Abstract

Regulated protein degradation by conserved ATP-dependent proteases plays a fundamental role for protein quality control in mitochondria and the biogenesis of the organelles. The inactivation of the membrane associated *m*-AAA protease causes severe pleiotropic phenotypes in various organisms including respiratory deficiencies, mitochondrial morphology defects and axonal degeneration in hereditary spastic paraplegia. The molecular basis of these defects, however, is not understood. Further insights into the regulatory role of the *m*-AAA protease within mitochondria can be obtained by the identification of endogenous substrates in yeast mitochondria. In a first approach, proteomes of wild type and *m*-AAA protease deficient mitochondria were compared by two-dimensional-PAGE to identify short-lived mitochondrial proteins with potential regulatory functions. These experiments revealed a remarkable stability of the mitochondrial proteome, but did not allow the identification of substrates of the *m*-AAA protease.

In a second approach, a proteolytically inactive variant of the yeast *m*-AAA protease was used to identify substrates irreversibly associated with the mutant protease via affinity purification. With this approach, MrpL32, a component of the large ribosomal subunit, could be identified. The *m*-AAA protease was shown to process MrpL32 both at the N- and C-terminus. This results in the tight association of MrpL32 with the inner mitochondrial membrane where it assembled with ribosomal particles. This mechanism permits the activation of the synthesis of mitochondrial encoded proteins in close proximity to the inner membrane. The expression of mature MrpL32 partially restored the respiratory competence of *m*-AAA protease deficient strains identifying the maturation of MrpL32 as a key function of the protease in yeast. The *m*-AAA protease-dependent processing of MrpL32 is conserved throughout evolution. The loss of paraplegin, a subunit of the homologous murine *m*-AAA protease, leads to a MrpL32 processing and a mitochondrial translation defect. The regulatory role of the *m*-AAA protease for mitochondrial ribosome assembly and mitochondrial translation may therefore help to understand why the loss of paraplegin results in axonal degeneration in hereditary spastic paraplegia.