Abstract

Dynamin-like GTPases maintain a tubular mitochondrial network mediating membrane fusion and fission. Two proteases in the mitochondrial inner membrane – OMA1 and YME1L – regulate fusion by proteolytic cleavage of the dynamin-like GTPase OPA1 leading to a balanced formation of long and short OPA1 forms. Remarkably, stress-induced activation of the zinc metalloprotease OMA1 results in a complete processing of long OPA1 forms, which leads to mitochondrial fragmentation and facilitates selective removal of extensively damaged mitochondria by mitophagy.

The loss of YME1L in mouse cardiomyocytes leads to activation of OMA1, induced OPA1 processing and mitochondrial fragmentation, which culminates in heart failure and death. Deletion of *Oma1* in cardiomyocytes stabilizes long OPA1 forms and restores tubular mitochondria and heart function. Therefore, OMA1 is emerging as a promising target to modulate mitochondrial stress response.

Here, we report that the stress-induced activity of OMA1 is mediated in a variety of different conditions such as depleted membrane potential, reactive oxygen species, ATP loss and elevated temperature. OMA1 activation is initiated by a positive charged sensor domain in its N-terminus and a C-terminal region is required for its stability. An autocatalytic cleavage mechanism stops OMA1 activity, thus allowing recovery of the mitochondrial network. We identified the scaffold SLP2 as an interactor of OMA1 possibly building a protease supercomplex with YME1L and the rhomboid protease PARL. Moreover, we established a cell-free reconstitution assay to monitor substrate cleavage by OMA1 *in vitro.* In addition, we developed a luciferase based indirect reporter assay to monitor its proteolytic activity and to identify chemical compounds that inhibit OMA1 function, potentially allowing to prevent mitochondrial damage in disease patients.