Substitution of PIM1 Protease in Mitochondria by Escherichia coli Lon Protease*

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PIM1 protease in mitochondria belongs to a conserved family of ATP-dependent proteases, which includes the Escherichia coli Lon protease. Yeast cells lacking PIM1 are largely defective in degrading misfolded proteins in the mitochondrial matrix, are respiratory deficient, and lose integrity of mitochondrial DNA. In order to analyze whether E. coli Lon protease is functionally equivalent to mitochondrial PIM1 protease, yeast cells lacking the PIM1 gene were transformed with a construct consisting of a mitochondrial targeting sequence fused onto the Lon protease. In these cells, the fusion protein was expressed and imported into mitochondria, and the targeting sequence was removed. In the absence of PIM1 protease, the E. coli Lon protease mediated the degradation of misfolded proteins in the matrix space in cooperation with the mitochondrial hsp70 system. These cells maintained the integrity of the mitochondrial genome and the respiratory function at 30°C but not at 37°C. Stabilization of mitochondrial DNA in Δpim1 cells depended on protein degradation by the E. coli Lon protease, as a proteolytically inactive Lon variant was not capable of substituting for a loss of PIM1 protease. These results demonstrate functional conservation of Lon-like proteases from prokaryotes to eukaryotes and shed new light on the role of Lon-like proteases in mitochondrial biogenesis.

Mitochondrial homeostasis depends on the coordinated synthesis and degradation of nuclear and mitochondrial encoded proteins. Energy-dependent proteases exist in various subcompartments of mitochondria that mediate the removal of abnormal proteins and ensure the proper stoichiometry of multienzyme complexes. In the mitochondrial matrix space of yeast and mammalian cells, an ATP-dependent protease has been identified that is very similar to the Escherichia coli Lon protease (Desautels and Goldberg, 1982; Watabe and Kimura, 1985; Wang et al., 1993; Kutejová et al., 1993; Van Dyck et al., 1994; Suzuki et al., 1994). The yeast homologue PIM1 protease, like the Lon protease, is a homo-oligomeric enzyme. It performs essential functions in mitochondrial biogenesis (Van Dyck et al., 1994; Suzuki et al., 1994). Cells lacking the PIM1 gene are respiratory deficient and accumulate mitochondrial DNA with extensive deletions (rho− phenotype). PIM1 protease controls selective mitochondrial protein turnover and the proteolytic breakdown of misfolded proteins in the matrix space (Suzuki et al., 1994; Wagner et al., 1994). Efficient proteolysis by the PIM1 protease depends on the mitochondrial hsp70 machinery that stabilizes abnormal polypeptides in a soluble state, susceptible to degradation (Wagner et al., 1994).

The Lon protease of E. coli shares 33% sequence identity with PIM1 protease (Van Dyck et al., 1994; Suzuki et al., 1994). It is required for the degradation of misfolded proteins and of specific proteins with regulatory functions in E. coli during cell division and cell wall synthesis (Gottesman and Maurizi, 1992; Goldberg, 1992). The single ATP-binding sequence is conserved between prokaryotic and eukaryotic Lon-like proteases. Studies on the E. coli Lon protease have demonstrated the requirement of ATP for proteolytic activity (Chung and Goldberg, 1981; Charette et al., 1981; Goldberg and Waxman, 1985; Waxman and Goldberg, 1986; Fischer and Glockshuber, 1994). ATP binding promotes the oligomerization of the Lon protease, whereas ATP hydrolysis is required for the proteolytic breakdown of polypeptides. The carboxy-terminal domain contains a conserved serine residue that most likely represents the nucleophile attacking the peptide bond of the substrate, as a mutation in Ser676 of E. coli Lon protease abolishes the proteolytic activity (Amerik et al., 1991; Fischer and Glockshuber, 1993).

Mature PIM1 protease and the E. coli Lon protease differ considerably in the molecular mass of their subunits (122 and 87 kDa, respectively). Differences in the sequences are apparent mainly in the amino-terminal region. The first 18 amino acid residues of PIM1 protease exhibit characteristics of mitochondrial targeting sequences and thus are believed to target the protein to mitochondria. However, the function of amino-terminal regions of the mature PIM1 protease, not present in E. coli Lon protease, is obscure. Interestingly, the sequences of this region in yeast and human PIM1 protease are rather similar (Wang et al., 1993, 1994; Amerik et al., 1994). Another characteristic of PIM1 protease, not found in other Lon-like proteases, is an extended spacer region between the ATPase and the carboxyl-terminal proteolytic domains.

In order to investigate the possible functional equivalence of the yeast and E. coli Lon-like proteases, we carried out a complementation analysis in Saccharomyces cerevisiae. A chimeric protein consisting of a mitochondrial targeting sequence and the E. coli Lon protease was constructed and expressed in a yeast strain lacking the PIM1 gene. This hybrid protein was imported into yeast mitochondria in vivo. Lon protease exhibited proteolytic activity within the mitochondrial matrix and...
was capable of replacing PIM1 protease in its function of maintaining mitochondrial DNA. Analysis of a Lon protease variant with an impaired proteolytic activity revealed that the peptidase activity is required for the stabilization of the mitochondrial genome. These results demonstrate the functional conservation of yeast PIM1 protease and E. coli Lon protease and thus provide further experimental support for the endosymbiotic origin of mitochondria. On the other hand, E. coli Lon protease did not support maintenance of respiratory function at high temperatures in the absence of PIM1 protease, pointing to functional differences between prokaryotic and mitochondrial Lon-like proteases.

MATERIALS AND METHODS

Cloning and Expression of the E. coli LON Gene in S. cerevisiae—Recombinant DNA techniques were used as described previously (Sambrook et al., 1989; Ausubel et al., 1992). To generate a hybrid protein with an amino-terminal mitochondrial targeting sequence, the E. coli LON gene was first modified by polymerase chain reaction amplification employing the following primers: N-terminal primer, GGCTTCGACTGGTAATCTGAGGCTTAC; C-terminal primer, CGGCCATATGTCAGCAAC. The polymerase chain reaction product was isolated as an XbaI-NdeI fragment and used to replace the amino-terminal portion of the E. coli LON gene from the plasmid YCP111::ADH1 site, which was filled in with Klenow, and the polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid spacer (GIHRG1) between the targeting sequence and the DHFR domain.

Yeast Strains and Methods—The following procedures were carried out according to published protocols: cultivation of yeast cells, yeast transformation (Schiessl et al., 1993), mating of yeast cells, sporulation, and tetrad dissection (Rose et al., 1993). For expression in yeast, the LON gene was transformed into W303–1B cells (MATα ade2–1 ura3–1 his3–11 trp1–1 can1–100 Gal–). Then the tetrads of transformants with Δpim1 cells (27T–1; pMAT Δpim1:URA3 ura3–52 trp1–Δ63 his3–Δ200) (Van Dyck et al., 1993) was carried out followed by random spore analysis. Wild type and mutant forms of pVT100-U-Su9 (1–69)-Lon were transformed into diploid yeast cells (YD028; PIMY′ pim1::6his3–Δ200 lys2–200/lys2–201 ade1–101 ade1–101 trp1–Δ63 trp1–Δ63 his3–Δ200) (Van Dyck et al., 1993) followed by tetrads dissection.

Assaying Protein Degradation within Mitochondria—To monitor the degradation of misfolded proteins in the mitochondrial matrix space, mutant protease proteins were synthesized by transcription and translation in the presence of [35S]methionine using rabbit reticulocyte lysate (Promega) according to published procedures (Söllner et al., 1991). Mitochondria were isolated as described previously (Herrmann et al., 1994). For import reactions, mitochondria were resuspended in import buffer (50 mM HEPES/KOH, pH 7.2, 0.5 mM sorbitol, 80 mM KCl, 10 mM magnesium acetate, 2 mM potassium phosphate, 2 mM manganese chloride, and 3% fatty-acid-free bovine serum albumin) at a concentration of 0.5 mg/ml suspended particles in 50 mM ATP, 0.1 mM EDTA, and 10 mM magnesium acetate (10 mM phosphocreatine, 10 μg/ml creatine kinase). Reticulocyte lysate was added to a final concentration of 5% of total volume. Import was performed for 10 min at 25 °C (Su9 DHFR (1–69)/NADH) or for 20 min at 15 °C (bovine DHFR) and stopped by the addition of 0.5 mM valinomycin and chilling on ice. Nonimported precursors were digested by incubation with protease K (150 μg/ml) for 30 min at 0 °C. After the addition of 1 mM phenylmethylsulfonyl fluoride, mitochondria were resolated by centrifugation for 10 min at 9,200 × g and washed with SHKCI buffer (0.6 mM sorbitol, 80 mM KCl, 50 mM HEPES/KOH, pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride. Mitochondria were resolated by centrifugation for 10 min at 9,200 × g and resuspended in import buffer in the presence of NADH, ATP, and an ATP-regenerating system, and further incubated for various time periods at 30 °C to allow proteolysis to occur. Mitochondria were then resolated by centrifugation for 10 min at 20,600 × g, washed with SHKCI buffer, and dissolved in SDS sample buffer. Samples were analyzed by SDS-PAGE and fluorography.

Biochemical Procedures—The following published procedures were used: SDS-polyacrylamide gel electrophoresis and fluorography of the resulting gels (Nicholson et al., 1987); quantification of the fluorographs on an Image Master densitometer (Pharmacia); blotting of proteins onto nitrocellulose and immunostaining of transferred proteins using the ECL chemiluminescence detection system (Amersham Corp.); and determination of protein concentration by the Coomassie dye binding assay (Bio-Rad).

RESULTS

Targeting of E. coli Lon Protease to Yeast Mitochondria in Vivo—The LON gene was fused in frame to the amino-terminal 69 amino acids of the ATPase subunit 9 of N. crassa to generate a species of the E. coli Lon protease that, when expressed in the cytosol, could become imported into mitochondria. The presence of subunit 9 has been used repeatedly to target heterologous proteins to mitochondria (Pfanner et al., 1987; Ungermann et al., 1994). The resulting hybrid gene Su9 (1–69)-Lon was cloned into a yeast expression vector under the control of the ADH1 promoter, allowing its constitutive expression, and transformed into the W303–1B strain. The hybrid genes were then isolated by tetrad dissection. In order to analyze the expression and localization of the Su9(1–69)-Lon gene were then isolated by tetrad dissection. In order to analyze the expression and localization of Su9 (1–69)-Lon in the mitochondrial fraction (Fig. 1, lanes 1–3), a hybrid protein was obtained containing a six-amino acid spacer (GIHRG1) between the targeting sequence and the DHFR domain.

2 The abbreviations used are: DHFR, dihydrofolate reductase; PAGE, polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid.
The temperature-dependent growth phenotype could be the consequence of a reduced affinity for specific amino acids in the sorting signal of cytochrome b_{6f}.

**Level of Lon protease might be limiting for growth under these conditions.** To distinguish between these possibilities, Su9 (1–69)-Lon was cloned into a multicopy yeast expression vector under the control of the ADH1 promoter and expressed in a Δpim1 mutant strain. E. coli Lon protease was recovered exclusively in the mitochondrial fraction upon cell fractionation (Fig. 1, lanes 4–6). When compared with the expression from a CEN-based plasmid, E. coli Lon protease accumulated at an approximately 50-fold higher level within yeast mitochondria under these conditions. The growth rate of the cells on nonfermentable carbon sources at 30 °C increased significantly (Fig. 2). Thus, when expressed from a CEN-based plasmid, the level of E. coli Lon protease within mitochondria is limiting for growth. Overexpression of Lon-like proteases, either of PIM1 protease in yeast or of Lon protease in E. coli, on the other hand, was observed to inhibit cell growth (Goff and Goldberg, 1987). A precise regulation of the cellular activity of Lon-like proteases must therefore exist. It remains to be determined whether this is achieved by regulation of protein synthesis or by posttranslational processes.

The presence of E. coli Lon protease even at high levels did not maintain mitochondrial respiration at higher temperature (Fig. 2). Δpim1 cells expressing Su9 (1–69)-Lon accumulated aberrations in the mitochondrial genome at 37 °C as demonstrated by mating with a mit− tester strain (data not shown). The temperature-dependent growth phenotype could be the consequence of a low specific activity or a low thermostability of Lon protease in the mitochondrial environment. Alternatively, functional differences between the prokaryotic and eukaryotic homologue have to be envisioned.

E. coli Lon Protease Mediates the Degradation of Misfolded Proteins within Mitochondria—The inability of E. coli Lon protease to support respiratory competence at high temperatures might be the consequence of a reduced affinity for specific substrate proteins or of an impaired interaction with other components of the proteolytic system in the mitochondrial matrix space. The PIM1 protease has recently been shown to mediate the degradation of misfolded proteins in cooperation with the mitochondrial hsp70 machinery, which maintains misfolded polypeptides in a conformation susceptible to degradation (Wagner et al., 1994).

In order to investigate whether E. coli Lon protease can also degrade polypeptides presented by mitochondrial hsp70, we employed two model proteins that fail to attain their native conformation after import into the mitochondrial matrix and thus are subject to proteolysis. Mouse DHFR, when fused to the amino acids 1–167 of cytochrome b_{6f}, is targeted to mitochondria and sorted to the intermembrane space. Exchange of specific amino acids in the sorting signal of cytochrome b_{6f} results in targeting of the hybrid protein to the matrix space (b_{6f}(1–167)R–DHFR; Schwarz et al. (1993)). The matrix targeting sequence is removed upon import by the matrix-localized proc-
cessing peptidase, yielding the so-called i-form, and a further, so far unidentified protease generates the slightly smaller i*-form. The unfolded cytchrome b$_2$ moiety becomes complexed by the mitochondrial hsp70 machinery and degraded by PIM1 protease. This results in the formation of a fragment f that corresponds to the folded DHFR domain (Wagner et al., 1994; Fig. 3). To investigate whether Lon protease can replace its mitochondrial homologue in this reaction, the stability of b$_2$(1–167)RIC-DHFR was analyzed in $\Delta$pim1 mutant mitochondria containing E. coli Lon protease. b$_2$(1–167)RIC-DHFR was found to be degraded in $\Delta$pim1 mutant mitochondria in the presence of Lon protease (Fig. 3). The efficiency of fragment formation was relatively low. However, fragment formation was strictly dependent on Lon protease, as b$_2$(1–167)RIC-DHFR was stable in mitochondria lacking PIM1 protease (Fig. 3). The product of proteolysis by Lon protease was indistinguishable in size from the fragment generated by PIM1 protease, suggesting a similar substrate specificity of both proteases.

The proteolytic breakdown of newly imported Su9 (1–69)-DHFR$^{7/42/49}$ in the mitochondrial matrix space was studied in similar experiments. The exchange of amino acid residues in the DHFR domain (Cys$^7$–Ser$^7$, Ser$^{42}$–Cys$^{42}$, and Asn$^{49}$–Cys$^{49}$) by site-directed mutagenesis results in the destabilization of the DHFR domain, preventing the binding of substrate molecules (Vestweber and Schatz, 1988). The mutated DHFR domain was fused to the 69 amino-terminal amino acid residues of subunit 9 of the F$_0$-ATPase of N. crassa, which comprise the matrix targeting signal (Su9 (1–69)-DHFR$^{7/42/49}$). Wild type Su9 (1–69)-DHFR was imported in parallel as a control. The imported, loosely folded DHFR domain was degraded with a half-time of about 15 min, whereas wild type DHFR was stable under these conditions (Fig. 4A). In contrast to wild type DHFR, the loosely folded DHFR was found in association with mitochondrial hsp70 upon communoprecipitation with antibodies against hsp70, reflecting the involvement of the mitochondrial hsp70 machinery in the degradation of this misfolded protein (data not shown). Proteolysis is mediated by PIM1 protease as demonstrated by the stability of the mutated DHFR domain within mitochondria lacking PIM1 protease when compared with wild type, similar to what was observed for b$_2$(1–167)RIC-DHFR (see above). To further characterize the

![Fig. 3. Degradation of b$_2$(1–167)$^{\text{RIC}}$-DHFR by E. coli Lon protease in the mitochondrial matrix. b$_2$(1–167)$^{\text{RIC}}$-DHFR was imported into mitochondria isolated from wild type, $\Delta$pim1, and $\Delta$pim1 cells expressing Su9 (1–69)-Lon from a CEN-based plasmid ($\Delta$pim1LON$^+$). After completion of import, proteolysis was assessed as described under “Materials and Methods.” i and i*, intermediate forms; f, fragment.](http://www.jbc.org/)

![Fig. 4. Degradation of the misfolded DHFR$^{7/42/49}$ by Lon-like proteases in the mitochondrial matrix. A, Su9 (1–69)-DHFR$^{7/42/49}$ and Su9 (1–69)-DHFR$^{7/42/49}$ were imported into wild type mitochondria. After completion of import, samples were further incubated at 30 °C to allow proteolysis to occur. B, Su9 (1–69)-DHFR$^{7/42/49}$ was imported into mitochondria isolated from wild type, $\Delta$pim1, and $\Delta$pim1 cells expressing Su9 (1–69)-Lon from a CEN-based plasmid ($\Delta$pim1LON$^+$), and proteolysis was analyzed upon further incubation of the samples at 30 °C. C, after import as in B, mitochondria were lysed at a concentration of 1 mg/ml by incubation in 0.1% Triton X-100, 10 mM MOPS/KOH, pH 7.2, 150 mM NaCl, 5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride for 10 min at 4 °C under vigorous mixing. Aggregation of the DHFR$^{7/42/49}$ domain was determined by centrifugation for 15 min at 25,000 × g. DHFR$^{7/42/49}$ in the supernatant was precipitated with 12.5% trichloroacetic acid. The pellet fraction was dissolved in SDS-PAGE sample buffer. Both fractions were then subjected to SDS-PAGE. In A, B, and C, total material after import was set to 100%.](http://www.jbc.org/)
fate of the nondegraded fraction, the solubility of mutated DHFR was analyzed in wild type mitochondria, in Δpim1 mitochondria and in Δpim1 mitochondria containing Lon protease. Aggregates of mutant DHFR accumulated in the pellet fraction in the absence of PIM1 protease (Fig. 4C). Release of misfolded polypeptide chains from mitochondrial hsp70 followed by aggregation in the absence of PIM1 protease has been reported earlier (Wagner et al., 1994). When compared with Δpim1 mitochondria, aggregation of mutant DHFR was diminished in the presence of E. coli Lon protease, as a fraction of the misfolded polypeptide chain was degraded (Fig. 4C). The nondegraded form of mutant DHFR, however, was apparently released from molecular chaperone proteins, leading to its aggregation.

These observations demonstrate that the E. coli Lon protease is active within yeast mitochondria and indicate that misfolded polypeptide chains associated with the mitochondrial hsp70 machinery are substrates of the E. coli Lon protease. Lon protease was also found to mediate the proteolysis of polypeptides stabilized by the hsp70 system at 37°C (data not shown). Therefore, the inability of the prokaryotic enzyme to maintain respiration at 37°C in the absence of PIM1 protease is apparently not caused by an impaired cooperation with molecular chaperones under these conditions.

**Conservation of Lon-like Proteases**

Each subunit of the E. coli Lon protease contains an ATPase and a proteolytic domain. Replacement of serine 679 by alanine was reported to abolish the proteolytic activity of the Lon protease without affecting its ability to hydrolyze ATP (Fischer and Glockshuber, 1993). On the other hand, the ATPase activity has been shown to be essential for the proteolytic function (Chung and Goldberg, 1981; Charette et al., 1981). Replacement of lysine 362 by alanine resulted not only in a 30-fold reduction of the ATPase activity, but also in a dramatic decrease in the peptidase activity of the E. coli Lon protease (Fischer and Glockshuber, 1994). In order to analyze whether both catalytic activities of the Lon protease are required for complementing the loss of PIM1 protease, mutants of Su9 (1–69)-Lon protease were constructed that carry a point mutation in serine 679 (S679A; Su9 (1–69)-LonS679A) or in lysine 362 (K362A; Su9 (1–69)-LonK362A). The hybrid proteins were expressed from a multicopy yeast expression vector in a E. coli isolate, containing Lon protease were synthesized in these cells, and they were imported into mitochondria at similar levels as observed by Western blotting (data not shown).

The expression of mutant Su9 (1–69)-LonK362A did not confer on Δpim1 mutant cells the ability to grow on nonfermentable carbon sources at 30°C, in contrast to the overexpression of the wild type form of Su9 (1–69)-Lon (Fig. 5A). Thus, the proteolytic activity of E. coli Lon protease is required for the maintenance of respiratory competence.

Cells expressing Su9 (1–69)-LonS679A were able to grow on nonfermentable carbon sources although at a strongly reduced rate (Fig. 5A). Most likely, this is due to a residual activity of the mutant Lon protease in ATP binding and hydrolysis. The K362A mutant of Lon protease was observed to exhibit a very weak proteolytic activity in vitro (about 2% of wild type activity; Fischer and Glockshuber (1994)).

The integrity of the mitochondrial genome was analyzed by crossing the Δpim1 strain complemented with mutant Su9 (1–69)-Lon with a mit− tester strain carrying a mutation in the mitochondrial OX12 gene (Fig. 5B). The respiratory defect of oxi2 mutants was not complemented by mating with a Δpim1 strain expressing Su9 (1–69)-LonK362A, pointing to aberrations in the mitochondrial DNA (Fig. 5B). Furthermore, replacement of serine 1015 by alanine in PIM1 protease was found to cause aberrations in mitochondrial DNA. In contrast, complementation was observed with Su9 (1–69)-LonK362A (Fig. 5B). Apparently, when overexpressed, a rather low specific proteolytic activity of Lon protease is sufficient to stabilize the mitochondrial genome.

**Discussion**

We demonstrate in the present study conservation of function of Lon-like proteases in prokaryotes and mitochondria. E. coli Lon protease can substitute for mitochondrial PIM1 protease in several respects; the prokaryotic homologue preserves the integrity of the mitochondrial genome and maintains respiration competence of the cells at 30°C in the absence of PIM1 protease. E. coli Lon protease exerts proteolytic activity within yeast mitochondria. Similar to PIM1 protease, it mediates the proteolytic breakdown of misfolded proteins in the mitochondrial matrix that are stabilized against aggregation by the hsp70 machinery. The interplay between folding and proteolytic reactions in mitochondria is not well understood. In particular, the question remains to be answered whether the transfer of polypeptides from molecular chaperone proteins to PIM1 protease occurs by direct interaction between the PIM1 protease and the chaperone proteins. The ability of E. coli Lon protease to degrade polypeptides presented by hsp70 is remarkable, as the cooperation of chaperone proteins with PIM1 protease appears to be rather specific. hsp78, a ClpB homologue in the mitochondrial matrix space has recently been demonstrated to stabilize misfolded proteins against aggregation. However, in contrast to polypeptides associated with hsp70, hsp78-bound proteins are not substrates of PIM1 protease (Schmitt et al., 1995).

PIM1 protease belongs to a group of nuclear encoded genes that are essential for mitochondrial genome stability including components of the replication system, the protein synthesis apparatus, and nucleotide biosynthesis. E. coli Lon protease can substitute for a loss of PIM1 protease and preserves the integrity of the mitochondrial genome at 30°C in the absence of PIM1 protease. Mutational analysis revealed that a proteolytically active Lon-like protease in the matrix is required for the maintenance of mitochondrial DNA. Proteolytic activation of a
mitochondrial protein through specific processing by PIM1 protease might be a process involved in preserving the integrity of the mitochondrial genome. Alternatively, PIM1 protease might regulate the turnover rate of a regulatory protein that negatively affects DNA stability. In any case, the requirement of the proteolytic activity for mitochondrial genome integrity is illustrated by two observations: low amounts of active protease as well as large amounts of Lon protease with a mutation in the ATPase domain are sufficient to stabilize mitochondrial DNA.

Interestingly, E. coli Lon protease does not entirely substitute for PIM1 protease. At 37°C, the respiratory competence of Δpim1 mutant cells and integrity of mitochondrial DNA were not restored upon expression of E. coli Lon protease. This might be the consequence of a low specific activity or a low thermostability of the prokaryotic homologue in the mitochondrial environment. Although the Lon protease was proteolytically active in the matrix space, it was rather inefficient in degrading misfolded proteins. An increase in the growth rate of Δpim1 mutant cells on nonfermentable carbon sources at 30°C was observed when high levels of Lon protease were accumulated within mitochondria. Respiratory competence of Δpim1 mutant cells at 37°C, on the other hand, was still not restored. As E. coli Lon protease was capable of degrading misfolded polypeptides in the mitochondrial matrix space under these conditions, it seems unlikely that this is the consequence of a low specific activity or a low thermostability of E. coli Lon protease within mitochondria. Rather, additional activities of PIM1 protease have to be envisioned. It might also include variations in the substrate specificity. It will be interesting to link these non-overlapping activities to protein domains that are not conserved between the mitochondrial and the prokaryotic homologue.

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