Abstract:

In order to identify new oligopeptidases in mitochondria of *S. cerevisiae* a database search was performed to identify genes encoding possible mitochondrial metalloproteases. The candidates were characterized with respect to their subcellular localization. To examine the localization of the peptidases in the different submitochondrial compartments radioactively labelled translation products of the identified proteins were imported into isolated mitochondria. These experiments demonstrate that Oma1 is part of the mitochondrial inner membrane, Prd1 and Ydr430c are located in the intermembrane space and Lap3, Qri7, Yer078c and Ynr020c are found within the matrix area.

Oma1 is the founding member of a conserved family of membrane bound metallopeptidases involved in the proteolysis of polytopic membrane proteins. The analysis of the degradation of an unstable variant of the innermembrane protein Oxa1 allowed to determine the approximate position of Oma1-cleavage sites in Oxa1. The misfolded model protein was cleaved on both sides of the mitochondrial inner membrane.

In further studies the function of the intermembrane space peptidases Prd1 and Ydr430c was examined. The participation of both peptidases in the degradation of oligopeptides in the intermembrane space was shown by chromatographic analysis of peptides exported from mitochondria. Prd1 and Ydr430c showed different substrate specificities depending on the length of the substrates. While peptides from the matrix space are degraded by both oligopeptidases, Ydr430c is mainly responsible for the oligopeptides produced by Yme1.

The growth phenotype of Δ*ydr430c*, which is even more severe in cells lacking the *i*-AAA protease (Δ*yme1*), indicates an important function of this oligopeptidase within mitochondria and an overlapping activity with the *i*-AAA protease. Mutational analysis suggests that the HXXEH motif is the proteolytically active center, identifying Ydr430c as a metallopeptidase.