Abstract

Mitochondria are critical for cellular survival and participate in a broad variety of cellular functions, ranging from ATP production to Ca^{2+} buffering. An elaborate surveillance system of chaperones and proteases preserves mitochondrial protein homeostasis and selectively removes damaged proteins. The *m*-AAA protease, composed of the subunits SPG7 (paraplegin) and AFG3L2, monitors protein homeostasis on the matrix side of the inner membrane and is required for mitochondrial morphology and efficient OXPHOS activity. Pathogenic mutations in *SPG7* and *AFG3L2* cause progressive neurodegenerative diseases, namely hereditary spastic paraplegia (HSP7), spinocerebellar ataxia (SCA28) and spastic ataxia neuropathy syndrome (SPAX5). Although several mouse models recapitulate human disease phenotypes, the underlying pathogenic mechanisms remained enigmatic.

This study identified the previously uncharacterized matrix protein C2ORF47, renamed MAIP1 (*m*-AAA protease interacting protein 1), as specific interacting protein of the *m*-AAA protease. MAIP1 assembles with *m*-AAA proteases and prohibitins into ~2.3 MDa complexes, which counteract cell death. The TIMM44-like domain containing protein MAIP1 exhibits chaperone-like activity and assists in the import and membrane insertion of EMRE, which is an essential subunit of the mitochondrial Ca^{2+} uniporter (MCU). Thus, MAIP1 links the *m*-AAA protease to mitochondrial protein import. Moreover, loss of the *m*-AAA protease or MAIP1 results in increased apoptotic sensitivity, which correlates with the loss of other essential components of the import machinery like TIMM23 and TIMM44. Interestingly, apoptotic sensitivity of cells devoid of *m*-AAA proteases or MAIP1 is restored by co-depletion of MCU, suggesting a physiological link between protein biogenesis and Ca^{2+} influx.

Moreover, this study demonstrates that the *m*-AAA protease degrades non-assembled EMRE, limiting its assembly with MCU. Loss of the *m*-AAA protease decreases ~1.1 MDa MCU holo-complexes containing the gatekeeper subunits MICU1-3 and results in the concomitant accumulation of constitutively active ~400 kDa MCU-EMRE channels, facilitating mitochondrial Ca^{2+} overload. Concordantly, depletion of the *m*-AAA protease increases propensity for mitochondrial permeability transition pore opening *in vitro* and *in vivo*, correlating with neuronal cell death. Together, these results suggest a new model for MCU assembly and explain neuronal loss in disease by deregulated mitochondrial Ca^{2+} homeostasis.