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Regulated protein degradation in mitochondria

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Abstract. Various adenosine triphosphate (ATP)-dependent proteases were identified within mitochondria which mediate selective mitochondrial protein degradation and fulfill crucial functions in mitochondrial biogenesis. The matrix-localized PIM1 protease, a homologue of the *Escherichia coli* Lon protease, is required for respiration and maintenance of mitochondrial genome integrity. Degradation of non-native polypeptides by PIM1 protease depends on the chaperone activity of the mitochondrial Hsp70 system, posing intriguing questions about the relation between the proteolytic system and the folding machinery in mitochondria. The mitochondrial inner membrane harbors two ATP-dependent metallopeptidases, the *m*- and the *i*-AAA protease, which expose their catalytic sites to opposite membrane surfaces and cooperate in the degradation of inner membrane proteins. In addition to its proteolytic activity, the *m*-AAA protease has chaperone-like activity during the assembly of respiratory and ATP-synthase complexes. It constitutes a quality control system in the inner membrane for membrane-embedded protein complexes.
Key words. Mitochondria; proteolysis; PIM1 protease; AAA protease; chaperone.

Introduction

Intracellular degradation of proteins is a process that facilitates the adaptation of cellular functions to changes in environmental conditions. Proteolysis of many cellular proteins occurs in lysosomal compartments, cellular organelles specialized for nonselective protein degradation in mammals and plants. In addition, proteases have been identified in the cytosol and in various organelles, including the endoplasmic reticulum, chloroplasts and mitochondria. Protein degradation by nonlysosomal proteases often depends on hydrolysis of ATP; this is thought to provide a link between the metabolic state of a cell and turnover of proteins [1, 2].

In recent years considerable progress has been made in the characterization of the proteolytic system of mitochondria. Similar to the turnover of cytosolic proteins, turnover of mitochondrial proteins can occur via the lysosomal pathway by autophagy of the whole organelle. This process is predominant under starvation conditions and results in the nonselective removal of mitochondrial proteins [3]. On the other hand, observed differences in the turnover rates of mitochondrial proteins located in different compartments and of individual proteins in the same compartment have suggested rather early on the existence of proteases within mitochondria [4–6]. More recently, a series of proteases have been identified in the mitochondrial matrix space and in the inner membrane which mediate the selective degradation of mitochondrial proteins. In all cases proteolytic activity depends on ATP hydrolysis and on the formation of high molecular weight assemblies. These proteases will be the focus of the present review.

ATP-dependent proteolysis of matrix-localized proteins

Proteins of the mitochondrial matrix were observed to be degraded at distinct rates, pointing to the existence of an independent proteolytic system in the mitochondrial matrix space. While the bulk of mitochondrial proteins are stable for 3.5–5 days in mammalian cells, δ-aminolevinulate synthase has a half-life of only 20–80 min [5, 7]. At least two ATP-dependent proteases seem to exist in this mitochondrial compartment; they represent homologues of the ATP-dependent Lon- and Clp-like proteases of prokaryotes.

PIM1 protease

Biochemical fractionation of mammalian mitochondria led to the identification of an ATP-dependent proteolytic activity in the matrix whose functional properties closely resemble those of *Escherichia coli* Lon protease [8–11]. Like the prokaryotic protein, the mitochondrial homologue is a serine protease and forms a high molecular weight oligomer, presumably a hexamer [12]. Cloning of the corresponding genes in humans and yeast, termed *PIM1* for *p*roteolysis *i*n *m*itochondria, revealed sequence identity of the mitochondrial proteins with the *E. coli* Lon protease of 36 and 33%, respectively [13–15]. Monomers of the mitochondrial Lon-like proteases, however, have a considerably higher molecular mass. They carry mitochondrial matrix-targeting sequences at the amino-terminus. In addition, sequence alignment reveals the presence of an amino-terminal

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extension in mitochondrial proteases whose function is presently unclear.

The functional conservation of Lon-like proteases from prokaryotes to eukaryotes has recently been demonstrated by a complementation study [16]. A deficiency in PIM1 protease could be rescued by expressing *E. coli* Lon protease in yeast. Sorting of *E. coli* Lon protease to mitochondria in vivo was ensured by fusing a mitochondrial targeting sequence to the amino-terminus of the protease. *E. coli* Lon protease was, however, incapable of substituting for PIM1 protease when cells were grown at high temperature. This points to functional differences between the proteases. It will be interesting to relate these nonoverlapping activities to specific substrates or protein domains that are not conserved between the mitochondrial and prokaryotic homologues.

PIM1 protease fulfills essential functions during mitochondrial biogenesis. Yeast cells lacking the *PIM1* gene are respiratory-deficient and accumulate deletions in mitochondrial DNA [14, 15]. Complementation studies in yeast using mutant forms of *E. coli* Lon protease revealed that a proteolytically active Lon-like protease is required for the maintenance of mitochondrial genome integrity [16]. Thus, PIM1 protease might modulate the accumulation of a regulatory protein within mitochondria which negatively affects DNA stability. Alternatively, proteolytic activation of a mitochondrial protein through specific processing by PIM1 protease might be involved in preserving the integrity of the mitochondrial genome.

Like other Lon-like proteases, the proteolytic activity of PIM1 protease strictly depends on ATP hydrolysis [12]. PIM1 protease is involved in regulated mitochondrial protein turnover [15]. The β-subunit of the mitochondrial processing peptidase (β-MPP) and the β-subunit of the F_1-ATPase were rapidly degraded in intact cells after inhibition of cytosolic protein synthesis. Proteolysis requires ATP and is mediated by the PIM1 protease as demonstrated by mutational analysis [15]. Similarly, PIM1 protease promotes the degradation of misfolded polypeptides in the mitochondrial matrix space which otherwise aggregate [17]. The accumulation of electron dense particles within mitochondria was observed in the absence of PIM1 protease in intact cells, which most likely reflect aggregated mitochondrial substrate proteins of the protease [15].

Cooperation of the mitochondrial Hsp70 system with PIM1 protease

Degradation of misfolded proteins by PIM1 protease depends on the chaperone function of the mitochondrial Hsp70 system [17] (fig. 1). Mt-Hsp70 interacts with misfolded polypeptides in an ATP-dependent manner, thereby stabilizing them against aggregation. Substrate binding of mt-Hsp70 is modulated by two components, Mdj1p and Mge1p [18–21]. In mitochondria lacking Mdj1p, degradation of misfolded polypeptides is impaired. Mdj1p is required for efficient binding of polypeptides to mt-Hsp70 [17, 22]. In analogy with the homologous *E. coli* DnaJ protein, Mdj1p presumably exerts chaperone activity on its own and thereby might ensure efficient polypeptide binding to mt-Hsp70. Mdj1p is also crucial for the release of misfolded polypeptides from mt-Hsp70. In the absence of Mdj1p, polypeptides remain associated with mt-Hsp70 and are not degraded by PIM1 protease [17]. Thus, release of mt-Hsp70 is required for proteolysis by PIM1 protease. In addition to Mdj1p, ATP-dependent binding of mt-Hsp70 to unfolded polypeptide chains is regulated by Mge1p, which acts as a nucleotide exchange factor of mt-Hsp70 [19–21, 23]. It seems therefore likely, though has not yet been demonstrated, that Mge1p function is also required for degradation of misfolded polypeptides by PIM1 protease. Mt-Hsp70 fulfills multiple functions in mitochondrial biogenesis in addition to its role in regulated proteolysis [24, 25]. Most importantly, it promotes the vectorial translocation of nuclear encoded mitochondrial preproteins across the mitochondrial membranes and, in cooperation with Mdj1p and Mge1p, mediates their subsequent folding in the matrix space. This raises the intriguing question of how a misfolded polypeptide chain, bound to mt-Hsp70 and prone to degradation by PIM1 protease, is distinguished from a folding-competent polypeptide which is on a productive folding pathway. Two mechanisms can be envisioned which are not mutually exclusive: (1) Misfolded polypeptide chains associated with mt-Hsp70 might be specifically recognized by PIM1 protease and directly bound to the protease. In *E. coli*, DnaK and Lon protease were detected in complexes which contain a nonsecreted mutant form of alkaline phosphatase [26]. Chaperone specificity of PIM1-mediated degradation in mitochondria is suggested by the observation that PIM1 protease does not mediate the proteolytic breakdown of misfolded polypeptides stabilized against aggregation by another chaperone protein of the mitochondrial matrix, the ClpB-homologue Hsp78 [27]. On the other hand, evidence for a physical interaction of PIM1 protease with chaperone proteins is lacking so far. (2) The fate of a chaperone-associated polypeptide might be determined by kinetic partitioning (fig. 1). Irreversibly misfolded proteins which fail to attain their native conformation upon release from mt-Hsp70 might re-bind to mt-Hsp70, resulting in a prolonged interaction with the chaperone protein. This might allow binding and, after dissociation of the chaperone, efficient degradation by the PIM1 protease. Evidence for a prolonged interaction of mt-Hsp70 with irreversibly misfolded protein segments was obtained upon analysing a chimeric protein which consists of the amino-terminal

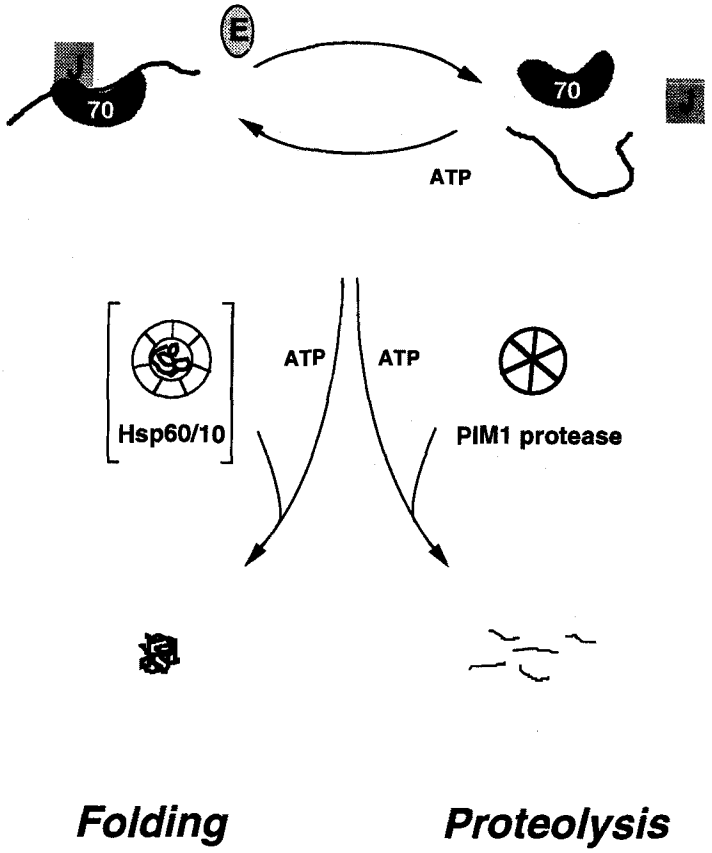

Figure 1. Role of the mt-Hsp70 system in folding and degradation of matrix-localized polypeptides. Newly imported mitochondrial preproteins are stabilized against aggregation by the mitochondrial chaperone proteins mt-Hsp70 and Mdj1p which cycle on and off the polypeptide. Folding to the native state occurs upon release from mt-Hsp70 and, depending on the preprotein, transfer to the mitochondrial Hsp60 system. Irreversibly misfolded polypeptides are degraded by PIM1 protease upon release from mt-Hsp70. 70 = mitochondrial Hsp70; J = Mdj1p; E = Mge1p; Hsp60/10 = Hsp60 and Hsp10.

portion of cytochrome b_2 fused to mouse dihydrofolate reductase (DHFR) [17]. This hybrid protein can be imported into isolated mitochondria. The process is mediated by mt-Hsp70, which binds to the cytochrome b_2 as well as the DHFR moiety of the fusion protein. After completion of membrane translocation in the mitochondrial matrix, mt-Hsp70 dissociates from the DHFR domain, which then folds into the native conformation. The amino-terminal cytochrome b_2 moiety, however, remains associated with mt-Hsp70 and is degraded by PIM1 protease in the presence of a folded DHFR domain.

Mitochondrial Clp protease

Clp proteases represent a second major class of ATP-dependent proteases in prokaryotes [2, 28]. They form cylinder-shaped, high molecular weight complexes [29] which are composed of multiple copies of two subunits: the serine protease ClpP and a regulatory subunit exerting ATPase and chaperone activity which apparently determines the substrate specificity of the protease [30–33]. Thus, cooperation of ATP-dependent proteases with molecular chaperone proteins may be a general principle of regulated protein degradation. Taking into consideration the endosymbiontic origin of mitochondria, the existence of homologous proteins involved in selective degradation of mitochondrial proteins appears likely. Indeed, a homologue of *E. coli* ClpP, whose amino-terminus displays characteristics of mitochondrial targeting sequences, has been identified in humans [34]. Its physiological function remains to be determined.

The identification of a homologue of the regulatory subunits of prokaryotic Clp proteases that exerts ATPase activity and interacts with human ClpP is still awaited. A candidate protein in yeast is Hsp78, which belongs to the Clp family of proteins and shares high amino acid sequence identity with *E. coli* ClpB [35]. Hsp78 has chaperone activity and can partially substitute for mt-Hsp70 in protein import and suppression of protein aggregation in the mitochondrial matrix space [27]. An obvious ClpP homologue is, however, not present in the yeast *Saccharomyces cerevisiae*, whose genome has been sequenced to completion. Moreover, cells lacking Hsp78 displayed no defects in proteolytic processes. Rather, Hsp78 is required for the protection of mitochondrial functions against heat damage [35a].

Regulated degradation of mitochondrial inner membrane proteins

Studies on the stability of mitochondrial translation products revealed initial evidence of proteolytic activity associated with the mitochondrial inner membrane [36, 37]. Eight proteins are encoded by mitochondrial DNA in yeast, seven of which are subunits of respiratory chain or ATP-synthase complexes and form integral parts of the mitochondrial inner membrane [38, 39]. In the absence of a supply of nuclear encoded subunits, nonassembled polypeptides are subject to rapid proteolysis. Biochemical characterization of this degradation process revealed a requirement for ATP and divalent metal ions, suggesting the involvement of an ATP-dependent metallopeptidase [40, 41].

Recently, two membrane-associated, ATP-dependent proteases have been identified within mitochondria, the so-called *m*- and *i*-AAA proteases, which mediate the degradation of nonassembled mitochondrial inner membrane proteins [42–45] (the prefixes *m* and *i* denote the presence of the active sites in the *m*atrix or *i*ntermembrane space, resectively). These proteases harbour a conserved ATPase domain of 230 amino acids which is characteristic of the AAA family of proteins (*A*TPases *a*ssociated with a variety of cellular *a*ctivities) [46, 47]. Members of this family exist in prokaryotic and eukaryotic cells and fulfill diverse cellular functions including organellar assembly, membrane fusion, membrane insertion of proteins and, as subunits of the 26S proteasome, protein degradation. AAA proteases comprise a distinct subfamily within the AAA family of proteins, as they exhibit two unique properties: (1) They are integral membrane proteins containing one or two membrane-spanning segments. (2) They contain a consensus binding motif for divalent metal ions, HEXXH, which is conserved between prokaryotic and eukaryotic AAA proteases and is characteristic of metalloproteases of the thermolysin family [48, 49]. Thus, AAA proteases define a fourth class of ATP-dependent proteases distinct from Lon- and Clp-like proteases in prokaryotes and mitochondria and from the eukaryotic 26S proteasome.

m-AAA protease

The mitochondrial *m*-AAA protease is composed of multiple copies of two highly homologous subunits, Yta10p (Afg3p) and Yta12p (Rca1p) [50]. These proteins constitute an 850-kDa complex in the mitochondrial inner membrane in a nucleotide-dependent manner. Components other than Yta10p and Yta12p were not detected in coimmunoprecipitation experiments after partial purification of the *m*-AAA protease. Yta10p and Yta12p are arranged in a similar fashion in the mitochondrial inner membrane [41, 50] with two transmembrane segments being present in both proteins. A small amino-terminal domain and a large carboxy-terminal domain containing the consensus binding sites for ATP and divalent metal ions face the mitochondrial matrix space. Both Yta10p and Yta12p exhibit ATPase and proteolytic activity [50, 51]. The integrity of the consensus binding motif, HEXXH, present in both subunits is essential for proteolysis, characterizing Yta10p and Yta12p as metallopeptidases. Protein degradation by the *m*-AAA protease requires ATP hydrolysis, though the integrity of the complex is preserved in the presence of nonhydrolysable ATP analogues [50].

The *m*-AAA protease mediates the degradation of newly synthesized, nonassembled mitochondrial translation products in the inner membrane [41, 50, 51]. In the absence of Yta10p or Yta12p, proteolysis is inhibited; this identifies the YTA10–12 complex as the active protease. Exchange of the active-site glutamate in the HEXXH binding motif of Yta10p or Yta12p by glutamine results in inhibition of the proteolytic breakdown of nonassembled inner membrane proteins despite the presence of an intact proteolytic center in the other subunit of the *m*-AAA protease [50]. Since the mutant subunits were observed to assemble into the YTA10–12 complex, the two subunits probably cooperate during proteolysis. On the other hand, analysis of a defined cleavage event mediated by the *m*-AAA proteases demonstrated that the subunits can act independently of each other, suggesting different substrate specificities of the Yta10p and Yta12p components [52]. The *m*-AAA protease can therefore be considered as a multicatalytic enzyme mediating the degradation of membrane proteins. In this regard, the *m*-AAA protease seems to be quite similar to the 26S proteasome in the eukaryotic cytosol.

Yta10p and Yta12p are essential for the maintenance of oxidative phosphorylation [43–45]. Yeast strains lacking either protein are incapable of growing on nonfermentable carbon sources. Surprisingly, respiratory competence of *Δyta10* and *Δyta12* mutant cells is restored upon expression of the proteolytically inactive subunit [50, 51]. This might be the consequence of a residual activity of the assembled, second subunit of the *m*-AAA protease. Alternatively, it could be that respiration does not depend on the proteolytic activity of the *m*-AAA protease, pointing to additional functions of the protease complex. Deletion of *YTA10* or *YTA12* results in a deficiency in mitochondrial respiratory complexes and ATP synthase, although synthesis of subunits of these complexes within the nucleus or mitochondria was not impaired [45, 53]. This has been taken as an indication of a role of Yta10p and Yta12p in assembly processes within the mitochondrial inner membrane. Analysis of the assembly of the membrane-embedded F_0 moiety of the ATP synthase indeed revealed a chaperone-like activity of the *m*-AAA protease

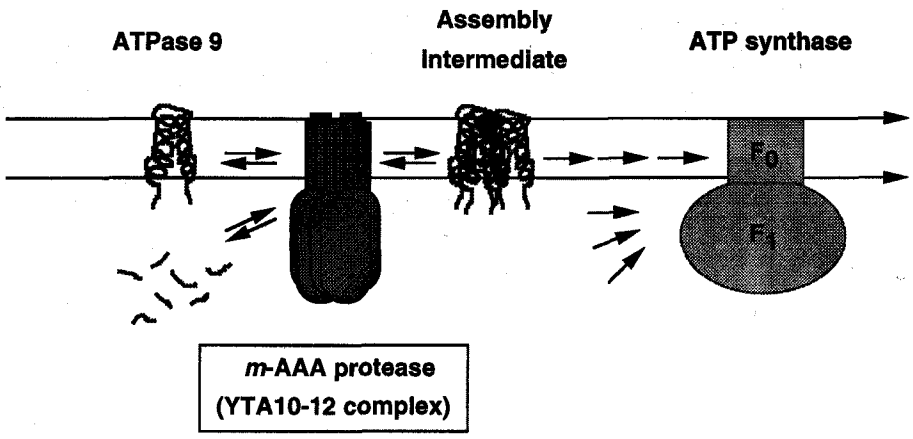

Figure 2. Proteolytic and chaperone-like activities of the *m*-AAA protease in the mitochondrial inner membrane. Newly synthesized ATPase9 forms an assembly intermediate of 48 kDa during the assembly of the F_0F_1-ATP synthase whose formation depends on the integrity of the YTA10–12 complex. Non-assembled subunits are degraded by the *m*-AAA protease in an ATP-dependent manner.

[50]. Assembly of subunit 9 of the ATP synthase (ATPase 9) proceeded normally in the presence of a proteolytically inactive subunit in the YTA10–12 complex but was impaired in the absence of Yta10p or Yta12p. Apparently, the *m*-AAA protease is required for ATP synthase assembly independent of its proteolytic activity. On the other hand, nonassembled ATPase 9 subunits were rapidly degraded, a process mediated by the *m*-AAA protease and strictly dependent on the proteolytic activity of both subunits [50]. Based on these observations, the proteolytic and chaperone-like activities of the *m*-AAA protease have been proposed to constitute a quality control system for the maintenance of functional protein complexes in the mitochondrial inner membrane (fig. 2).

i-AAA protease

The mitochondrial *i*-AAA protease, similar to the *m*-AAA protease, forms an 850-kDa complex in the mitochondrial inner membrane [52]. It is active in the mitochondrial intermembrane space in contrast to the *m*-AAA protease. Yme1p [42] (also termed Yta11p [54] and Osd1p [55]) is the sole subunit of the *i*-AAA protease identified so far in yeast, but the existence of other subunits has not been excluded. Yme1p is arranged differently in the inner membrane as compared to Yta10p and Yta12p, although these three AAA protease subunits show high sequence similarity. It exposes a large carboxy-terminal domain containing the binding sites for ATP and divalent metal ions to the mitochondrial intermembrane space, and is anchored within the membrane by only one membrane-spanning segment [52].

First evidence for a proteolytic activity of Yme1p resulted from studies on the stability of the subunit II of cytochrome oxidase (COXIIp). Rapid proteolysis of COXIIp was observed in strains which do not assemble the cytochrome oxidase complex or lack cytochrome c [56–58]. Deletion of *YME1* results in the stabilization of nonassembled COXIIp, demonstrating the requirement of Yme1p for proteolysis [56–58]. Yme1p was characterized as an ATP-dependent metallopeptidase by mutational analysis [52, 58]. Mutations in the consensus binding sites for ATP or divalent metal ions abolish proteolytic activity of Yme1p and result in the stabilization of nonassembled COXIIp.

Several distinct phenotypes have been observed upon inactivation of Yme1p, suggesting important functions for the *i*-AAA protease during mitochondrial biogenesis [42, 59]. Yme1p was originally identified in a genetic screen as a mutant having an increased rate of DNA escape from mitochondria to the nucleus [60]. Yeast cells lacking *YME1* or expressing a proteolytically inactive mutant form of Yme1p are respiratory-deficient at high temperature, exhibit a cold-sensitive growth defect on complete glucose media and grow only very poorly when mitochondrial DNA is deleted or lost entirely. In addition, accumulation of punctate and grossly swollen mitochondria was observed in the absence of Yme1p, suggesting a role for Yme1p in the maintenance of mitochondrial morphology [59]. The molecular basis of the various phenotypes caused by inactivation of Yme1p is presently unclear. Yme1p seems to act mainly as a metallopeptidase in mitochondria, as disturbance of the proteolytic activity of Yme1p by site-directed mutagenesis causes similar phenotypes as a deletion of the entire *YME1* gene [58]. Most likely, multiple proteolytic substrates of Yme1p exist in mitochondria, reflecting the various defects associated with a *yme1*

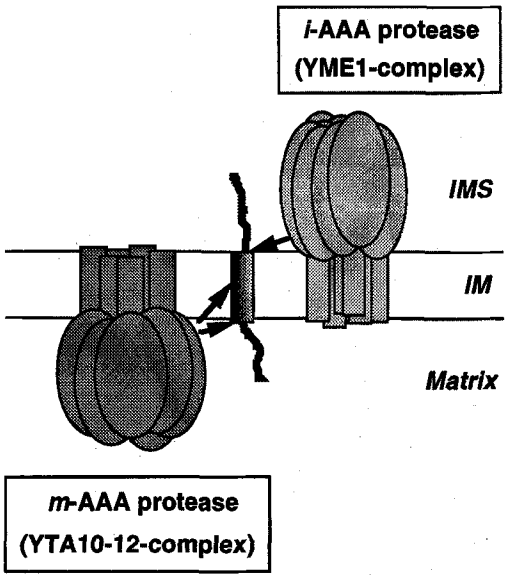

Figure 3. Topology of the *m*-AAA protease and *i*-AAA protease in the mitochondrial inner membrane. The two proteases constitute a proteolytic system in the mitochondrial inner membrane which mediates the degradation of inner membrane proteins. The arrows indicate the estimated position of cleavage sites in a nonassembled model polypeptide containing one membrane-spanning segment [52]. IMS = mitochondrial intermembrane space; IM = mitochondrial inner membrane.

mutation. Each of the various phenotypes can apparently be suppressed individually. For example, mutations in the mitochondrial ATP synthase γ-subunit suppress the slow growth phenotype of *yme1* mutants lacking mitochondrial DNA [61]. On the other hand, the cold-sensitive phenotype of *Δyme1* mutant cells can be suppressed by mutation in *YME2*, another mitochondrial inner membrane protein, structurally unrelated to Yme1p [62]. Thus, analysis of the complex phenotype associated with a *yme1* mutation might provide interesting clues as to the role of Yme1p during mitochondrial biogenesis.

Cooperation of *m*- and *i*-AAA protease in the degradation of inner membrane proteins

A hallmark of the AAA proteolytic system in the inner membrane is the membrane arrangement of the proteases. The topology of the *m*- and *i*-AAA proteases in the inner membrane suggests degradation of hydrophilic segments of integral membrane proteins on either side of the membrane. Indeed, cleavage of a model polypeptide by the *m*- and *i*-AAA proteases on opposite membrane surfaces has recently been reported, providing an important clue as to how inner membrane proteins are degraded [52] (fig. 3). Interestingly, the model polypeptide was also cleaved by the *m*-AAA protease within its membrane-spanning domain. It is presently unclear how the transmembrane region of a protein is transferred from the hydrophobic bilayer into a hydrophilic, proteinaceous environment, allowing its degradation. However, in view of its chaperone-like activity, a direct role of the *m*-AAA protease in this process is an intriguing possibility.

Perspectives

Insights into regulated protein degradation within mitochondria have grown considerably over recent years. ATP-dependent proteases have been identified in the mitochondrial matrix and in the inner membrane. Information on their role during the biogenesis of mitochondria has been obtained. Still very little is known about substrate proteins of the various proteases. The characterization of these substrate proteins will unravel specific functions of ATP-dependent proteases in mitochondrial homeostasis and in the clearing of nonfunctional polypeptides. Analysis of regulated proteolytic processes in mitochondria will also provide answers to questions of more general relevance, e.g. concerning the mechanisms of how membrane proteins are degraded in comparison to soluble proteins, or the functional interplay between molecular chaperone proteins and proteases.

1 Goldberg A. L. and Rock K. L. (1992) Proteolysis, proteasomes and antigen presentation. Nature **357:** 375–379
2 Gottesman S. and Maurizi M. R. (1992) Regulation by proteolysis: energy-dependent proteases and their targets. Microbiol. Rev. **56:** 592–621
3 Takeshige K., Baba M., Tsuboi S., Noda T. and Ohsumi Y. (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for induction. J. Cell Biol. **119:** 301–311
4 Walker J. H., Burgess R. L. and Mayer R. J. (1978) Relative rate of turnover of subunits of mitochondrial proteins. Biochem. J. **176:** 927–932
5 Russel S. M., Burgess R. J. and Mayer R. J. (1980) Protein degradation in rat liver during post-natal development. Biochem. J. **192:** 321–330
6 Lipsky N. G. and Pedersen P. L. (1981) Protein turnover in animal cells: half-lives of mitochondria and mitochondrial subfractions of rat liver based on [^{14}C] bicarbonate incorporation. J. Biol. Chem. **256:** 8652–8657
7 Dice J. F. and Goldberg A. L. (1975) Relationship between in vivo degradative rates and isoelectric points of proteins. Proc. Natl. Acad. Sci. USA **72:** 3893–3897
8 Desautels M. and Goldberg A. L. (1982) Demonstration of an ATP-dependent, vanadate-sensitive endoprotease in the matrix of rat liver mitochondria. J. Biol. Chem. **257:** 11673–11679
9 Desautels M. and Goldberg A. L. (1982) Liver mitochondria contain an ATP-dependent, vanadate-sensitive pathway for the degradation of proteins. Proc. Natl. Acad. Sci. USA **79:** 1869–1873
10 Watabe S. and Kimura T. (1985) Adrenal cortex mitochondrial enzyme with ATP-dependent protease and protein-dependent ATPase activities. J. Biol. Chem. **260:** 14498–14504
11 Watabe S. and Kimura T. (1985) ATP-dependent protease in bovine adrenal cortex. J. Biol. Chem. **260:** 5511–5517
12 Kutejová E., Durcová G., Surovková E. and Kuzela S. (1993) Yeast mitochondrial ATP-dependent protease: purification and comparison with the homologous rat enzyme and the bacterial ATP-dependent protease La. FEBS Lett. **329:** 47–50
13 Wang N., Gottesman S., Willingham M. C., Gottesman M. M. and Maurizi M. R. (1993) A human mitochondrial ATP-dependent protease that is highly homologous to bacterial Lon protease. Proc. Natl. Acad. Sci. USA **90:** 11247–11251

14 Van Dyck L., Pearce D. A. and Sherman F. (1994) PIM1 encodes a mitochondrial ATP-dependent protease that is required for mitochondrial function in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **269:** 238–242
15 Suzuki C. K., Suda K., Wang N. and Schatz, G. (1994) Requirement for the yeast gene LON in intramitochondrial proteolysis and maintenance of respiration. Science **264:** 273–276
16 Teichmann U., van Dyck L., Guiard B., Fischer H., Glockshuber R., Neupert W. et al. (1996) Substitution of PIM1 protease in mitochondria by *Escherichia coli* Lon protease. J. Biol. Chem. **271:** 10137–10142
17 Wagner I., Arlt H., van Dyck L., Langer T. and Neupert W. (1994) Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. EMBO J. **13:** 5135–5145
18 Rowley N., Prip B. C., Westermann B., Brown C., Schwarz E., Barrell B. et al. (1994) Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding. Cell **77:** 249–259
19 Bolliger L., Deloche O., Glick B. S., Georgopoulos C., Jeno P., Kronidou N. et al. (1994) A mitochondrial homolog of bacterial GrpE interacts with mitochondrial hsp70 and is essential for viability. EMBO J. **13:** 1998–2006
20 Laloraya S., Dekker P. J. T., Voos W., Craig E. A. and Pfanner N. (1995) Mitochondrial GrpE modulates the function of matrix Hsp70 in translocation and maturation of preproteins. Mol. Cell. Biol. **15:** 7098–7105
21 Westermann B., Prip-Buus C., Neupert W. and Schwarz E. (1995) The role of the GrpE homologue, Mge1p, in mediating protein import and protein folding in mitochondria. EMBO J. **14:** 3452–3460
22 Prip-Buus C., Westermann B., Schmitt M., Langer T., Neupert W. and Schwarz E. (1996) Role of the mitochondrial DnaJ homologue, Mdj1p, in the prevention of heat-induced protein aggregation. FEBS Lett. **380:** 142–146
23 Ikeda E., Yoshida S., Mitsuzawa H., Uno I. and Toh-e, A. (1994) YGE1 is a yeast homolog of *Escherichia coli* grpE and is required for maintenance of mitochondrial functions. FEBS Lett. **339:** 265–268
24 Stuart R. A., Cyr D. M., Craig E. A. and Neupert W. (1994) Mitochondrial molecular chaperones: their role in protein translocation. Trends Biochem. Sci. **19:** 87–92
25 Langer T. and Neupert W. (1994) Chaperoning mitochondrial biogenesis. In: The Biology of Heat Shock Proteins and Molecular Chaperones, pp. 53–83, Morimoto R., Tissières A. and Georgopoulos C. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor
26 Sherman M. and Goldberg A. L. (1992) Involvement of the chaperonin dnaK in the rapid degradation of a mutant protein in *Escherichia coli*. EMBO J. **11:** 71–77
27 Schmitt M., Neupert W. and Langer T. (1995) Hsp78, a Clp homologue within mitochondria, can substitute for chaperone functions of mt-hsp70. EMBO J. **14:** 3434–3444
28 Horwich A. L. (1995) Resurrection or destruction? Curr. Biol. **5:** 455–458
29 Kessel M., Maurizi M. R., Kim B., Kocsis E., Trus B. L., Singh S. K. et al. (1995) Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26 S proteasome. J. Mol. Biol. **250:** 587–594
30 Gottesman S., Clark W. P., de Crecy-Lagard V. and Maurizi M. R. (1993) ClpX, an alternative subunit for the ATP-dependent Clp protease of *Escherichia coli*: sequence and in vivo activities. J. Biol. Chem. **268:** 22618–22626
31 Wojtkowiak D., Georgopoulos C. and Zylicz M. (1993) Isolation and characterization of ClpX, a new ATP-dependent specificity component of the Clp protease of *Escherichia coli*. J. Biol. Chem. **268:** 22609–22617
32 Wickner S., Gottesman S., Skowyra D., Hoskins J., McKenney K. and Maurizi M. R. (1994) A molecular chaperone, ClpA, functions like DnaK and DnaJ. Proc. Natl. Acad. Sci. USA **91:** 12218–12222
33 Wawrzynow A., Wojtkowiak D., Marszalek J., Banecki B., Jonsen M., Graves B. et al. (1995) The ClpX heat-shock protein of *Escherichia coli*, the ATP-dependent substrate specificity component of the ClpP-ClpX protease, is a novel molecular chaperone. EMBO J. **14:** 1867–1877
34 Bross P., Andresen B. S., Knudsen I., Kruse T. A. and Gregersen, N. (1995) Human ClpP protease: cDNA sequence, tissue-specific expression and chromosomal assignment of the gene. FEBS Lett. **377:** 249–252
35 Leonhardt S. A., Fearon K., Danese P. N. and Mason T. L. (1993) Hsp78 encodes a yeast mitochondrial heat shock protein in the Clp family of ATP-dependent proteases. Mol. Cell. Biol. **13:** 6304–6313.
35a Schmitt M., Neupert W. and Langer T. (1996) The molecular chaperone Hsp78 confers compartment-specific thermotolerance to mitochondria. J. Cell Biol. **134:** 1375–1386
36 Wheeldon L. W., Dianoux A. C., Bof M. and Vignais P. V. (1974) Stable and labile products of mitochondrial protein synthesis in vitro. Eur. J. Biochem. **46:** 189–199
37 Kalnov S. L., Novikova L. A., Zubatov A. S. and Luzikov V. N. (1979) Proteolysis of the products of mitochondrial protein synthesis in yeast mitochondria and submitochondrial particles. Biochem. J. **182:** 195–202
38 Tzagoloff A. and Myers A. M. (1986) Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55:** 249–285
39 Grivell L. A. (1989) Nucleo-mitochondrial interactions in yeast mitochondrial biogenesis. Eur. J. Biochem. **182:** 477–493
40 Yasuhara T., Mera Y., Nakai T., and Ohashi A. (1994) ATP-dependent proteolysis in yeast mitochondria. J. Biochem. **115:** 1166–1171
41 Pajic A., Tauer R., Feldmann H., Neupert W., and Langer T. (1994) Yta10p is required for the ATP-dependent degradation of polypeptides in the inner membrane of mitochondria. FEBS Lett. **353:** 201–206
42 Thorsness P. E., White K. H. and Fox T. D. (1993) Inactivation of *YME1*, a member of the ftsH-SEC18–PAS1–CDC48 family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 5418–5426
43 Guélin E., Rep M. and Grivell L. A. (1994) Sequence of the AFG3 gene encoding a new member of the FtsH/Yme1/Tma subfamily of the AAA-protein family. Yeast **10:** 1389–1394
44 Tauer R., Mannhaupt G., Schnall R., Pajic A., Langer T. and Feldmann H. (1994) Yta10p, a member of a novel ATPase family in yeast, is essential for mitochondrial function. FEBS Lett. **353:** 197–200
45 Tzagoloff A., Yue J., Jang J. and Paul M. F. (1994) A new member of a family of ATPases is essential for assembly of mitochondrial respiratory chain and ATP synthetase complexes in *Saccharomyces cerevisiae*. J. Biol. Chem. **269:** 26144–26151
46 Kunau W. H., Beyer A., Franken T., Gotte K., Marzioch M., Saidowsky J. et al. (1993) Two complementary approaches to study peroxisome biogenesis in *Saccharomyces cerevisiae*: forward and reversed genetics. Biochimie **75:** 209–224
47 Confalonieri F. and Duguet M. (1995) A 200-amino acid ATPase module in search of a basic function. BioEssays **17:** 639–650
48 Rawlings N. D. and Barrett A. J. (1993) Evolutionary families of peptidases. Biochem. J. **290:** 205–218
49 Stöcker W. and Bode W. (1995) Structural features of a superfamily of zinc-endopeptidases: the metzincins. Curr. Op. Struct. Biol. **5:** 383–390.
50 Arlt H., Tauer R., Feldmann H., Neupert W. and Langer T. (1996) The YTA10–12-complex, an AAA protease with chaperone-like activity in the inner membrane of mitochondria. Cell **85:** 875–885
51 Guélin E., Rep M. and Grivell L. A. (1996) Afg3p, a mitochondrial ATP-dependent metalloprotease, is involved in the degradation of mitochondrially-encoded Cox1, Cox3, Cob, Su6, Su8 and Su9 subunits of the inner membrane complexes III, IV and V. FEBS Lett. **381:** 42–46
52 Leonhard K., Herrmann J. M., Stuart R. A., Mannhaupt G., Neupert W. and Langer T. (1996) AAA proteases with catalytic sites on opposite membrane surfaces comprise a proteolytic system for the ATP-dependent degradation of inner membrane proteins in mitochondria. EMBO J. **15:** 4218–4229

53 Paul M. F. and Tzagoloff A. (1995) Mutations in *RCA1* and *AFG3* inhibit F_1-ATPase assembly in *Saccharomyces cerevisiae*. FEBS Lett. **373:** 66–70

54 Schnall R., Mannhaupt G., Stucka R., Tauer R., Ehnle S., Schwarzlose C. et al. (1994) Identification of a set of yeast genes coding for a novel family of putative ATPases with high similarity to constituents of the 26S protease complex. Yeast **10:** 1141–1155

55 Nakai T., Yasuhara T., Fujiki Y. and Ohashi A. (1995) Multiple genes, including a member of the AAA family, are essential for degradation of unassembled subunit 2 of cytochrome *c* oxidase in yeast mitochondria. Mol. Cell. Biol. **15:** 4441–4452

56 Nakai T., Mera Y., Yasuhara T. and Ohashi A. (1994) Divalent metal ion-dependent mitochondrial degradation of unassembled subunits 2 and 3 of cytochrome c oxidase. J. Biochem. **116:** 752–758

57 Pearce D. A. and Sherman F. (1995) Degradation of cytochrome oxidase subunits in mutants of yeast lacking cytochrome c and suppression of the degradation by mutation of *yme1*. J. Biol. Chem. **270:** 1–4

58 Weber E. R., Hanekamp T. and Thorsness P. E. (1996) Biochemical and functional analysis of the *YME1* gene product, an ATP and zinc-dependent mitochondrial protease from *S. cerevisiae*. Mol. Biol. Cell **7:** 307–317.

59 Campbell C. L., Tanaka N., White K. H. and Thorsness, P. E. (1994) Mitochondrial morphological and functional defects in yeast caused by *yme1* are suppressed by mutation of a 26S protease subunit homologue. Mol. Biol. Cell. **5:** 899–905

60 Thorsness P. E. and Fox T. D. (1993) Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. Genetics **134:** 21–28

61 Weber E. R., Rooks R. S., Shafer K. S., Chase J. W. and Thorsness P. E. (1995) Mutations in the mitochondrial ATP synthase gamma subunit suppress a slow-growth phenotype of *yme1* yeast lacking mitochondrial DNA. Genetics **140:** 435–442

62 Hanekamp T. and Thorsness P. E. (1996) Inactivation of *YME2/RNA12*, which encodes an integral inner mitochondrial membrane protein, causes increased escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2764–2771