Role of the Novel Metallopeptidase MoP112 and Saccharolysin for the Complete Degradation of Proteins Residing in Different Subcompartments of Mitochondria*

Melanie Kambacheld‡, Steffen Augustin‡, Takashi Tatsuta‡, Stefan Müller§, and Thomas Langer‡§¶

From the ‡Institut für Genetik and §Zentrum für Molekulare Medizin (ZMMK), Universität zu Köln, 50674 Köln, Germany

Mitochondria harbor a conserved proteolytic system that mediates the complete degradation of organellar proteins. ATP-dependent proteases, like a Lon protease in the matrix space and m- and i-AAA proteases in the inner membrane, degrade malfolded proteins within mitochondria and thereby protect the cell against mitochondrial damage. Proteolytic breakdown products include peptides and free amino acids, which are constantly released from mitochondria. It remained unclear, however, whether the turnover of malfolded proteins involves only ATP-dependent proteases or also oligopeptidases within mitochondria. Here we describe the identification of Mop112, a novel metallopeptidase of the pitrilysin family M16 localized in the intermembrane space of yeast mitochondria. This peptidase exerts important functions for the maintenance of the respiratory competence of the cells that overlap with the i-AAA protease. Deletion of MOP112 did not affect the stability of malfolded proteins in mitochondria, but resulted in an increased release from the organelle of peptides, generated upon proteolysis of mitochondrial proteins. We find that the previously described metallopeptidase saccharolysin (or Prd1) exerts a similar function in the intermembrane space. The identification of peptides released from peptidase-deficient mitochondria by mass spectrometry indicates a dual function of Mop112 and saccharolysin: they degrade peptides generated upon proteolysis of proteins both in the intermembrane and matrix space and presequence peptides cleaved off by specific processing peptidases in both compartments. These results suggest that the turnover of mitochondrial proteins is mediated by the sequential action of ATP-dependent proteases and oligopeptidases, some of them localized in the intermembrane space.

Mitochondria are essential organelles with central anabolic and catabolic functions. To maintain their homeostasis and thereby avoid cell damage, a precise control of the steady state levels of mitochondrial proteins is required. First, evidence for the presence of an independent proteolytic system within mitochondria came from early studies that revealed different turnover rates of proteins residing in different mitochondrial subcompartments (1, 2). Many components of this system, often highly conserved throughout evolution, have been identified since then and found to exert crucial functions within mitochondria (3, 4). They control distinct steps in the biogenesis of mitochondria and selectively degrade malfolded and non-assembled polypeptides accumulating in the organelle. These could be non-assembled proteins, which accumulate in case of an imperfect coordination of nuclear and mitochondrial gene expression, or oxidatively damaged proteins progressively generated in aging cells. Quantitative measurements of mitochondrial protein turnover in logarithmically growing yeast cells suggested the degradation of up to 10% of the mitochondrial proteome per hour, most likely reflecting to a large extent malfolded or damaged proteins (5).

Central components of the proteolytic system of mitochondria are conserved ATP-dependent proteases that ensure the quality control of mitochondrial proteins in various subcompartments (3, 4). These include the ubiquitously present Lon protease in the matrix space (also termed PIM1 protease in yeast), which has been proposed to exert a protective function against oxidative damage (6), and two AAA proteases in the inner membrane mediating the degradation of non-assembled membrane proteins (7): the i-AAA protease containing Yme1 subunits in yeast and human is active in the intermembrane space (8–10), whereas the m-AAA protease, composed of Yta10 and Yta12 subunits in yeast and AFG3L2 and paraplegin subunits in human, degrades proteins from the matrix side (11–13). Inactivation of ATP-dependent proteases causes severe phenotypes in various organisms including neurodegeneration in humans (13–15). These deficiencies might reflect the deleterious effect of malfolded polypeptides accumulating in the absence of the peptidases or impaired proteolysis of mitochondrial proteins with crucial regulatory functions.

Proteolysis initiated by ATP-dependent proteases can lead to the complete degradation of mitochondrial proteins to amino acid residues. This was demonstrated by analyzing proteolytic breakdown products of non-assembled mitochondrial translation products by AAA proteases in the inner membrane of the mitochondria (16, 17). However, ~30% of the proteolytic products represent peptides composed of up to ~20 amino acid residues that are released from the organelle (17). Two pathways for the export of peptides can be distinguished. The majority of mitochondrial translation products is degraded by the m-AAA protease on the matrix side of the inner membrane and subsequently transported across the inner membrane by the ABC-transporter Md11. Peptides generated upon proteolysis by the i-AAA protease accumulate in the intermembrane space from where they are set free from the organelle. It should be
noted that peptide export is not restricted to proteolytic breakdown products of mitochondrially encoded proteins. Rather, peptides generated upon the ongoing turnover of mitochondrial proteins in various subcompartments appear to be permanently released from mitochondria (5).

The formation of peptides and amino acid residues upon proteolysis of mitochondrial proteins raises the question, which peptidases mediate the proteolytic breakdown? As ATP-dependent proteases are generally thought to degrade proteins only to peptides, the involvement of oligopeptidases appears likely. Several oligopeptidases have been identified but their function has been exclusively studied in vitro. These include mammalian neurolysin (18, 19) and yeast saccharolysin (20, 21), both metallopeptidases localized in the mitochondrial intermembrane space, and a novel metallopeptidase, zincc-MP, which is present in mitochondria and chloroplasts and was shown to degrade targeting peptides of nuclear-encoded preproteins in vitro (22). Moreover, recent characterization of the mitochondrial proteome of various organisms revealed the presence of additional oligopeptidases in mitochondria that include the pleomycyl hydrase Lap3 (23), however, their functional characterization remains to be elucidated. Here, we have identified a novel metallopeptidase, termed Mop112, in the intermembrane space of yeast mitochondria and characterized its role for the turnover of mitochondrial proteins.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions—** Yeast cells were grown according to standard procedures at 30 °C in YP medium (1% yeast extract, 2% peptone) containing galactose (2%) or on lactate medium. All strains used in this study were derivatives of W303. The Δyae1 and Δoma1 strains were described previously (8, 24).

The MOP112 gene was disrupted by PCR-mediated homologous recombination in W303-1B, Δaul1 and Δoma1 cells using a disruption cassette harboring the KanMX6 gene (25). PRD1 was disrupted in W303-1A, Δmop112, Δyae1, and Δyae1Δmop112 cells using a HIS3MX6-containing cassette for homologous recombination (25).

For expression in yeast, the MOP12 gene was amplified by PCR and cloned as a Smal-Pet1 DNA fragment into the centromer-based plasmid YCPlac11 harboring the ADH1 promoter. The resulting plasmid was used as a template to modify the MOP112 by PCR-based site-directed mutagenesis employing the QuikChange mutagenesis kit (Stratagene). The following primer pairs were used: for generation of mop112

| Primer Name | Sequence |
|-------------|----------|
| 5'-CCAGATTCACCAGGGAGCCCTATTACCTTCAGACATAACG-3' | and 5'-CGTGGTGTAGTCGTGAGTAGGTTGACTAATTCATACGAG-3' for generation of mop112

5'-GGGGTCTCCTCATATCTTACCTAGATGCGGTTGGCTCACAAGTG-3' and 5'-GGGGTCTCCTCATATCTTACCTAGATGCGGTTGGCTCACAAGTG-3' for generation of mop112

5'-AACCCACACTTGTCTGCTTTATAGAAGAGGAGGGCCAC-3' for generation of mop112

5'-AACCCACACTTGTCTGCTTTATAGAAGAGGAGGGCCAC-3' for generation of mop112

5'-GGGGTCTCCTCATATCTTACCTAGATGCGGTTGGCTCACAAGTG-3' and 5'-GGGGTCTCCTCATATCTTACCTAGATGCGGTTGGCTCACAAGTG-3' for generation of mop112

Stability of Newly Imported Polypeptides in Isolated Mitochondria—Mitochondria were isolated from yeast cells grown on lactate medium by differential centrifugation and further purified by sucrose density centrifugation (26, 28). Released peptides were isolated by sizing chromatography and further analyzed by LC-MS/MS as described before (5). All assignments with a probability score larger than 30 and average mass deviations of fragment ions <0.25 Da were further analyzed. If more than one peptide of a given protein was identified probability scores between 40 and 30 were added. Each experiment was performed four times and proteins were only included in the data set if corresponding peptides were identified in at least two of four experiments.

**Antibody Production—** For generation of a Mop112-specific polyclonal antiserum, the peptide C-SGVTTDDMRQARE corresponding to amino acid residues 931-943 of Mop112 was coupled with maleimide-activated carrier protein (Inject, Pierce) to keyhole limpet hemocyanin and used for generation of antibodies in rabbits.

**RESULTS**

Mop112 Is Localized in the Intermembrane Space of Mitochondria—The open reading frame YDR430c encodes a putative metallopeptidase with a predicted molecular mass of 112 kDa in yeast that has been localized to mitochondria in genome-wide studies (23, 29) and which we named Mop112 (for mitochondrial oligopeptidase 112 kDa). In agreement with a mitochondrial localization, amino acids 1–20 have the propensity to form an amphipathic helix, a characteristic of mitochondrial targeting sequences. To determine the submitochondrial localization of the protein, radiolabeled Mop112 was incubated with isolated mitochondria. Mop112 was imported into mitochondria in a membrane potential (ΔΨ) dependent manner and accumulated in a protease-protected location in the organelle (Fig. 1A). We did not observe proteolytic processing of Mop112 upon import into mitochondria. Osmotic disruption of the outer membrane made the newly imported protein accessible to proteinase K indicating a localization of Mop112 to the intermembrane space (Fig. 1A).

The ΔΨ-dependent import of Mop112 is reminiscent of pre-
sequence-containing mitochondrial matrix and inner membrane proteins that require the TIM23 translocase for import (30). We therefore examined the import of Mop112 into mitochondria harboring a conditional allele of Tim23 (Tim23fs) (31).
motif, HXXEH, at position 84–88, which represents the proteolytic center in many metallopeptidases (Fig. 2B). Whereas the histidine residues serve as binding ligands for the metal ion, the glutamate residue fulfills a catalytic function. We replaced both histidine residues by tyrosine and exchanged also glutamate 87 with glutamine by site-directed mutagenesis. The mutant proteins were expressed in Δmop112 cells and examined for their ability to suppress the growth defect of Δmop112 cells on non-fermentable carbon sources. The growth of mop112H84Y, mop112E87Q, or mop112H84E mutant cells on glycerol-containing media at high temperature was impaired as was growth of Δmop112 cells (Fig. 2A). Mutant and wild type proteins accumulated at similar levels in these cells as revealed by immunoblotting of cellular extracts with a Mop112-specific antiserum (Fig. 2C). Thus, the integrity of the consensus metal binding motif is essential for respiring growth at high temperatures, suggesting that Mop112 has metallopeptidase activity.

The growth phenotype of Δmop112 cells is reminiscent of Δyme1 cells that lack the i-AAA protease acting on the intermembrane space side of the inner membrane (Fig. 2D) (32). To examine a potential functional relationship of Mop112 and the i-AAA protease, we deleted MOP112 in Δyme1 cells by homologous recombination and analyzed the growth of Δyme1Δmop112 mutant cells on glucose- and glycerol-containing media. Strikingly, deletion of MOP112 impaired both respiratory and fermentative growth of Δyme1 cells (Fig. 2D). This synthetic growth defect of mutations in MOP112 and YME1 points to a functional relationship of both proteins and therefore is in agreement with a proteolytic activity of Mop112 suggested by our mutational analysis. Notably, the requirement of Mop112 for efficient growth of Δyme1 cells appears to be rather specific as we did not observe any synthetic growth defect upon deletion of PRD1, encoding the oligopeptidase saccharolysin in the intermembrane space (21, 33), in Δyme1 cells (data not shown).

Mop112 Is Not Essential for the Degradation of Mitochondrial Proteins—The i-AAA protease mediates the degradation of inner membrane proteins with large domains protruding into the intermembrane space. We therefore analyzed the stability of various non-assembled inner membrane proteins in Δmop112 mutant cells. Phb1 and Phb2, which constitute a large multisubunit complex in the inner membrane (34, 35), were synthesized in a cell-free system and imported post-translationally into isolated wild type, Δyme1, Δmop112, and Δyme1Δmop112 mitochondria (Fig. 3, A and B). Newly imported prohibitin subunits assemble only inefficiently with pre-existing subunits (36) and were therefore rapidly degraded when mitochondria were further incubated at 37 °C after completion of import (Fig. 3). Proteolysis was impaired in mitochondria lacking Yme1 demonstrating the involvement of the i-AAA protease in the degradation of non-assembled prohibitin subunits (Fig. 3). In contrast, deletion of MOP112 in wild type and Δyme1 cells did not stabilize newly imported Phb1 or Phb2 subunits (Fig. 3). We made similar observations in mitochondria lacking saccharolysin (data not shown). Moreover, proteolysis of Yta10-(1–161)-DHFR(out), another known substrate of the i-AAA protease (37), was not affected in mitochondria lacking either Mop112 or saccharolysin (data not shown). We conclude that Mop112 is not essential for the degradation of misfolded polypeptides in mitochondria including substrates of the i-AAA protease.

Mop112 Degrades Peptides Generated upon Proteolysis of Mitochondrial Translation Products—Whereas these experiments demonstrated the degradation of mitochondrial encoded proteins in the absence of Mop112, different degradation products may accumulate in Δmop112 mitochondria. The proteolytic breakdown of mitochondrial proteins results in the formation of free amino acid residues and a heterogeneous spectrum of peptides that are released from the organelle (5, 17). To examine whether Mop112 affects the stability of peptides within mitochondria, we analyzed the pool of peptides released from wild type and Δmop112 mitochondria upon proteolysis of non-assembled mitochondrial translation products.

Mitochondrial encoded polypeptides were synthesized in the presence of [35S]methionine and subjected to proteolysis by further incubating mitochondria at 37 °C. In agreement with the experiments described before, non-assembled mitochondrial...
The means of multiple experiments (S.E.): WT (n) are shown (open circles) and subjected to sizing chromatography. In the indicated strains were subjected to sizing chromatography and radioactivity present in eluate fractions was determined. B, eluate fractions of wild type (closed squares) and Δmop112 (open squares) mitochondria. C and D, supernatant fractions of mitochondria isolated from the indicated strains were subjected to sizing chromatography. A, the radioactive material in eluate fractions corresponding to peak 1 is given as percentage of wild type (WT). In D, peak 1 was further subdivided and radioactive material in eluate fractions corresponding to a molecular mass of 2100−1500 Da (dark gray) and 1500−800 Da (light gray) is given as percentage of WT.

The means of multiple experiments (n) are shown (±S.E.): WT (n = 21), Δprd1 (n = 9), Δmop112 (n = 11), Δprd1Δmop112 (n = 6), Δyme1Δmop112 (n = 3), Δoma1 (n = 4).

Mitochondrial translation products were not stabilized in the absence of Mop112 (Fig. 4A). Rather, we observed a slightly increased accumulation of trichloroacetic acid-soluble degradation products in Δmop112 mitochondria (Fig. 4A).

To analyze these proteolytic products, mitochondria were removed from the reaction prior to trichloroacetic acid precipitation. The supernatant fractions containing degradation products were collected and fractionated by gel filtration allowing separation of free amino acid residues (peak 3), peptides composed of up to six amino acids (peak 2), and those composed of more than six amino acids (peak 1). Strikingly, a significantly increased amount of large peptides was released from Δmop112 mitochondria when compared with wild type mitochondria (Fig. 4, B and C). In contrast, a similar amount of smaller peptides was detected in the supernatant fraction of both wild type and Δmop112 mitochondria (Fig. 4B). Deletion of OMA1 or YER078c, encoding metalloproteases localized in the inner membrane or matrix space (24), respectively, did not significantly affect peptide release from mitochondria under these conditions (Fig. 4C). These experiments are consistent with oligopeptidase activity of Mop112 and suggest that Mop112 degrades peptides generated upon proteolysis of non-assembled mitochondrial translation products.

The function of Mop112 might overlap with saccharoylins (or Prd1), which is also localized in the intermembrane space of the mitochondria. We therefore generated double mutant cells lacking both Mop112 and Prd1 and examined the release of peptides formed upon proteolysis of mitochondrial encoded polypeptides from these mitochondria. The deletion of PRD1 did not affect the growth of Δmop112 cells (data not shown). However, we observed a slight increase of large peptides released from Δprd1 mitochondria (Fig. 4C). Thus, both Prd1 and Mop112 appear to degrade peptides derived from mitochondrial translation products in the intermembrane space. Notably, peptides affected by Mop112 appear to be larger than those degraded by Prd1. Whereas deletion of PRD1 appeared to affect all fractions corresponding to peak 1 equally, large peptides within peak 1 accumulated preferentially in the absence of Mop112 (Fig. 4D).

Mop112 Degradation Peptides Generated in the Matrix or Intermembrane Space—Released peptides derived from mitochondrial translation products are generated either by the i-AAA protease in the intermembrane space or by the m-AAA protease in the matrix space and subsequently transported by the ABC transporter Mdl1 across the inner membrane (17). To examine whether Mop112 affects the stability of peptides generated in the intermembrane space upon proteolysis by the i-AAA protease, the export of peptides from the matrix was inhibited by deleting MDL1 in Δmop112 cells. Mitochondrial encoded polypeptides were synthesized in the presence of [35S]methionine in isolated mitochondria and the release of peptides upon proteolysis of the translation products was monitored as before. The amount of peptides composed of more than six amino acid residues increased significantly in the supernatant of Δmdl1Δmop112 mitochondria when compared with Δmdl1 mitochondria (Fig. 5A) demonstrating that Mop112 affects the stability of peptides generated by the i-AAA protease.

Further experiments assessed the role of Mop112 for the stability of peptides generated upon proteolysis of mitochondrial encoded polypeptides by the m-AAA protease in the matrix space. Degradation of these substrates in the intermembrane space is inhibited in Δyme1 mitochondria lacking the i-AAA protease. After labeling of mitochondrial encoded proteins with [35S]methionine, wild type, Δyme1, and Δyme1Δmop112 mitochondria were incubated at 37 °C and the accumulation of proteolytic breakdown products in the supernatant fraction was analyzed. In agreement with previous findings (17), a reduced amount of peptides composed of more than six amino acids was set free from the Δyme1 mitochondria (Fig. 5B). Significantly more peptides were released from Δyme1 mitochondria lacking Mop112 (Fig. 5B). Deletion of PRD1 in Δyme1 cells resulted in a similar increase of large peptides in the mitochondrial supernatant, whereas their amount further increased in Δyme1Δmop112 Δprd1 cells (Fig. 5B). We therefore conclude that both Mop112 and Prd1, although localized in the intermembrane space, affect the stability of at least some peptides generated upon proteolysis of mitochondrial translation products by the m-AAA protease in the matrix.

Mop112 and Prd1 Affect Peptide Efflux from Mitochondria—To examine whether Mop112 and Prd1 are generally involved in peptide turnover within mitochondria, peptides released from wild type, Δmop112, Δprd1, and Δmop112Δprd1 mitochondria were purified and identified by mass spectrometry. In agreement with previous findings (5), a heterogeneous spectrum of peptides was detected in the supernatant of wild type mitochondria that are derived from a large variety of mitochondria.
Oligopeptidases in the Mitochondrial Intermembrane Space

Fig. 5. Mop112 degrades peptides generated in the matrix and the intermembrane space of mitochondria. Proteolysis of mitochondrial translation products and peptide release from mitochondria isolated from the indicated yeast strains was analyzed as described in the legend to Fig. 4. Radioactive material eluting in peak 1 was quantified. The means of multiple experiments (n) are shown (±S.E.): WT (n = 21), Δmdl1 (n = 3), Δmdl1Δmop112 (n = 3), Δyme1 (n = 8), Δyme1Δmop112 (n = 6), Δyme1Δprd1 (n = 3), and Δyme1Δmop112Δprd1 (n = 6). A, peptide release from mitochondria lacking Mdl1. B, peptide release from mitochondria lacking peptidases of the intermembrane space.

Fig. 6. Released peptides and sources of peptides. A, total number of identified peptides exported from wild type (WT; 314 peptides), Δprd1 (251 peptides), Δmop112 (273 peptides), and Δprd1Δmop112 (560 peptides) mitochondria. Peptides were only included in the dataset if peptides of the corresponding protein were identified in at least two of four experiments. B, number of proteins that represent peptide sources.

| Protein | Submitochondrial location | Δmdl1 | Δmop112 | Δmdl1Δmop112 |
|---------|---------------------------|-------|---------|--------------|
| Atp16   | M                         | +     | +       |              |
| Atp2    | M                         | +     | +       |              |
| Atp6    | M                         |       | +       |              |
| Cox4    | M                         |       | +       |              |
| Cox5a   | IM                        |       | +       |              |
| Cox6    | M                         |       | +       |              |
| Cox7    | IM                        |       | +       |              |
| Cyb2    | IMS                       |       | +       |              |
| Hem1    | M                         |       | +       |              |
| Ilv5    | M                         |       | +       |              |
| Mrpl32  | M                         |       | +       |              |
| Pda1    | M                         |       | +       |              |
| Qcr10   | IM                        | +     | +       |              |
| Rpm19   | M                         |       | +       |              |
| Sdh2    | M                         |       | +       |              |
| Ymr002w | n                         |       | +       |              |

a M, matrix; IM, inner membrane; IMS, intermembrane space; n, non-mitochondrial (‘). b +, identified peptides.

Prd1, both localized in the intermembrane space, are involved in the complete degradation of a large number of mitochondrial proteins to amino acids and therefore broadly influence the peptide efflux from mitochondria.

Interestingly, we also identified 45 peptides in the supernatant...
tient of the Δprd1Δmop112 mitochondria that are derived from mitochondrial targeting sequences of seven proteins localized in various subcompartments, namely Atp2, Cox6, Cyb2, Gut2, MrpL32, Pdb1, and Yta10 (Table III). Peptides derived from Gut2 were also released from Δprd1 mitochondria, but we did not detect any presequence-derived peptides in the supernatant of wild type mitochondria. These findings indicate that both oligopeptidases are involved in the turnover of mitochondrial targeting sequences that are cleaved off by specific processing peptidases in various subcompartments of the organelle.

### DISCUSSION

Although the capability of the proteolytic system of mitochondria to degrade proteins to free amino acid residues has been recognized from early on, mammalian neurolysin and yeast saccharolysin remained for a long time the only known mitochondrial oligopeptidases. Here we began for the first time to analyze the role of oligopeptidases within mitochondria and describe Mop112 as a novel metallopeptidase of the mitochondrial intermembrane space.

The observed synthetic growth defect of deletions of MOP112 and YME1 suggests a functional relationship of Mop112 with the ATP-dependent i-AAA protease. We did not observe a stabilization of various misfolded polypeptides, including known substrates of the i-AAA protease, in mitochondria lacking Mop112, suggesting that Mop112 is not an essential component of the quality control system in mitochondria. However, peptides generated upon proteolysis by ATP-dependent proteases are stabilized in the absence of Mop112 and can be detected in the mitochondrial supernatant. Its activity overlaps with saccharolysin (Prd1), which is also shown to act on proteolytic products generated in the matrix space, being it peptides or free amino acid residues, can be extruded from the matrix for further degradation by oligopeptidases in the intermembrane space. This raises the possibility that the degradation of peptides to amino acid residues occurs exclusively in this mitochondrial compartment. However, we consider this hypothesis as highly unlikely for several reasons. First, peptides did not accumulate in the matrix space when peptide export from mitochondria is impaired (17). Second, several putative oligopeptidases have been identified in the mitochondrial proteome and were localized to the matrix space (23). A similar activity of at least some of these peptides during proteolysis of matrix proteins is favorable. Therefore, parallel proteolytic pathways composed of ATP-dependent proteases and oligopeptidases degrading peptides to amino acids may exist in both the intermembrane and matrix space of mitochondria. Proteolytic products generated in the matrix space, being it peptides or free amino acid residues, can be extruded from the matrix for further degradation in the intermembrane space or release from mitochondria.

Notably, such peptides also include presequences of nuclear-encoded mitochondrial preproteins that are cleaved off by specific processing peptidases present in various subcompartments (38). Peptides derived from mitochondrial targeting sequences were observed to be released from mitochondria lacking both Mop112 and Prd1 but not from wild type mitochondria. This finding assigns a function to both peptidases in the degradation of presequences within mitochondria and illustrates again their overlapping substrate specificities.

Whereas our results reveal a role of Mop112 for the stability of peptides within mitochondria, it remains to be determined...
whether the growth defect associated with mop112 mutants reflects solely the loss of a general oligopeptidase activity. Although exerting an overlapping substrate specificity, deletion of PRD1 did not impair cell growth, nor did we observe a synthetic growth defect associated with mutations in PRD1 and YME1. Therefore, Mop112 is likely to exert specific functions within mitochondria. However, as both cells lacking Mop112 or Yme1 exhibit a similar growth defect on non-fermentable carbon sources at high temperature, we favor the hypothesis that the impaired respiratory competence of Δmop112 cells is caused by the impaired proteolysis of specific substrate protein(s) of both peptidases. Future studies will aim at the identification of these substrates to further characterize proteolytic functions of Mop112 within mitochondria.

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Melanie Kambacheld, Steffen Augustin, Takashi Tatsuta, Stefan Müller and Thomas Langer

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