging owing to the increased mitochondrial dimensions and then be further worsened by energetic deficiency, creating a vicious cycle. Several evidences suggest that *m*-AAA protease-depleted mitochondria may have a reduced capability to adapt to high energetic demands. Fibroblasts from some *SPG7* mutated HSP patients had depolarized mitochondria, reduced complex I activity and ability to synthesize ATP, together with an increased susceptibility to oxidative damage [24,42]. *Spg7* knock-out mice showed a defect in ATP synthesis only at 23–26 months of age [34]. Reduction of the levels of complexes I and III were observed in mitochondria isolated from brain of *Afg3l2* $^{-/-}$ mice or from cerebellum and spinal cord of *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice [35,38]. In the last model, instability of respiratory complexes rather than decreased assembly suggested a peculiar alteration of the mitochondrial membranes. This would also be consistent with the tendency of mitochondria with reduced levels of *m*-AAA protease subunits to lose mt-DNA [38]. In conclusion, many independent data suggest that *m*-AAA protease-depleted mitochondria have a mild impairment of respiratory function. This mild impairment may become however extremely relevant in situations of high metabolic demands or in cellular districts where energy production should rapidly adapt to cell function, without the possibility of fast mitochondrial protein replenishment and turnover as it happens in synaptic terminals or dendritic spines of neurons.

Understanding the cause for the alteration of mitochondrial morphology and function is crucial to unravel the first molecular steps of the pathogenic process. Both incomplete removal of misfolded

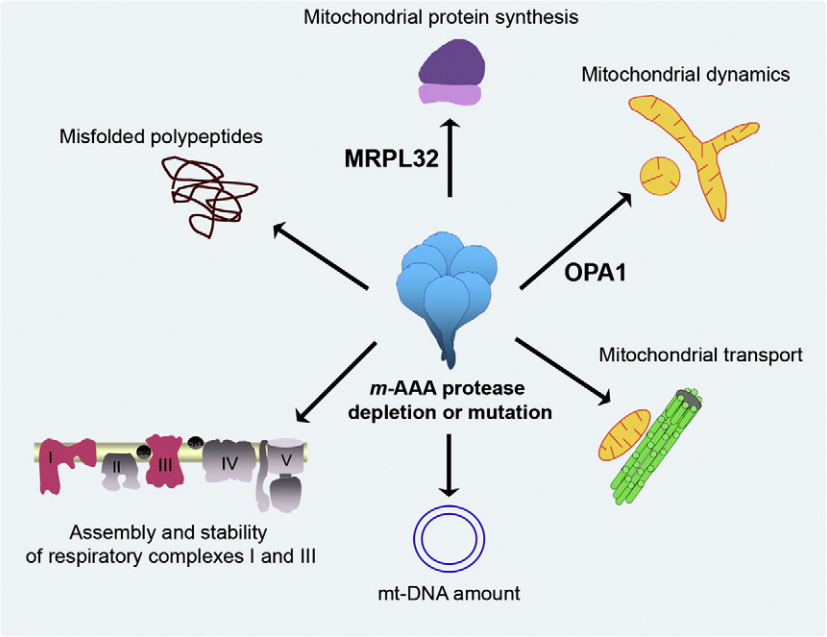

Fig. 3. Possible pathogenic pathways associated with dysfunctional *m*-AAA proteases. Mutations or depletion of *m*-AAA protease subunits can lead to neurodegeneration through several mechanisms, including accumulation of misfolded polypeptides; decreased mitochondrial protein synthesis, due to impaired processing of MRPL32; abnormal mitochondria dynamics, via OPA1; defective mitochondrial transport; mt-DNA depletion; respiratory deficiency owing to reduced assembly and stability of complexes I and III.

proteins and impaired processing of specific substrates regulating mitochondrial dynamics and/or the membrane structure might be postulated. Accumulation of misfolded polypeptides in *m*-AAA protease deficient mitochondria has not been formally demonstrated yet. Moreover, our knowledge on the endogenous neuronal substrates of the *m*-AAA protease is still rather limited. The only substrate so far identified that is unambigously cleaved by both the yeast and the mammalian *m*-AAA protease is the mitochondrial ribosome component MRPL32 [18]. Impairment of MRPL32 processing and accumulation of its precursor has been found in the liver of paraplegin-deficient mice [18]. Furthermore, accumulation of the MRPL32 precursor was observed in cerebellar mitochondria of *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice (P.M. and E.I.R., unpublished observations). However, sufficient mature MRPL32 is still present in these mitochondria and no defects of mitochondrial protein synthesis have been observed in *Afg3l2* null mutants [35]. This result can be explained by the efficient cleavage of MRPL32 from the residual AFG3L1-containing *m*-AAA protease, or by the presence of a redundant protease in mammals. So it appears unlikely that abnormal processing of MRPL32 underlies neurodegeneration. Recent studies have suggested that the *m*-AAA protease may process OPA1 [43,44]. OPA1 is a particularly appealing substrate since it has a role in mitochondrial fusion, cristae morphology, and protection from apoptosis [45–49] and an OPA1-mediated pathogenic pathway could potentially explain many aspects of the observed phenotypes. Moreover, OPA1 itself is mutated in a human neurodegenerative disease [50]. The implications and controversy surrounding this finding is discussed extensively in the next chapter.

3. Processing of OPA1, mutated in autosomal dominant optic atrophy, by mitochondrial proteases

Besides exerting a crucial role in quality control of mitochondrial proteins, mitochondrial proteases cleave specific substrates, allowing their maturation, and thereby controlling a number of tasks. OPA1 is a key regulator of mitochondrial function, whose activity strictly depends on regulated or induced processing. As its yeast homolog, Mgm1, OPA1 is a dynamin-like GTPase involved in mitochondrial inner membrane fusion. In addition, mammalian OPA1 plays a crucial role in the regulation of mitochondrial cristae morphology, and in protecting cells from apoptosis [45–49].

OPA1 is mutated in autosomal dominant optic atrophy (ADOA), a progressive neurological disease characterized by degeneration of the retinal ganglion cells and atrophy of the optic nerve [51,52]. Recently, the spectrum of OPA1-associated neurological conditions has expanded to include atypical clinical presentations and the so called “OPA1 plus” phenotype, in which optic atrophy associates with chronic progressive ophtalmoplegia, ataxia, sensironeuronal deafness, sensory-motor neuropathy and myopathy, and with mt-DNA multiple deletions [53]. The spectrum of OPA1 mutations in patients covers almost the entire gene, and ranges from missense mutations to loss-of-function alleles, strongly suggesting that haploinsufficiency is at the basis of the disease. Cell lines from OPA1 patients have a fragmented mitochondrial network, pointing to impairment of mitochondrial dynamics in the degenerative process [53].

OPA1 resides in the mitochondrial intermembrane space, in tight association with the membrane. Mitochondria contain various mature OPA1 isoforms that arise through the combinations of alternative splicing events (eight alternative *OPA1* transcripts have been described in humans) and proteolytic maturation [43,44,54]. All OPA1 protein variants contain a mitochondrial targeting sequence, followed by a transmembrane segment, the GTPase dynamin-like domain, a central domain of unknown function, and a C-terminal coiled coil region. Alternative splicing involves exons 4, 4b, and 5b that can be present or absent from the transcript in different combinations. Interestingly, exons 4b and 5b are vertebrate specific, and encode for additional hydrophobic stretches. Several evidences suggest that the pro-fusion and the anti-apoptotic activities of OPA1 are distinct and independent from each other and can be ascribed to different isoforms [55]. OPA1 variants containing exon 4 are required to maintain the mitochondrial network fusion ability. In contrast, OPA1 isoforms containing exon 5b appear to be dispensable for maintenance of a fused mitochondrial network, but are crucial for the anti-apoptotic role.

After removal of the mitochondrial targeting sequence by the mitochondrial processing peptidase (MPP), long OPA1 isoforms can undergo additional cleavage events at sites S1 and S2 to generate shorter isoforms, lacking the first transmembrane domain, which are

only peripherally attached to the membrane [43,56] (Fig. 4A). All *OPA1* transcripts encode polypeptides containing the S1 cleavage site located in exon 5, while only transcripts containing exon 5b bear the S2 site in addition. As result of this complex transcriptional and post-translation regulation, in most human and murine tissues, five main OPA1 forms are recognizable, two long membrane-bound OPA1 isoforms (L1 and L2) and three short soluble ones (S3–5) (Fig. 4A).

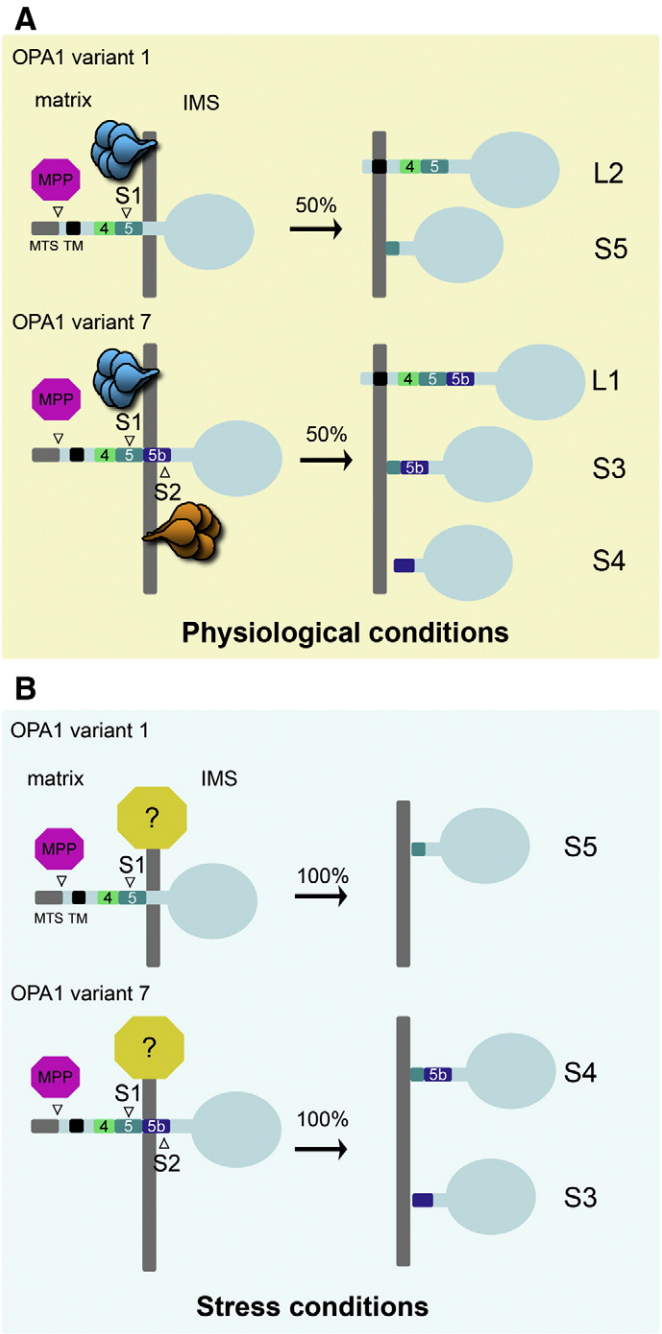

Fig. 4. OPA1 undergoes proteolytic cleavage under physiological and stress conditions. A. In physiological conditions, most tissues express two OPA1 splicing variants (variant 1 and 7), which differ for the absence or presence of exon 5b, respectively. Both variants are first processed by the matrix metallopeptidase (MPP) that removes the mitochondrial targeting signal (MTS) and then by AAA proteases that lead to the formation of long and short OPA1 isoforms in similar amounts. The *m*-AAA protease cleaves OPA1 at site S1, located in exon 5. The *i*-AAA protease instead cleaves OPA1 at S2 within exon 5b. As a result, five main isoforms of OPA1 are produced, L1 and L2, and S3-5. B. Under stress conditions, when ATP levels are low, a yet to be identified protease cleaves OPA1 at S1 and accumulates short OPA1 isoforms.

OPA1 processing is a key step for its function. A combination of long and short OPA1 isoforms is required for proper mitochondrial fusion [57]. Interestingly, all OPA1 isoforms containing exon 4b are efficiently processed and are not able to rescue fusion defects of OPA1 null MEF. On the other hand, OPA1 fusion-competent isoforms appear to be only partially processed and to produce a combination of long and short variants in approximately equimolar amount [57]. OPA1 complexes composed of soluble short isoforms and membrane-bound long isoforms mediate tightening of cristae junctions and protect cells from apoptosis [45].

The relevance of OPA1 processing for ADOA pathogenesis is still unclear, since in most cases patients carry heterozygous mutations that reduce the total level of the protein but do not impair processing. However, several pathological conditions have been linked to abnormal OPA1 processing: low mitochondrial ATP levels, membrane potential dissipation, and apoptosis induction stimulate OPA1 processing leading to the disappearance of long OPA1 variants and the accumulations of short stable isoforms [43,58]. This so-called inducible OPA1 processing may play a protective role in several conditions characterized by mitochondrial dysfunction, by leading to mitochondrial fragmentation and preventing fusion of dysfunctional organelles with the functional network. Notably, increased OPA1 processing was observed in a cybrid cell line of a patient with MERRF syndrome, a severe mitochondrial myopathy associated with mutations in the mt-DNA, in fibroblasts from the mutator mouse, carrying a mutation in the proof-reading domain of the mitochondrial polymerase gamma and thereby accumulating mt-DNA mutations, and finally in the heart of a heart-specific knock-out of the TFAM transcription factor [56]. Induced OPA1 processing can be prevented by incubation with o-phenanthroline, a metalloprotease inhibitor, and zinc, whereas it is stimulated by iron [43,56,58].

Increased OPA1 processing in absence of a measurable mitochondrial dysfunction or alteration of membrane potential occurred in Prohibitin-2 KO cells [59]. These cells had abnormal mitochondria with absent lamellar shaped cristae and accumulation of vesicular shaped cristae. Moreover, they displayed an increased susceptibility to a wide range of apoptotic stimuli. These phenotypes could be rescued by the expression of a long uncleavable OPA1 isoform, demonstrating that prohibitins regulate mitochondrial morphology through OPA1. The prohibitins are large ring-shaped complexes in the inner membrane, composed of two homologous and interdependent subunits, with a putative membrane scaffolding function [60]. In yeast they form a supercomplex together with the *m*-AAA protease, and negatively regulate its activity [61]. The lack of prohibitins may therefore increase the activity of this and other mitochondrial proteases.

The identification of the protease or proteases implicated in regulating OPA1 processing has received great attention in the last few years, but has remained somewhat elusive. The *i*-AAA protease YME1L was found to regulate OPA1 cleavage at S2 in downregulation experiments [57,62] (Fig. 4A). This cleavage site is present in alternative splice forms important for the anti-apoptotic OPA1 function. Debate instead still exists about the mammalian protease responsible for OPA1 cleavage at S1.

PARL is a mitochondrial rhomboid-like protease located in the inner mitochondrial membrane, with seven membrane-spanning regions (Fig. 1A, B). As other rhomboids, PARL is an intramembrane serine protease. Since the *S. cerevisiae* mitochondrial rhomboid Pcp1 is the protease responsible for maturation of Mgm1 [63], PARL was considered a prime candidate for mediating OPA1 cleavage. Albeit sequence and functional homology between Mgm1 and OPA1, the yeast and the mammalian systems display consistent differences. Neither Pcp1 nor PARL affect processing of OPA1 in yeast, although yeast cells are able to conduct proteolytic processing of OPA1 [44]. Moreover, OPA1 processing occurs normally in rhomboid-7 (the PARL homolog) Drosophila mutants and was even slightly enhanced in MEF derived from *Parl* knock-out mice [62,64].

Remarkably, the *m*-AAA protease has been implicated in OPA1 processing at the S1 site [43,44] (Fig. 4A). The formation of OPA1 short isoforms is in fact impaired in yeast cells lacking the *m*-AAA subunits, but is restored upon expression of the mammalian protease. Interestingly, the homo-oligomeric AFG3L2 or AFG3L1 isoenzymes appear to be more efficient than the hetero-oligomeric paraplegin-containing complex [44]. Although overexpression of paraplegin in mammalian cells stimulate OPA1 processing, paraplegin-deficient cell lines and tissues show normal OPA1 processing, consistent with the redundant role of the homo-oligomeric complex [43,44]. It cannot be excluded, however, that absence of paraplegin affects OPA1 processing in a subset of mitochondria located distally in long axons, explaining the specific phenotype observed in the mouse model. Due to technical reasons, this hypothesis is difficult to test. A possible role of the *m*-AAA protease in OPA1 cleavage opens up the intriguing possibility that the diseases linked to paraplegin and AFG3L2 may in part be due to impairment of regulated processing of this crucial GTPase.

Although several data are consistent with a role of the ATP-dependent *m*- and *i*-AAA proteases in the regulated cleavage of OPA1 molecules, it is unlikely that they might be implicated in processing OPA1 in conditions of mitochondrial stress associated with decreased ATP levels. It is instead conceivable that another yet to be identified protease might account for the inducible OPA1 cleavage (Fig. 4B). The identification of this protease will certainly shed new light on a very important pathway by which mitochondria respond to energy dysfunction, apoptotic stimuli and membrane potential dissipation.

4. Mitochondrial proteases protect neurons from apoptotic death: HTRA2/OMI and PARL

Two other mitochondrial proteases have received great attention in the last few years for their potential role in neurodegeneration: the HRTA2/OMI and the PARL proteases. They will be here discussed together because of some similarities in the way they affect neuronal cell death and of a recently discovered functional connection between them.

HTRA2/OMI is a mitochondrial serine protease, homologous to the bacterial HtrA stress responsive genes, DegP and DegS (for extensive review see [65]). DegP acts as a chaperone at normal temperatures but exerts non-selective proteolytic activity against misfolded polypetides in the periplasmic space at high temperatures, while DegS cleaves a specific substrate to initiate a transcriptional stress-response [66,67]. The mammalian HTRA2 is a trimer characterized by an N-terminal mitochondrial targeting sequence, followed by a transmembrane domain, an inhibitor of apoptosis (IAP)-binding motif (IBM), a trypsin-like protease domain, and a C-terminal PDZ domain (Fig. 1A). HTRA2 is attached to the inner mitochondrial membrane by the transmembrane domain and resides in the intermembrane space [68] (Fig. 1B). The protease activity of HTRA2 has been assessed in vitro using β-casein as a substrate. In this assay, the absence of the PDZ domain or the presence of peptides that bind the PDZ domain, significantly augments HTRA2 proteolytic activity, suggesting a regulatory role for this domain [69,70]. It is worth stressing that whether HTRA2 has a protease and/or chaperone activity within mitochondria under physiological or stress conditions is largely unclear. The endogenous substrates of HTRA2 in the mitochondria are still unknown. Recently, loss of HTRA2 has been shown to result in the accumulation of unfolded proteins in the mitochondria, with defective respiration and enhanced production of reactive oxygen species [71].

Upon mitochondrial import, HTRA2 is further processed into a more soluble form that is devoid of the transmembrane domain and exposes the IBM domain in the N-terminal part (Fig. 1B). This domain is homologous to those found in the Drosophila IAP inhibitors Reaper, Hid and Grim, and the mammalian Smac/Diablo. Upon apoptotic stimuli, HTRA2 is released from the mitochondria and binds to IAPs, inactivating their caspase-inhibitor activity. Consistently, RNA interference of HTRA2 increased the resistance of cell lines against apoptotic stimuli [72–76].

The pro-apoptotic role of HTRA2 was questioned when a spontaneous mutation in the murine *Htra2* gene was found to be at the basis of the *mnd2* mutant mouse [77,78]. These mice displayed a progressive neurodegenerative phenotype, characterized by abnormal gait, ataxia, repetitive movements and akinesis. The mutation in the *mnd2* mouse is a homozygous S276C substitution, which affects the proteolytic activity of the protein using the β-casein assay. These data strongly indicate that a mutation inactivating the protease activity of HTRA2 does not induce resistance to apoptosis, but rather increases neuronal death. A virtually identical phenotype was later reported for mice carrying a targeted deletion of most of the coding region, producing a complete loss-of-function allele [79]. Cells derived from this mouse model had an increased susceptibility to cell death stimuli. Moreover, mitochondria from HTRA2-deficient mouse embryonic fibroblasts showed an increased susceptibility to mitochondrial stress and calcium-induced permeability transition and mitochondrial membrane permeabilization [78,79].

Thus, mouse genetics shifted the focus towards an important role of HTRA2 in neurodegeneration. The phenotypic characterization of *mnd2* and *Htra2* knock-out mice showed a peculiar pattern of neuronal loss. The brain region most affected by lack of HTRA2 was the striatum. Focal loss of neurons, reactive astrogliosis, and occasional apoptotic cells were described, together with a selective loss of terminals in the nigrostriatal pathway [78–80]. No loss of dopaminergic neurons in the substantia nigra was however detected [79]. Neurodegeneration was also found in other brain regions, in the brainstem, in the cerebellum, and in the spinal motoneurons. In addition, *Htra2* knock-out mice showed a dramatic decrease of the size of heart, thymus and spleen [78–80].

Interestingly, a very similar phenotype was described for mice lacking PARL [81]. *Parl* knock-out mice show degeneration of striatal and thalamic neurons, lymphocyte loss in the thymus and spleen, growth retardation and cachexia. This phenotype can be ascribed to massive apoptosis, without a substantial impairment of respiratory function [81]. $Parl^{-/-}$ cells were susceptible to a series of intrinsic apoptotic stimuli, released cytochrome *c* faster than control cells, and failed to be protected against apoptosis by OPA1 expression [81]. Thus two proteases, PARL and HTRA2/Omi seem to protect striatal neurons and lymphocytes from apoptotic cell death.

A possible link between these two proteases was given by the finding of a common interactor, the BCL2-family related mitochondrial protein HAX1 [82]. These interactions facilitate the mitochondrial processing of HTRA2 by PARL [82]. Again, mice lacking HAX1 showed striatal neurodegeneration, loss of lymphocytes in thymus and spleen, and increased apoptosis [82]. In Drosophila, the rhomboid-7 protease (the PARL orthologue) has also been involved in Htra2/Omi cleavage, stressing a direct link between these two proteases [64]. All together, these data strengthen the concept that mitochondrial proteases, through regulation of specific substrates or via quality control mechanisms in response to stress, play a key role for protecting neurons from apoptotic death.

The akinesia of HTRA2-deficient mice and *mnd2* mutants, together with the involvement of nigrostriatal connections, fostered the idea of a possible link between the protease and Parkinson disease (PD). Parkinson disease is one of the most common neurodegenerative diseases in the aging population. It is characterized by the clinical triad of rigidity, bradikinesia, and tremor, and by the neuropathological loss of dopaminergic neurons in the substantia nigra with typical intracytoplasmatic ubiquitin- and α-synuclein positive inclusions, the Lewy bodies. Although in large part sporadic, a number of PARK loci have been involved in rare inherited forms of the disease [83]. The HTRA2–Parkinson disease connection is still a matter of debate, since different studies have supported or rejected the hypothesis of a functional association. Genetic data and a functional link between HTRA2 and PINK1, a mitochondrial kinase responsible for an autosomal recessive

form of PD, have been reported by some groups and disputed by others. These conflicting studies are summarized in Table 1. HTRA2 has been found as a component of α-synuclein-containing inclusions in brains with PD, dementia with Lewy bodies, and multiple-system atrophy [84,85].

Recently, one study has shown a role of *Drosophila* rhomboid-7 in proteolytic cleavage of PINK1, a maturation event required for the production of a soluble cytoplasmic released form [64]. Although so far there is no evidence for conservation of this process in humans, this result highlights once again the complex interactions existing between mitochondrial proteases and molecules involved in neurodegenerative phenotypes.

5. Common themes and future perspectives

Increasing evidence highlights the protective role of mitochondrial proteases against axonal degeneration and neuronal death. Proteolytic-dependent quality control and protein processing is subject to special challenges and demands in neurons, due to their strict requirement for mitochondrial ATP production and Ca^{++} buffering, and to the need of rapid response to changing local physiological conditions. Owing to the extreme polarized organization of neurons, dysfunctional, old mitochondria located at a great distance from the cell body presumably need to be retrogradely transported to import newly synthesized proteins, or to be eliminated by mitophagy. Neurons are thus highly dependent on efficient intra-organellar systems, which deal with protein misfolding and unbalance of protein synthesis, and allow the immediate local regulation of the mitochondrial proteome in response to functional needs. Although the neuronal selectivity observed in several of the discussed pathologies is still a missing piece of the puzzle, strikingly the neurons that are more sensitive to defective proteolytic function are those with longer axons, the most elaborate dendritic trees, or special arrangement of mitochondrial distribution, such as the optic nerve [86], again emphasizing the strict relation existing in neurons between function and distribution of mitochondria.

As illustrated by the previous examples, proteases may be directly mutated in neurodegenerative diseases, may increase the susceptibility of neurons to apoptotic cell death, and may regulate mitochondrial proteins which in turn are involved in neurodegenerative phenotypes or in mitochondrial dysfunction. The elucidation of these pathways has important implications for the disclosure of pathogenic pathways in specific neurodegenerative diseases, and for future therapeutic strategies. For example, the identification of the protease responsible for OPA1 induced processing in conditions of low ATP production may pave the way to treatments with a potential impact in a broad collection of pathologies characterized by mitochondrial dysfunction. Moreover, the identification of specific substrates of the *m*-AAA proteases may provide novel therapeutic targets for the associated diseases.

A still, rather unexplored field is the involvement of mitochondrial proteases in the mitochondrial unfolded protein response (mtUPR), in which the accumulation of unfolded proteins in the mitochondrial matrix results in the transcriptional upregulation of nuclear genes encoding mitochondrial stress proteins [87]. Among these are Yme1, the component of the *i*-AAA protease, and ClpP, a matrix ATP-dependent protease [88]. Upregulation of the levels of the ClpP and Lon ATP-dependent proteases occurs in the heart of a Friedreich ataxia heart-specific conditional mouse model [89]. The expression and the stability of mitochondrial proteases may therefore modulate the phenotype of several neurodegenerative conditions associated to impaired mitochondrial function.

In conclusion, mitochondrial proteases are emerging as important actors on the stage of mitochondrial function and dysfunction in neurons, and as attractive therapeutic targets for the prevention of neuronal cell death and axonal degeneration.

Table 1
Contradictory studies on the role of HTRA2 in Parkinson disease.

	In favor	Against
Genetic evidence in human patients	A141S polymorphism in HTRA2 associated with sporadic PD (414 PD patients of German origin and 331 healthy controls); G399S mutation found in PD patients (518 PD patients, 370 controls) [84].	No association between the A141S polymorphism and PD; G399S change also found in normal controls at the same frequency (644 PD cases and 828 North American normal controls) [91].
	R404W mutation found in PD patients; a number of mutations were found in regulatory regions of the gene only in PD patients and not controls (266 Belgian PD patients, 273 controls [90].	No pathogenic HTRA2 variants found in a study on a Poland populations of 95 PD probands [92].
Functional link between HTRA2 and PINK1	In Drosophila, PINK1 overexpression phenotype is partially suppressed by loss of HtrA2 [64]; while HtrA2 overexpression can partially substitute for loss of PINK1 [93].	Genetic analysis in Drosophila does not support function of HtrA2 and PINK1 in the same pathway [95].
	HTRA2 is phosphorylated by the p38 pathway in a PINK-1 dependent manner. Phosphorylated HTRA2 is increased in the brain of sporadic PD patients, and decreased in PINK1 mutated patients [94].	

Note added in proof

Late-onset cerebellar degeneration in mice heterozygous for Afg3l2 has been recently reported (Maltecca F., Magnoni R., Cerri F., Cox G.A., Quattrini A., Casari G. J. Neurosci., 2009, 29:9244–9254).

Acknowledgements

We wish to thank Franco Taroni for sharing unpublished observations, and Thomas Langer for many stimulating discussions. This work was supported by grants from the UMDF, MDA, and CARIPLO foundations.

References

[1] M.P. Mattson, M. Gleichmann, A. Cheng, Mitochondria in neuroplasticity and neurological disorders, Neuron 60 (2008) 748–766.
[2] O. Kann, R. Kovacs, Mitochondria and neuronal activity, Am. J. Physiol., Cell Physiol. 292 (2007) C641–C657.
[3] D.C. Chan, Mitochondria: dynamic organelles in disease, aging, and development, Cell 125 (2006) 1241–1252.
[4] P.J. Hollenbeck, W.M. Saxton, The axonal transport of mitochondria, J. Cell Sci. 118 (2005) 5411–5419.
[5] J.Q. Kwong, M.F. Beal, G. Manfredi, The role of mitochondria in inherited neurodegenerative diseases, J. Neurochem. 97 (2006) 1659–1675.
[6] M.F. Beal, Mitochondria and neurodegeneration, Novartis Found. Symp. 287 (2007) 183–192 (discussion 192–186).
[7] M.T. Ryan, N.J. Hoogenraad, Mitochondrial–nuclear communications, Annu. Rev. Biochem. 76 (2007) 701–722.
[8] W. Voos, K. Rottgers, Molecular chaperones as essential mediators of mitochondrial biogenesis, Biochim. Biophys. Acta 1592 (2002) 51–62.
[9] T. Tatsuta, T. Langer, Quality control of mitochondria: protection against neurodegeneration and ageing, EMBO J. 27 (2008) 306–314.
[10] H. Arlt, R. Tauer, H. Feldmann, W. Neupert, T. Langer, The YTA10–12 complex, an AAA protease with chaperone-like activity in the inner membrane of mitochondria, Cell 85 (1996) 875–885.
[11] S. Banfi, M.T. Bassi, G. Andolfi, A. Marchitiello, S. Zanotta, A. Ballabio, G. Casari, B. Franco, Identification and characterization of AFG3L2, a novel paraplegin-related gene, Genomics 59 (1999) 51–58.

[12] G. Casari, M. De Fusco, S. Ciarmatori, M. Zeviani, M. Mora, P. Fernandez, G. De Michele, A. Filla, S. Cocozza, R. Marconi, A. Dürr, B. Fontaine, A. Ballabio, Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease, Cell 93 (1998) 973–983.
[13] A.F. Neuwald, L. Aravind, J.L. Spouge, E.V. Koonin, AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes, Genome Res. 9 (1999) 27–43.
[14] K. Leonhard, B. Guiard, G. Pellecchia, A. Tzagoloff, W. Neupert, T. Langer, Membrane protein degradation by AAA proteases in mitochondria: extraction of substrates from either membrane surface, Mol. Cell 5 (2000) 629–638.
[15] T. Tatsuta, S. Augustin, M. Nolden, B. Friedrichs, T. Langer, m-AAA protease-driven membrane dislocation allows intramembrane cleavage by rhomboid in mitochondria, EMBO J. 26 (2007) 325–335.
[16] K. Leonhard, J.M. Herrmann, R.A. Stuart, G. Mannhaupt, W. Neupert, T. Langer, AAA proteases with catalytic sites on opposite membrane surfaces comprise a proteolytic system for the ATP-dependent degradation of inner membrane proteins in mitochondria, EMBO J. 15 (1996) 4218–4229.
[17] H. Arlt, G. Steglich, R. Perryman, B. Guiard, W. Neupert, T. Langer, The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease, EMBO J. 17 (1998) 4837–4847.
[18] M. Nolden, S. Ehses, M. Koppen, A. Bernacchia, E.I. Rugarli, T. Langer, The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria, Cell 123 (2005) 277–289.
[19] M. Koppen, M.D. Metodiev, G. Casari, E.I. Rugarli, T. Langer, Variable and tissue-specific subunit composition of mitochondrial m-AAA protease complexes linked to hereditary spastic paraplegia, Mol. Cell. Biol. 27 (2007) 758–767.
[20] A.E. Harding, Hereditary "pure" spastic paraplegia, The hereditary ataxias and related disorders, Churchill Livingstone, New York, 1984, pp. 174–191.
[21] J.K. Fink, Hereditary spastic paraplegia, Curr. Neurol. Neurosci. Rep. 6 (2006) 65–76.
[22] N. Elleuch, C. Depienne, A. Benomar, A.M. Hernandez, X. Ferrer, B. Fontaine, D. Grid, C.M. Tallaksen, R. Zemmouri, G. Stevanin, A. Durr, A. Brice, Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia, Neurology 66 (2006) 654–659.
[23] F. Brugman, H. Scheffer, J.H. Wokke, W.M. Nillesen, M. de Visser, E. Aronica, J.H. Veldink, L.H. van den Berg, Paraplegin mutations in sporadic adult-onset upper motor neuron syndromes, Neurology 71 (2008) 1500–1505.
[24] A. Arnoldi, A. Tonelli, F. Crippa, G. Villani, C. Pacelli, M. Sironi, U. Pozzoli, M.G. D'Angelo, G. Meola, A. Martinuzzi, C. Crimella, F. Redaelli, C. Panzeri, A. Renieri, G.P. Comi, A.C. Turconi, N. Bresolin, M.T. Bassi, A clinical, genetic, and biochemical characterization of SPG7 mutations in a large cohort of patients with hereditary spastic paraplegia, Human Mutat. 29 (2008) 522–531.
[25] C.J. McDermott, R.K. Dayaratne, J. Tomkins, M.E. Lusher, J.C. Lindsey, M.A. Johnson, G. Casari, D.M. Turnbull, K. Bushby, P.J. Shaw, Paraplegin gene analysis in hereditary spastic paraparesis (HSP) pedigrees in northeast England, Neurology 56 (2001) 467–471.
[26] P.A. Wilkinson, A.H. Crosby, C. Turner, L.J. Bradley, L. Ginsberg, N.W. Wood, A.H. Schapira, T.T. Warner, A clinical, genetic and biochemical study of SPG7 mutations in hereditary spastic paraplegia, Brain 127 (2004) 973–980.
[27] T. Warnecke, T. Duning, A. Schwan, H. Lohmann, J.T. Epplen, P. Young, A novel form of autosomal recessive hereditary spastic paraplegia caused by a new SPG7 mutation, Neurology 69 (2007) 368–375.
[28] C. Tzoulis, P.S. Denora, F.M. Santorelli, L.A. Bindoff, Hereditary spastic paraplegia caused by the novel mutation 1047insC in the SPG7 gene, J. Neurol. 255 (2008) 1142–1144.
[29] C. Cagnoli, G. Stevanin, A. Durr, P. Ribai, S. Forlani, A. Brussino, P. Pappi, L. Pugliese, M. Barberis, R.L. Margolis, S.E. Holmes, S. Padovan, N. Migone, D. Di Bella, F. Taroni, A. Brice, A. Brusco, Mutations in AFG3L2 gene (SCA28) in autosomal dominant cerebellar ataxias, presented at the Annual meeting of the American Society of Human Genetics, Philadelphia, Pennsylvania, 2008.
[30] D. DiBella, F. Lazzaro, A. Brusco, G. Battaglia, P.A.A. Finardi, V. Fracasso, M. Plumari, C. Cagnoli, F. Tempia, A. Brussino, C. Gellera, C. Mariotti, P. Plevani, S. DiDonato, T. Langer, M. Muzi-Falconi, F. Taroni, AFG3L2 mutations cause autosomal dominant ataxia SCA28 and reveal an essential role of the m-AAA AFG3L2 homocomplex in the cerebellum, presented at the Annual meeting of the American Society of Human Genetics, Philadelphia, Pennsylvania, 2008.
[31] F. Taroni, S. DiDonato, Pathways to motor incoordination: the inherited ataxias, Nat. Rev., Neurosci. 5 (2004) 641–655.
[32] C. Cagnoli, C. Mariotti, F. Taroni, M. Seri, A. Brussino, C. Michielotto, M. Grisoli, D. Di Bella, N. Migone, C. Gellera, S. Di Donato, A. Brusco, SCA28, a novel form of autosomal dominant cerebellar ataxia on chromosome 18p11.22–q11.2, Brain 129 (2006) 235–242.
[33] C. Mariotti, A. Brusco, D. Di Bella, C. Cagnoli, M. Seri, C. Gellera, S. Di Donato, F. Taroni, Spinocerebellar ataxia type 28: a novel autosomal dominant cerebellar ataxia characterized by slow progression and ophthalmoparesis, Cerebellum 7 (2008) 184–188.
[34] F. Ferreirinha, A. Quattrini, M. Pirozzi, V. Valsecchi, G. Dina, V. Broccoli, A. Auricchio, F. Piemonte, G. Tozzi, L. Gaeta, G. Casari, A. Ballabio, E.I. Rugarli, Axonal degeneration in paraplegin-deficient mice is associated with abnormal mitochondria and impairment of axonal transport, J. Clin. Invest. 113 (2004) 231–242.
[35] F. Maltecca, A. Aghaie, D.G. Schroeder, L. Cassina, B.A. Taylor, S.J. Phillips, M. Malaguti, S. Previtali, J.L. Guenet, A. Quattrini, G.A. Cox, G. Casari, The mitochondrial protease AFG3L2 is essential for axonal development, J. Neurosci. 28 (2008) 2827–2836.
[36] L.W. Duchen, S. Gomez, J.L. Guénet, S. Love, Paralysé: a new neurological mutant mouse with progressive atrophy and loss of motor nerve terminals, J. Physiol. 345 (1983) 166P.
[37] B. Blondet, M.J. Duxson, A.J. Harris, J. Melki, J.L. Guenet, M. Pincon-Raymond, F. Rieger, Nerve and muscle development in paralyse mutant mice, Dev. Biol. 132 (1989) 153–166.
[38] P. Martinelli, V. La Mattina, A. Bernacchia, R. Magnoni, F. Cerri, G. Cox, A. Quattrini, G. Casari, E.I. Rugarli, Genetic interaction between the m-AAA protease isoenzymes reveals novel roles in cerebellar degeneration, Hum. Mol. Genet. 18 (2009) 2001–2013.
[39] G. Kremmidiotis, A.E. Gardner, C. Settasatian, A. Savoia, G.R. Sutherland, D.F. Callen, Molecular and functional analyses of the human and mouse genes encoding AFG3L1, a mitochondrial metalloprotease homologous to the human spastic paraplegia protein, Genomics 76 (2001) 58–65.
[40] M. Pirozzi, A. Quattrini, G. Andolfi, G. Dina, M.C. Malaguti, A. Auricchio, E.I. Rugarli, Intramuscular viral delivery of paraplegin rescues peripheral axonopathy in a model of hereditary spastic paraplegia, J. Clin. Invest. 116 (2006) 202–208.
[41] C. Soderblom, C. Blackstone, Traffic accidents: molecular genetic insights into the pathogenesis of the hereditary spastic paraplegias, Pharmacol. Ther. 109 (2006) 42–56.
[42] L. Atorino, L. Silvestri, M. Koppen, L. Cassina, A. Ballabio, R. Marconi, T. Langer, G. Casari, Loss of m-AAA protease in mitochondria causes complex I deficiency and increased sensitivity to oxidative stress in hereditary spastic paraplegia, J. Cell Biol. 163 (2003) 777–787.
[43] N. Ishihara, Y. Fujita, T. Oka, K. Mihara, Regulation of mitochondrial morphology through proteolytic cleavage of OPA1, EMBO J. 25 (2006) 2966–2977.
[44] S. Duvezin-Caubet, M. Koppen, J. Wagener, M. Zick, L. Israel, A. Bernacchia, R. Jagasia, E.I. Rugarli, A. Imhof, W. Neupert, T. Langer, A.S. Reichert, OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria, Mol. Biol. Cell 18 (2007) 3582–3590.
[45] C. Frezza, S. Cipolat, O. Martins, M. de Brito, G.V.Beznoussenko Micaroni, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, L. Scorrano, OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion, Cell 126 (2006) 177–189.
[46] K.L. Cerveny, Y. Tamura, Z. Zhang, R.E. Jensen, H. Sesaki, Regulation of mitochondrial fusion and division, Trends Cell Biol. 17 (2007) 563–569.
[47] B. Westermann, Molecular machinery of mitochondrial fusion and fission, J. Biol. Chem. 283 (2008) 13501–13505.
[48] G. Lenaers, P. Reynier, G. Elachouri, C. Soukkarieh, A. Olichon, P. Belenguer, L. Baricault, B. Ducommun, C. Hamel, C. Delettre, OPA1 functions in mitochondria and dysfunctions in optic nerve, Int. J. Biochem. Cell Biol. (2009), doi:10.1016/j.biocel.2009.04.013.
[49] R. Yamaguchi, L. Lartigue, G. Perkins, R.T. Scott, A. Dixit, Y. Kushnareva, T. Kuwana, M.H. Ellisman, D.D. Newmeyer, Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization, Mol. Cell 31 (2008) 557–569.
[50] A. Olichon, E. Guillou, C. Delettre, T. Landes, L. Arnaune-Pelloquin, L.J. Emorine, V. Mils, M. Daloyau, C. Hamel, P. Amati-Bonneau, D. Bonneau, P. Reynier, G. Lenaers, P. Belenguer, Mitochondrial dynamics and disease, OPA1, Biochim. Biophys. Acta 1763 (2006) 500–509.
[51] C. Alexander, M. Votruba, U.E. Pesch, D.L. Thiselton, S. Mayer, A. Moore, M. Rodriguez, U. Kellner, B. Leo-Kottler, G. Auburger, S.S. Bhattacharya, B. Wissinger, OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28, Nat. Genet. 26 (2000) 211–215.
[52] C. Delettre, G. Lenaers, J.M. Griffoin, N. Gigarel, C. Lorenzo, P. Belenguer, L. Pelloquin, J. Grosgeorge, C. Turc-Carel, E. Perret, C. Astarie-Dequeker, L. Lasquellec, B. Arnaud, B. Ducommun, J. Kaplan, C.P. Hamel, Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy, Nat. Genet. 26 (2000) 207–210.
[53] P. Amati-Bonneau, D. Milea, D. Bonneau, A. Chevrollier, M. Ferre, V. Guillet, N. Gueguen, D. Loiseau, M.A. Crescenzo, C. Verny, V. Procaccio, G. Lenaers, P. Reynier, OPA1-associated disorders: phenotypes and pathophysiology, Int. J. Biochem. Cell Biol. (2009), doi:10.1016/j.biocel.2009.04.012.
[54] V.R. Akepati, E.C. Muller, A. Otto, H.M. Strauss, M. Portwich, C. Alexander, Characterization of OPA1 isoforms isolated from mouse tissues, J. Neurochem. 106 (2008) 372–383.
[55] A. Olichon, G. Elachouri, L. Baricault, C. Delettre, P. Belenguer, G. Lenaers, OPA1 alternate splicing uncouples an evolutionary conserved function in mitochondrial fusion from a vertebrate restricted function in apoptosis, Cell Death Differ. 14 (2007) 682–692.
[56] S. Duvezin-Caubet, R. Jagasia, J. Wagener, S. Hofmann, A. Trifunovic, A. Hansson, A. Chomyn, M.F. Bauer, G. Attardi, N.G. Larsson, W. Neupert, A.S. Reichert, Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology, J. Biol. Chem. 281 (2006) 37972–37979.
[57] Z. Song, H. Chen, M. Fiket, C. Alexander, D.C. Chan, OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L, J. Cell Biol. 178 (2007) 749–755.
[58] L. Baricault, B. Segui, L. Guegand, A. Olichon, A. Valette, F. Larminat, G. Lenaers, OPA1 cleavage depends on decreased mitochondrial ATP level and bivalent metals, Exp. Cell Res. 313 (2007) 3800–3808.
[59] C. Merkwirth, S. Dargazanli, T. Tatsuta, S. Geimer, B. Lower, F.T. Wunderlich, J.C. von Kleist-Retzow, A. Waisman, B. Westermann, T. Langer, Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria, Genes Dev. 22 (2008) 476–488.
[60] C. Merkwirth, T. Langer, Prohibitin function within mitochondria: essential roles for cell proliferation and cristae morphogenesis, Biochim. Biophys. Acta 1793 (2009) 27–32.
[61] G. Steglich, W. Neupert, T. Langer, Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria, Mol. Cell. Biol. 19 (1999) 3435–3442.

[62] L. Griparic, T. Kanazawa, A.M. van der Bliek, Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage, J. Cell Biol. 178 (2007) 757–764.
[63] M. Herlan, F. Vogel, C. Bornhovd, W. Neupert, A.S. Reichert, Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA, J. Biol. Chem. 278 (2003) 27781–27788.
[64] A.J. Whitworth, J.R. Lee, V.M. Ho, R. Flick, R. Chowdhury, G.A. McQuibban, Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin, Dis Model Mech 1 (2008) 168–174 (discussion 173).
[65] L. Vande Walle, M. Lamkanfi, P. Vandenabeele, The mitochondrial serine protease HtrA2/Omi: an overview, Cell Death Differ. 15 (2008) 453–460.
[66] C. Spiess, A. Beil, M. Ehrmann, A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein, Cell 97 (1999) 339–347.
[67] N.P. Walsh, B.M. Alba, B. Bose, C.A. Gross, R.T. Sauer, OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain, Cell 113 (2003) 61–71.
[68] T. Kadomatsu, M. Mori, K. Terada, Mitochondrial import of Omi: the definitive role of the putative transmembrane region and multiple processing sites in the amino-terminal segment, Biochem. Biophys. Res. Commun. 361 (2007) 516–521.
[69] L.M. Martins, B.E. Turk, V. Cowling, A. Borg, E.T. Jarrell, L.C. Cantley, J. Downward, Binding specificity and regulation of the serine protease and PDZ domains of HtrA2/Omi, J. Biol. Chem. 278 (2003) 49417–49427.
[70] W. Li, S.M. Srinivasula, J. Chai, P. Li, J.W. Wu, Z. Zhang, E.S. Alnemri, Y. Shi, Structural insights into the pro-apoptotic function of mitochondrial serine protease HtrA2/Omi, Nat. Struct. Biol. 9 (2002) 436–441.
[71] N. Moisoi, K. Klupsch, V. Fedele, P. East, S. Sharma, A. Renton, H. Plun-Favreau, R.E. Edwards, P. Teismann, M.D. Esposti, A.D. Morrison, N.W. Wood, J. Downward, L.M. Martins, Mitochondrial dysfunction triggered by loss of HtrA2 results in the activation of a brain-specific transcriptional stress response, Cell Death Differ. 16 (2009) 449–464.
[72] R. Hegde, S.M. Srinivasula, Z. Zhang, R. Wassell, R. Mukattash, L. Cilenti, G. DuBois, Y. Lazebnik, A.S. Zervos, T. Fernandes-Alnemri, E.S. Alnemri, Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction, J. Biol. Chem. 277 (2002) 432–438.
[73] L.M. Martins, I. Iaccarino, T. Tenev, S. Gschmeissner, N.F. Totty, N.R. Lemoine, J. Savopoulos, C.W. Gray, C.L. Creasy, C. Dingwall, J. Downward, The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif, J. Biol. Chem. 277 (2002) 439–444.
[74] Y. Suzuki, Y. Imai, H. Nakayama, K. Takahashi, K. Takio, R. Takahashi, A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death, Mol. Cell 8 (2001) 613–621.
[75] A.M. Verhagen, J. Silke, P.G. Ekert, M. Pakusch, H. Kaufmann, L.M. Connolly, C.L. Day, A. Tikoo, R. Burke, C. Wrobel, R.L. Moritz, R.J. Simpson, D.L. Vaux, HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins, J. Biol. Chem. 277 (2002) 445–454.
[76] G. van Loo, M. van Gurp, B. Depuydt, S.M. Srinivasula, I. Rodriguez, E.S. Alnemri, K. Gevaert, J. Vandekerckhove, W. Declercq, P. Vandenabeele, The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi interacts with caspase-inhibitor XIAP and induces enhanced caspase activity, Cell Death Differ. 9 (2002) 20–26.
[77] J.M. Jones, R.L. Albin, E.L. Feldman, K. Simin, T.G. Schuster, W.A. Dunnick, J.T. Collins, C.E. Chrisp, B.A. Taylor, M.H. Meisler, mnd2: a new mouse model of inherited motor neuron disease, Genomics 16 (1993) 669–677.
[78] J.M. Jones, P. Datta, S.M. Srinivasula, W. Ji, S. Gupta, Z. Zhang, E. Davies, G. Hajnoczky, T.L. Saunders, M.L. Van Keuren, T. Fernandes-Alnemri, M.H. Meisler, E.S. Alnemri, Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice, Nature 425 (2003) 721–727.
[79] L.M. Martins, A. Morrison, K. Klupsch, V. Fedele, N. Moisoi, P. Teismann, A. Abuin, E. Grau, M. Geppert, G.P. Livi, C.L. Creasy, A. Martin, I. Hargreaves, S.J. Heales, H. Okada, S. Brandner, J.B. Schulz, T. Mak, J. Downward, Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice, Mol. Cell. Biol. 24 (2004) 9848–9862.
[80] S. Rathke-Hartlieb, U. Schlomann, P. Heimann, M.H. Meisler, H. Jockusch, J.W. Bartsch, Progressive loss of striatal neurons causes motor dysfunction in MND2 mutant mice and is not prevented by Bcl-2, Exp. Neurol. 175 (2002) 87–97.
[81] S. Cipolat, T. Rudka, D. Hartmann, V. Costa, L. Serneels, K. Craessaerts, K. Metzger, C. Frezza, W. Annaert, L. D'Adamio, C. Derks, T. Dejaegere, L. Pellegrini, R. D'Hooge, L. Scorrano, B. De Strooper, Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling, Cell 126 (2006) 163–175.
[82] J.R. Chao, E. Parganas, K. Boyd, C.Y. Hong, J.T. Opferman, J.N. Ihle, Hax1-mediated processing of HtrA2 by Parl allows survival of lymphocytes and neurons, Nature 452 (2008) 98–102.
[83] S. Lesage, A. Brice, Parkinson's disease: from monogenic forms to genetic susceptibility factors, Hum. Mol. Genet. 18 (2009) R48–R59.
[84] K.M. Strauss, L.M. Martins, H. Plun-Favreau, F.P. Marx, S. Kautzmann, D. Berg, T. Gasser, Z. Wszolek, T. Muller, A. Bornemann, H. Wolburg, J. Downward, O. Riess, J.B. Schulz, R. Kruger, Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease, Hum. Mol. Genet. 14 (2005) 2099–2111.
[85] Y. Kawamoto, Y. Kobayashi, Y. Suzuki, H. Inoue, H. Tomimoto, I. Akiguchi, H. Budka, L.M. Martins, J. Downward, R. Takahashi, Accumulation of HtrA2/Omi in neuronal and glial inclusions in brains with alpha-synucleinopathies, J. Neuropathol. Exp. Neurol. 67 (2008) 984–993.
[86] C.Y. Yu Wai Man, P.F. Chinnery, P.G. Griffiths, Optic neuropathies—importance of spatial distribution of mitochondria as well as function, Med. Hypotheses 65 (2005) 1038–1042.
[87] Q. Zhao, J. Wang, I.V. Levichkin, S. Stasinopoulos, M.T. Ryan, N.J. Hoogenraad, A mitochondrial specific stress response in mammalian cells, EMBO J. 21 (2002) 4411–4419.
[88] J.E. Aldridge, T. Horibe, N.J. Hoogenraad, Discovery of genes activated by the mitochondrial unfolded protein response (mtUPR) and cognate promoter elements, PLoS ONE 2 (2007) e874.
[89] B. Guillon, A.L. Bulteau, M. Wattenhofer-Donze, S. Schmucker, B. Friguet, H. Puccio, J.C. Drapier, C. Bouton, Frataxin deficiency causes upregulation of mitochondrial Lon and ClpP proteases and severe loss of mitochondrial Fe-S proteins, FEBS J. 276 (2009) 1036–1047.
[90] V. Bogaerts, K. Nuytemans, J. Reumers, P. Pals, S. Engelborghs, B. Pickut, E. Corsmit, K. Peeters, J. Schymkowitz, P.P. De Deyn, P. Cras, F. Rousseau, J. Theuns, C. Van Broeckhoven, Genetic variability in the mitochondrial serine protease HTRA2 contributes to risk for Parkinson disease, Human Mutat. 29 (2008) 832–840.
[91] J. Simon-Sanchez, A.B. Singleton, Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls, Hum. Mol. Genet. 17 (2008) 1988–1993.
[92] O.A. Ross, A.I. Soto, C. Vilarino-Guell, M.G. Heckman, N.N. Diehl, M.M. Hulihan, J.O. Aasly, S. Sando, J.M. Gibson, T. Lynch, A. Krygowska-Wajs, G. Opala, M. Barcikowska, K. Czyzewski, R.J. Uitti, Z.K. Wszolek, M.J. Farrer, Genetic variation of Omi/HtrA2 and Parkinson's disease, Parkinsonism Relat. Disord. 14 (2008) 539–543.
[93] L.S. Tain, R.B. Chowdhury, R.N. Tao, H. Plun-Favreau, N. Moisoi, L.M. Martins, J. Downward, A.J. Whitworth, N. Tapon, Drosophila HtrA2 is dispensable for apoptosis but acts downstream of PINK1 independently from Parkin, Cell Death Differ. (2009).
[94] H. Plun-Favreau, K. Klupsch, N. Moisoi, S. Gandhi, S. Kjaer, D. Frith, K. Harvey, E. Deas, R.J. Harvey, N. McDonald, N.W. Wood, L.M. Martins, J. Downward, The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1, Nat. Cell Biol. 9 (2007) 1243–1252.
[95] J. Yun, J.H. Cao, M.W. Dodson, I.E. Clark, P. Kapahi, R.B. Chowdhury, M. Guo, Loss-of-function analysis suggests that Omi/HtrA2 is not an essential component of the PINK1/PARKIN pathway in vivo, J. Neurosci. 28 (2008) 14500–14510.