The mitochondrial *m*-AAA protease regulates mitochondrial fusion: implications for hereditary spastic paraplegia and spinocerebellar ataxia

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The mitochondrial *m*-AAA protease has a crucial role in axonal development and maintenance. Human mitochondria possess two *m*-AAA protease isoenzymes: a hetero-oligomeric complex, composed of paraplegin and AFG3L2, and a homo-oligomeric AFG3L2 complex. Loss of function of paraplegin (encoded by the *SPG7* gene) causes hereditary spastic paraplegia, a disease characterized by retrograde degeneration of cortical motor axons, while missense mutations in AFG3L2 are responsible for spinocerebellar ataxia SCA28. *Spg7*$^{-/-}$ mice show a late-onset degeneration of long spinal and peripheral axons with accumulation of abnormal mitochondria. In contrast, *Afg3l2*$^{Emv66/Emv66}$ mutant mice, lacking the AFG3L2 protein, are affected by a severe neuromuscular phenotype, due to defects in motor axon development. The role of the homo-oligomeric *m*-AAA protease and the extent of cooperation and redundancy between the two isoenzymes in adult neurons are still unclear. By crossing *Spg7* knock out mice and *Afg3l2*, we found an early-onset severe neurological phenotype in *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice, characterized by loss of balance, tremor, and ataxia. *Spg7*$^{-/-}$ *Afg3l2*$^{Emv66/+}$ mice display acceleration and worsening of the axonopathy observed in paraplegin-deficient mice. In addition, they show prominent cerebellar degeneration with loss of Purkinje cells and parallel fibers, and reactive astrogliosis. Mitochondria from affected tissues are prone to lose mt-DNA, and have unstable respiratory complexes. At late stages neurons contain structural abnormal mitochondria defective in COX-SDH reaction. Our data demonstrate genetic interaction between the *m*-AAA isoenzymes and suggest that different neuronal populations have variable thresholds of susceptibility to reduced levels of the *m*-AAA protease. To unravel the possible mechanism of neuronal degeneration, we investigated possible substrates of the *m*-AAA protease in the

different mouse models. Mitochondrial fusion depends on the dynamin-like GTPase OPA1, whose activity is controlled by proteolytic cleavage. Some studies have proposed that the *m*-AAA protease might be involved in OPA1 processing. We found that the loss of AFG3L2 in mouse tissues, downregulation of AFG3L1 and AFG3L2 in MEFs, or the expression of a dominant negative AFG3L2 variant in human cells decreases the stability of long OPA1 isoforms and induces OPA1 processing. We identified the mitochondrial protease OMA1 as the protease responsible for this cleavage. Moreover, cleavage by OMA1 causes the accumulation of short OPA1 variants, if mtDNA is depleted or mitochondrial activities are impaired. Our findings link distinct peptidases to constitutive and induced OPA1 processing and suggest that the pathogenesis of neurodegenerative disorders associated with mutations in *m*-AAA protease subunits might involve impaired mitochondrial fusion.