



**UvA-DARE (Digital Academic Repository)**

**The role of protein degradation in mitochondrial function and biogenesis**

Rep, M.; Grivell, L.A.

*Published in:*  
Current Genetics

[Link to publication](#)

*Citation for published version (APA):*

Rep, M., & Grivell, L. A. (1996). The role of protein degradation in mitochondrial function and biogenesis. *Current Genetics*, 30, 267-380.

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <http://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

## REVIEW

M. Rep · L. A. Grivell

**The role of protein degradation in mitochondrial function and biogenesis**

Received: 14 May 1996

**Abstract** It has been known for a long time that mitochondria contain their own protein-degradation systems. Only recently, however, have genes for mitochondrial proteases been identified and the powerful techniques of molecular biology been applied to gain insight into the role of protein degradation in mitochondrial biogenesis. It is now clear that the mitochondrial proteases that are involved in the initial stages of degradation are similar to prokaryotic ATP-dependent proteases, and that a division of labour exists between soluble and membrane-bound systems. These systems are essential for the biogenesis of fully functional mitochondria. Their natural targets are currently being identified, and their co-operation with chaperones and possible dual functions as chaperones/proteases are being investigated.

**Key words** Mitochondrial proteases · Protein turnover · Proteolysis · Chaperones

**Introduction**

Biogenesis of mitochondria has been intensively studied for more than two decades. In particular, the mitochondrial system for gene expression, and the co-ordination of this system with the nucleo-cytoplasmic one, have received much attention (see Grivell 1995 for a review). While the bulk of mitochondrial research has concentrated on the synthesis of mitochondrial components, it has not gone unnoticed that protein turnover and degradation are also an important part of mitochondrial biogenesis (Luzikov 1985; Desautels 1986). In general, protein degradation may serve

several distinct purposes: (1) protective, by removing polypeptides that are potentially harmful to the cell, (2) regulatory, by controlling the concentrations of enzymes or regulatory proteins, and (3) metabolic, by releasing amino acids to be used for other purposes. Like other supermolecular structures, the degradation of mitochondrial fragments is mediated by lysosomal or, in the case of yeast, vacuolar proteases via the process of autophagocytosis (Takehige et al. 1992). This process falls into the third category. For the first two functions, however, mitochondrial proteases are responsible (Desautels 1986). This review summarizes what is known to-date on protein degradation in mitochondria, the proteases involved, and the significance of protein degradation for mitochondrial biogenesis.

**Targets for turnover**

It is well established that half-lives are widely divergent among proteins of every mitochondrial compartment (Desautels 1986; Hare 1990). A major class of targets for proteolysis are subunits of enzymes that carry out electron transport and oxidative phosphorylation. These enzymes constitute about 50% of the protein content of the mitochondrial inner membrane (Hatefi 1985) and, generally speaking, each consists of a combination of subunits that are encoded by either the mitochondrial or the nuclear genome. The regulation of the rate of subunit synthesis through transcriptional, and perhaps translational, control may assure the production of roughly comparable amounts of subunits belonging to a particular complex; but it is clear that this is not (and perhaps cannot be) achieved with high precision, especially since two different genetic systems are involved. Turnover of subunits is therefore necessary to prevent accumulation of single subunits and sub-complexes in the mitochondrial inner membrane, which may disturb assembly processes or change the properties of the inner membrane (Manoil and Traxler 1995). Indeed, many subunits of mitochondrial inner membrane enzyme complexes are quickly degraded when assembled into a com-

M. Rep · L. A. Grivell (✉)  
Section for Molecular Biology,  
Department of Molecular Cell Biology,  
University of Amsterdam,  
Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Communicated by H. T. Jacobs

**Table 1** Sensitivity towards degradation of unassembled subunits of mitochondrial inner membrane complexes<sup>a</sup>. Hs: *Homo sapiens*; Nc: *Neurospora crassa* (all other data were obtained with *Saccha-*

*romyces cerevisiae*); FP: flavoprotein; IP: iron-sulfur protein; FeS: Rieske iron-sulfur protein; Cob: cytochrome *b*; Cyt<sub>c</sub><sub>1</sub>: cytochrome *c*<sub>1</sub>

Subunit	Lower levels due to loss or mutation of:	References
<b>Complex I</b>		
All mitochondrially encoded subunits (Hs)	ND4	Hofhaus and Attardi 1993
21 and 21.3 of peripheral arm (Nc)	12.3 and 29.9 of the peripheral arm 51 of the peripheral arm	Duarte et al. 1995 Fecke et al. 1994
<b>Complex II</b>		
FP	IP	Sagbini et al. 1994
<b>Complex III</b>		
Core I	<i>Cob</i>	Sen and Beattie 1985; Chevillotte-Brivet et al. 1987
Core II	<i>Cob</i>	Sen and Beattie 1985
<i>Cob</i> *	Core I Core I, core II, Qcr7p, Qcr8p	Gatti and Tzagoloff 1990 De Haan et al. 1984; Berden et al. 1988; Crivellone et al. 1988; Schoppink et al. 1989
Cyt <sub>c</sub> <sub>1</sub>	Cbp3p**, Cbp4p**	Wu and Tzagoloff 1989; Crivellone 1994
FeS	Qcr6p and <i>Cob</i> (simultaneously) All complex III subunits except Qcr6p and Qcr10p	Schoppink et al. 1988 De Haan et al. 1984; Sen and Beattie 1985; Berden et al. 1988; Crivellone et al. 1988; Schoppink et al. 1989
Qcr7p	Bcs1p** Core I, core II, Qcr7p, <i>Cob</i>	Nobrega et al. 1992 De Haan et al. 1984; Berden et al. 1988; Crivellone et al. 1988; Schoppink et al. 1989
Qcr8p	Qcr6p Cbp3p**, Cbp4p** Core I, core II, Qcr8p, <i>Cob</i>	Yang and Trumppower 1994 Wu and Tzagoloff 1989; Crivellone 1994 De Haan et al. 1984; Berden et al. 1988; Crivellone et al. 1988; Schoppink et al. 1989
Qcr9p	Cbp3p**, Cbp4p** Qcr6p	Wu and Tzagoloff 1989; Crivellone 1994 Yang and Trumppower 1994
<b>Complex IV</b>		
CoxI*	CoxIV, CoxVa, CoxIX CoxVII <sup>b</sup> Sco1p**	McEwen et al. 1986 Calder and McEwen 1991 Krummeck and Rödel 1990
CoxII*	Cytochrome <i>c</i> ** CoxI, CoxIV, CoxVa, CoxIX Sco1p**	Pearce and Sherman 1995 a, b Dowhan et al. 1985; McEwen et al. 1986; Nakai et al. 1994 Schulze und Rödel 1989; Krummeck and Rödel 1990
CoxIII*	Cytochrome <i>c</i> ** CoxIV Sco1p**	Pearce and Sherman 1995 a Dowhan et al. 1985; Nakai et al. 1994 Krummeck and Rödel 1990
CoxIV	Cytochrome <i>c</i> **	Pearce and Sherman 1995 a
CoxV	CoxI, CoxII, Sco1p** CoxI CoxII CoxIV	Krummeck and Rödel 1990 McEwen et al. 1986; Krummeck and Rödel 1990 Krummeck and Rödel 1990 Dowhan et al. 1985
CoxVI	Cytochrome <i>c</i> **	Pearce and Sherman 1995 a
CoxVII	CoxI, CoxII CoxIX	Krummeck and Rödel 1990 McEwen et al. 1986
CoxVIII	CoxI, CoxII CoxIX	Krummeck and Rödel 1990 McEwen et al. 1986
<b>Complex V</b>		
Atp3p (γ)	Atp14p (δ) and Atp6,8,9p (simultaneously) Atp11p**, Atp12p**	Giraud and Velours 1994 Weber et al. 1996
Atp6p*	Atp4p (b) Atp7p (d)	Paul et al. 1989 Norais et al. 1991
Atp7p (d)	Atp8p, Atp9p Atp4p (b)	Jean-Francois et al. 1986 a Norais et al. 1994

<sup>a</sup> In most cases, sensitivity to degradation is inferred from low steady state amounts (see text). Data on the degradation of mitochondrial translation products caused by a general lack of cytoplasmically synthesized subunits (due to translation *in organello* or cycloheximide treatment of cells) are not included in this table

<sup>b</sup> The low level of CoxI in a strain disrupted for *COX7* could be due either to reduced synthesis or very fast (co-translational) turnover

\* Mitochondrially encoded subunits

\*\* Proteins not part of the complex

plex is made impossible by the lack of (an)other subunit(s) or of assembly-assisting proteins (Table 1, see also Hare 1990). Proteolytic systems responsible for the turnover of subunits in such artificial situations are likely to be also involved in maintaining the stoichiometry of subunits and the removal of non-functional proteins under natural conditions.

In addition to unassembled but otherwise normal membrane proteins, other polypeptides may threaten the integrity of the membrane. These include: (1) hydrophobic pre-sequences, produced by proteolytic processing of cytochrome *b*<sub>2</sub>, cytochrome *c*<sub>1</sub> and cytochrome *c* peroxidase (Kaput et al. 1982; Nunnari et al. 1993), (2) denatured matrix proteins that partition to the membrane due to the exposure of hydrophobic amino-acid sequences, as suggested for bacteria (Voellmy and Goldberg 1981), and (3) membrane proteins that have suffered oxygen damage. Substantial research has shown that, in diverse cell types, proteins damaged by oxygen radicals become sensitive to degradation (Davies et al. 1987; Pacifici and Davies 1990). Recently, membrane-bound proteases were shown to preferentially degrade oxidatively damaged erythrocyte membrane proteins. Inhibition studies point to the involvement of both serine- and metallo-proteases (Beppu et al. 1994). In rat liver mitochondria, conditions of enhanced radical flux indeed increased proteolysis by 20% (Dean and Pollak 1985). However, no such effect was seen in yeast mitochondria (Yasuhara et al. 1994).

The turnover of matrix proteins has been studied mainly in mammalian mitochondria. These studies have shown that half-lives vary among proteins of this class (Nicoletti et al. 1977; Hare and Hodges 1982b). Several studies indicate that a high turnover rate of key enzymes in biosynthesis pathways may facilitate the control of mitochondrial function. Cholesterol side-chain desmolase and p450, two enzymes involved in steroid biosynthesis in adrenal mitochondria and whose levels are regulated by ACTH (adrenocorticotrophic hormone), have relatively short half-lives (Kimura 1969; Purvis et al. 1973). In the case of p450, ACTH may inhibit the rate of its degradation (Purvis et al. 1973). Other inducible mitochondrial enzymes that are characterized by high turnover rates are  $\delta$ -aminolevulinic acid synthetase, a key enzyme for heme synthesis (Marver et al. 1966), and alanine- and ornithine-aminotransferase (Swick et al. 1968).

### Degradation of mitochondrial translation products

Much of the information on mitochondrial protein degradation has come from studies of the turnover of mitochondrial translation products. This section summarizes the main results of these studies. It should be remembered, however, that most of these proteins are highly hydrophobic. The mechanism(s) of turnover may therefore not be applicable to mitochondrial proteins in general.

The degradation of mitochondrial proteins synthesized within the organelle can be conveniently analysed either by

blocking cytoplasmic translation with cycloheximide or by studying protein synthesis and turnover in isolated mitochondria. A high turnover of mitochondrial translation products (half-life approximately 60 min) in isolated yeast mitochondria was observed for the first time by Bakalkin et al. (1978). In 1979, Kalnov et al. reported that one-third to one-half of the proteins synthesized in isolated yeast mitochondria were degraded with a half-life of about 35 min (Kalnov et al. 1979b). This degradation occurred more efficiently in mitochondria isolated from cells growing in logarithmic phase than in stationary phase (Kalnov et al. 1979a) and is inhibited by the addition of glucose to stationary cells (Luzikov et al. 1983). These differences in the rate of proteolysis under different growth conditions were suggested to be caused by changes in the fluidity of the inner membrane (Luzikov et al. 1983, 1984, see also Luzikov 1986). Black-Schaefer et al. (1991) also observed two pools with different rates of turnover, one with a half-life of minutes, the other with a half-life of hours. Instability of (a subset of) mitochondrial translation products has also been observed in rat liver mitochondria (Wheeldon et al. 1974; Desautels and Goldberg 1982b), as well as in HeLa cells and rat hepatoma cells in the presence of cycloheximide (Constantino and Attardi 1977; Hall and Hare 1990). As assembly of mitochondrially encoded subunits of inner membrane complexes depends on the presence of imported subunits, and these imported subunits will become limiting at some point in isolated mitochondria or when cytoplasmic translation is inhibited, the rapidly degraded mitochondrial translation products probably represent unassembled subunits.

### Incomplete translation products

Incomplete mitochondrial translation products produced by carrying out translation in the presence of puromycin are rapidly degraded in yeast (Kalnov et al. 1979b; Pajic et al. 1994) and rat liver mitochondria (Desautels and Goldberg 1982b). In rat heart mitochondria, degradation of 50–60% of newly made polypeptides was observed in the absence of the membrane potential. Most of the proteins subject to the observed degradation were of abnormal size, and were presumed to result from premature chain-termination (Cote et al. 1990). C-terminal truncation as a result of nonsense mutations can also result in instability. This has been reported for cytochrome *b* (di Rago et al. 1993) and implied for CoxII and CoxIII by the observation that mutations in the *COX2* and *COX3* genes (of which most are nonsense mutations) in the majority of cases result in the complete absence of the corresponding translation product (Weiss-Brummer et al. 1979; Baranowska et al. 1983). In several cases, however, truncated translation products of variable lengths were detected. Apparently, the recognition of truncated CoxII and CoxIII proteins by a proteolytic system depends on the exact C-terminus, which may influence the degree of folding and/or the accessibility of 'sensitive' sequences.

## Characterization of the proteolytic system

In all systems investigated thus far, degradation of mitochondrial translation products is dependent on ATP. Recently, several groups have reported that this process is also dependent on divalent metal-ions (Nakai et al. 1994; Pajic et al. 1994; Yasuhara et al. 1994). These reports differ, however, as to the ability of  $Zn^{2+}$  to restore proteolysis after treatment with the divalent metal-ion chelator o-phenantroline. Whereas Yasuhara et al. (1994) found inhibition by  $Zn^{2+}$ , Nakai et al. (1994) reported stimulation by the same ion. Both groups found reversal of inhibition by  $Mn^{2+}$  and  $Co^{2+}$ . Looking at the degradation of incomplete mitochondrial translation products induced by puromycin, Pajic et al. (1994) also report stimulation by  $Zn^{2+}$ , although  $Co^{2+}$ ,  $Fe^{2+}$  and  $Mn^{2+}$  yield higher activities. Interestingly, only  $Co^{2+}$ ,  $Fe^{2+}$  and  $Mn^{2+}$  can replace  $Zn^{2+}$  in the metalloprotease thermolysin to yield an active enzyme ( $Mn^{2+}$  restores only 10% of the activity), while a high amount of  $Zn^{2+}$  inhibits thermolysin activity (Holland et al. 1995). High concentrations of  $Zn^{2+}$  also inhibit mitochondrial-processing peptidases (Kalousek et al. 1992). A similar inhibitory effect of  $Zn^{2+}$  on other mitochondrial metalloproteases may explain the ambiguity concerning the effect of  $Zn^{2+}$  on mitochondrial protein degradation.

In addition to metal chelators, vanadate (an inhibitor of various ATPases) inhibits the degradation of mitochondrial translation products (Desautels and Goldberg 1982b). The same holds for chloramphenicol, an inhibitor of mitochondrial translation (Wheeldon et al. 1974; Kalnov et al. 1979b; Black-Schaefer et al. 1991), and hemin (Yasuhara et al. 1994). It is unclear at present why chloramphenicol should inhibit degradation, as other inhibitors of translation do not have this effect (Langer et al. 1995). Inhibition by hemin may reflect a positive regulation of heme on the production of respiratory chain components. However, such an interpretation is weakened by the fact that hemin also inhibits proteases from other sources (Tanaka et al. 1983; Waxman et al. 1985).

The inhibition by the protease inhibitors phenylmethylsulphonyl fluoride (PMSF), leupeptin, antipain and chymostatin as shown by Kalnov et al. (1979b) could not be reproduced in later studies, and may be related to the experimental set-up these authors used (discussed in Yasuhara et al. 1994).

---

### Degradation of nuclear-encoded subunits of membrane complexes

In addition to mitochondrial translation products, many nuclear-encoded subunits of mitochondrial inner membrane complexes in yeast are known to be degraded rapidly when not assembled (Table 1). Increased turnover of imported subunits of complex IV was also observed in human and mouse cell lines in the absence of mitochondrial protein synthesis (Hayashi et al. 1990; Chrzanowska-Lightowlers et al. 1993; Nijtmans et al. 1995). It should be noted, how-

ever, that in most cases only steady state amounts of subunits were measured, high turnover being inferred from the assumption that synthesis and import were not affected. In only a few cases has subunit turnover actually been shown by pulse-chase experiments (De Haan et al. 1984; Nijtmans et al. 1995).

For some subunits, reports differ as to their stability in the absence of complex assembly in yeast. For example, in some studies core I and II were found to be stable in strains disrupted for other subunits of complex III (Berden et al. 1988; Crivellone et al. 1988), while other investigators have reported the instability of core I in *cob* mutants (Sen and Beattie 1985; Chevillotte-Brivet et al. 1987) and of core II in a core-I mutant (Gatti and Tzagoloff 1990). Nuclear-encoded subunits of complex IV accumulate in the absence of mitochondrially encoded subunits in yeast and *N. crassa*, even though they do not form stable sub-complexes (Sebald et al. 1972; Bertrand and Werner 1977; Cabral and Schatz 1978). However, strongly reduced levels of some of these subunits have been found in *cox1*, *cox4*, *cox9* and cytochrome *c* mutants (Dowhan et al. 1985; McEwen et al. 1986; Pearce and Sherman 1995a, see Table 1). The observed differences are possibly due to variations in experimental conditions and/or strain differences.

Although cytochrome *c* is not part of any complex (it shuttles between complex III and IV transferring electrons down the respiratory chain), in terms of stability it does, under certain circumstances, behave like a subunit of these complexes. Certain variants are rapidly degraded when cytochromes  $aa_3$  or  $c_1$ , its physiological partners, are absent (Pearce and Sherman 1995b). Conversely, cytochrome  $aa_3$  is absent in *cyc1/cyc7* double mutants, lacking both isoforms of cytochrome *c* (Pearce and Sherman 1995a). In fact, these mutants had diminished amounts of both nuclear- and mitochondrially encoded subunits of complex IV, with the latter group being virtually undetectable by Western analysis.

---

### Can fully assembled complexes also be targets for degradation?

Generally, intact enzyme complexes of the mitochondrial inner membrane are found to be quite stable in mammals (Hare 1990) and yeast (represented by the 'stable' fraction of mitochondrial translation products, see above). However, several reports mention either turnover of subunits that are already in a complex, or turnover of whole complexes. In rat hepatoma cells, all subunits of complex IV and V turn over very slowly (half-life >100 h). For complex III, however, four of eight subunits (core II, cytochrome *b* and two smaller subunits) turned over more rapidly (half-life 35–42 h) (Hare and Hodges 1982a). Presumably, the subunits with short half-lives are replaced by newly synthesized subunits after the complex has fallen apart. The question remains whether disassembly of the complex simply represents a random process or whether

(damaged?) complexes are actively disassembled by "quality control" factors.

Active turnover of complete complexes has received experimental support. Part of the cytochromes *b*, *c*, *c*<sub>1</sub> and *aa*<sub>3</sub> that accumulate in yeast cells during exponential growth on galactose disappears in the course of further culture growth without loss of respiratory rate (Galkin et al. 1975). This loss of cytochromes is inhibited by the protease inhibitors PMSF and pepstatin (Luzikov et al. 1976). Administration of these inhibitors also increases the accumulation of cytochromes in glucose-grown cells (Galkin et al. 1980). These changes in cytochrome content are not accompanied by changes in the activity of the respiratory chain complexes, indicating that the 'surplus' cytochromes accumulated during exponential growth are not functional (Galkin et al. 1979a, b, 1980). The nature of these non-functional cytochromes is unclear. One possibility is that they represent incomplete complexes, and are "edited out" by specific recognition/protease systems, as occurs with plasma-membrane complexes (Klausner 1989). This is not likely, however, since solubilization of mitochondria led to the apparent activation of the extra cytochromes (Galkin et al. 1979a, b, 1980). Moreover, surplus cytochrome *c* is made and degraded in a similar way as the complex-bound cytochromes. This suggests the presence of whole mitochondrial compartments which for some reason are non-functional and so may be degraded by autophagocytosis (discussed in Luzikov 1986).

Co-ordinated loss of all cytochromes is also observed when yeast cells are transferred from aerobic to anaerobic growth conditions. In this case, however, the loss is complete and appears not to be due to lysosomal degradation, as mutations that alter the stability of iso-1-cytochrome *c* in vitro and its steady state level in vivo also alter its half-life after the shift to anaerobic conditions (Pearce and Sherman 1995c). Another indication that complete complexes may be actively and selectively degraded in mitochondria is the loss of cytochrome *c* oxidase in cytochrome *c*-less yeast (Pearce and Sherman 1995a) and *N. crassa* (Bottorff et al. 1994). If cytochrome *c* has no role in the assembly of cytochrome *c* oxidase, one has to conclude that cytochrome *c* protects the complex from proteolytic breakdown. The initial attack by the responsible protease may then be on the 'unprotected' subunit 2, the cytochrome *c*-binding subunit (Hatefi 1985). If this is true, interpretations of gene disruption experiments designed to uncover the role of many 'accessory' subunits of mitochondrial respiratory chain complexes (see Table 1 for references) need to take into account the possibility that these subunits are not involved in complex assembly, but protect an already assembled complex from proteolytic attack (in addition to having possible functional roles).

### Mitochondrial proteases

Early attempts to characterize mitochondrial proteases in mammalian cells led to the identification of soluble (Al-

berti and Bartley 1969; Subramanian et al. 1975) and membrane-bound (Aoki 1978; Haas and Heinrich 1978; Hare 1978) proteases. However, it has been difficult to exclude the contamination of mitochondrial preparations with proteases from lysosomes or mast cell granules (see, for instance, Rubio and Grisolia 1977; Duque-Magalhães 1979; Haas et al. 1979). A major breakthrough was the demonstration of an ATP-dependent protease with a similarity to *E. coli* Lon (or La) in the matrix of mitochondria from rat liver (Desautels and Goldberg 1982a, 1985), counterparts of which were later found in bovine adrenal cortex (Watabe and Kimura 1985a, b) and yeast (Kutejová et al. 1993). In addition, non-ATP-dependent metalloproteases involved in the processing of precursor proteins were identified in mitochondria from rat liver (Conboy et al. 1982; Miura et al. 1982), bovine adrenal cortex (Kumamoto et al. 1986), yeast (McAda and Douglas 1982; Böhni et al. 1983), and *N. crassa* (Hawlitchev et al. 1988). Only in the last few years have genes that encode these and other (putative) mitochondrial proteases been isolated from *S. cerevisiae* and other organisms (Table 2).

As shown in Table 2, mitochondrial proteases can be divided into two groups: proteases involved in processing precursor proteins and proteases involved in degradation. The first group falls outside the scope of this review and will not be considered further. To the second group belong Pim1p(Lon), a matrix-localized protease of which the human gene has also been cloned (Wang et al. 1993; Amerik et al. 1994), and the related proteins Yme1p(Yta11p), Rca1p(Yta12p) and Afg3p(Yta10p) that belong to a subgroup of the AAA family of ATPases (Confalonieri and Duguet 1995) and are associated with the inner membrane (see Table 2 for references). Pim1p is related to *E. coli* Lon/La (Charette et al. 1981; Chung and Goldberg 1981), while Afg3p, Rca1p and Yme1p are all related to *E. coli* FtsH (Tomoyasu et al. 1993a, b).

The members of this group are (putative) ATP-dependent proteases. Such proteases are involved in catalyzing the first step of degradation: cleaving the substrate into peptides that are subsequently degraded by non-ATP-dependent proteases. As ATP hydrolysis is only required for the degradation of large proteins, the energy from ATP hydrolysis probably allows the protease to function in a processive manner (Goldberg 1992; Gottesman and Maurizi 1992) and/or to unfold proteins so as to allow entry of the polypeptide chain into the proteolytic 'core' of the protease (see below).

Are all mitochondrial ATP-dependent proteases multimers?

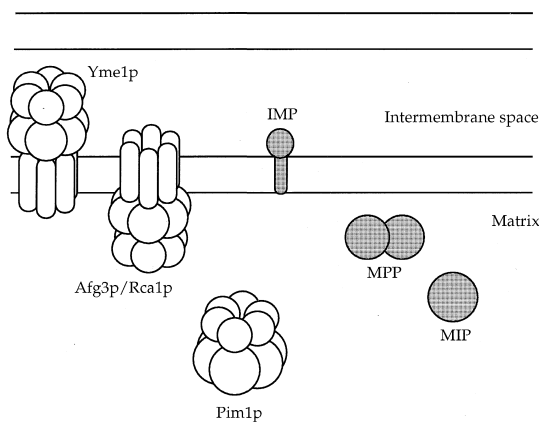
The formation of multimers is common among ATP-dependent proteases (Rechsteiner et al. 1993). Bacterial Lon is a homotetramer (Chung and Goldberg 1981), while its homologues in mammalian and yeast mitochondria appear to be hexamers (Watabe and Kimura 1985a; Kutejová et al. 1993). Homo-multimerization of FtsH has been shown by Akiyama et al. (1995). There have been several indica-

**Table 2** Mitochondrial proteases of *S. cerevisiae* for which the genes have been cloned

Item	Type	<i>E. coli</i> homologue	Location	Composition/structure	Proteolytic function	References <sup>a</sup>
Processing peptidases						
MPP	Metallo		Matrix	Heterodimer of protease and protease enhancer	Cleavage of mitochondrial targeting sequence	Jensen and Yaffe 1988; Pollock et al. 1988
MIP	Metallo		Matrix	Monomer	Cleavage of octapeptide of bipartite presequence	Isaya et al. 1994
IMP	Serine	Lep	Inner membrane, facing IMS	Dimer of two homologous subunits with different substrate specificities	Cleavage of N-terminal (sorting) sequence of some inner membrane and IMS proteins	Behrens et al. 1991; Nunnari et al. 1993
ATP-dependent proteases						
Pim1p/Lon	Serine	Lon(La)	Matrix	Homo-hexamamer	Degrades (unfolded) matrix proteins	Van Dyck et al. 1994; Suzuki et al. 1994
Afg3p/Yta10p	Metallo	FtsH	Inner membrane, facing matrix	In a complex with Rca1p/Yta12p	Degrades incomplete and mature mitochondrial translation products	Schnall et al. 1994; Guélin et al. 1994; Tauer et al. 1994
Rca1p/Yta12p	Metallo	FtsH	Inner membrane, facing matrix	In a complex with Afg3p/Yta10p	Degrades incomplete and mature mitochondrial translation products	Schnall et al. 1994; Tzagoloff et al. 1994
Yme1p/Yta11p	Metallo	FtsH	Inner membrane	In a complex	Degrades CoxII	Thorsness et al. 1993; Nakai et al. 1995

<sup>a</sup> Reference is made to the cloning of *S. cerevisiae* genes; see text for references on biochemical characterization of the proteases from yeast and other organisms.

MPP: mitochondrial processing peptidase; MIP: mitochondrial intermediate peptidase; IMP: inner membrane protease; IMS: intermembrane space



**Fig. 1** Schematic picture of proteases identified in yeast mitochondria. Drawn approximately to scale, ATP-dependent proteases involved in protein degradation are in *white* and processing peptidases required for maturation of imported proteins and/or proteins translocated into the intermembrane space are in *grey*. The bipartite structure of the (extra-membrane part of) ATP-dependent proteases reflects the presence in the primary sequence of ATPase and protease domains. The hexameric nature of the Afg3p/Rca1p and Yme1p proteases in the picture is solely to indicate their oligomeric structure; the exact number of subunits present in the complexes is not known. The orientation of the Yme1p complex is controversial. The picture shows an orientation towards the inter-membrane space (Thomas Langer, personal communication). Weber et al. (1996), however, reported an orientation towards the matrix side. See Table 2 and text for details on the different proteases

tions that mitochondrial membrane-bound proteases are also present as homo- or hetero-multimers. Over-expression of *YME1* does not increase the amount of Yme1p protein, presumably because Yme1p which is not associated with other proteins in a complex is unstable (Thorsness et al. 1993). Candidates for Yme1p-associated proteins from genetic screens are the products of *YME2* (Thorsness and Fox 1993), *OSD2* and *OSD3* (Nakai et al. 1995). In addition, homo-multimerization of Yme1p was suggested by intragenic complementation between two *yme1* alleles (Thorsness and Fox 1993). Recently, a high-molecular-weight complex containing Yme1p has been identified, as well as a complex involving both Afg3p and Rca1p (Arlt et al. 1996; Thomas Langer, personal communication). Formation of homo-multimers is also seen with other members of the AAA family (Peters et al. 1990, 1993; Whiteheart et al. 1994; Fröhlich et al. 1995) and may be a common feature of these proteins.

Figure 1 shows the location and (putative) quaternary structure of the mitochondrial proteases the yeast genes of which have been cloned. In this picture, non-ATP-dependent proteases responsible for breaking down oligopeptides to amino acids are strikingly absent. Such proteases may be among proteases reported for the mitochondrial matrix (Beer et al. 1982; Yasuhara and Ohashi 1987; Yasuhara et al. 1994) and inner membrane (Novikova et al. 1981; Zubatov et al. 1984). However, their physiological functions remain to be established. Identification of the

corresponding genes will be an important step towards that end.

---

### Division of labour

The division in the group of ATP-dependent proteases into matrix-localized (Pim1p) and membrane-localized (Afg3p, Rca1p, Yme1p) members parallels a division in substrate proteins. Until now, Pim1p has been implicated in (1) the degradation of imported proteins that fail to fold into a native structure (Wagner et al. 1994) and (2) the turnover of MPP $\beta$  and F<sub>1</sub> $\beta$ , two matrix-localized proteins (Suzuki et al. 1994). Furthermore, the *PIM1* gene is induced four-fold by heat-shock (Van Dyck et al. 1994) and electron-dense material, presumably consisting of protein-aggregates, accumulates in the mitochondrial matrix of *lon*<sup>-</sup> (*pim1*) mutants (Suzuki et al. 1994). Targets of the Pim1p counterpart in bovine adrenal cortex include SP-22, a matrix-localized oxygen-radical scavenger (Watabe et al. 1994, 1995).

That degradation of matrix proteins is essential for mitochondrial biogenesis in yeast is apparent from the phenotype lacking the matrix protease. Such strains are respiratory deficient and quickly accumulate deletions in mtDNA ("rho<sup>-</sup> induction") (Suzuki et al. 1994; Van Dyck et al. 1994). In *pim1* cells, rho<sup>-</sup> induction may be related to over-accumulation of a particular protein(s) or perhaps to a disturbing effect of aggregated mitochondrial proteins. In contrast to Pim1p, Afg3p(Yta10p) is not involved in the degradation of misfolded proteins in the matrix but is necessary for the degradation of incomplete, membrane-bound mitochondrial translation products (Pajic et al. 1994). Recently, it has been shown that Afg3p is also involved in the degradation of most mature mitochondrial translation products destined for the inner membrane, with the notable exception of CoxII (Guélin et al. 1996). The latter appears to be degraded by Yme1p. Mutation of *YME1* has been found to attenuate degradation of CoxII in the absence of CoxIV (Nakai et al. 1995; Weber et al. 1996), and a stabilizing effect of *YME1*-inactivation on both CoxII and CoxIII has been observed in a strain lacking cytochrome *c* (Pearce and Sherman 1995a). The presence of ATP- and zinc-binding motifs typical of metalloproteases in Yme1p, Rca1p and Afg3p supports the notion that these three proteins are indeed responsible for the degradation of mitochondrial translation products destined for the inner membrane, which, as described above, is both ATP- and divalent metal ion-dependent.

The division of labour between soluble and membrane-bound proteases does not completely coincide with the division between soluble and membrane-bound polypeptides. Bovine p450scc and adrenodoxin reductase, both present on the matrix side of the inner membrane, can be degraded by the matrix ATP-dependent protease (Watabe et al. 1993). Possibly, the mitochondrial membrane-bound system is specific for integral membrane proteins. However, although no soluble targets for Afg3p, Rca1p or

Yme1p have yet been identified, their existence should not be excluded at this point. The homologue of these proteins in *E. coli*, FtsH, governs the degradation of the soluble proteins  $\lambda$ -cII (Herman et al. 1993),  $\lambda$ -cIII and  $\sigma^{32}$  (Herman et al. 1995; Tomoyasu et al. 1995) in addition to being involved in degrading the integral membrane protein SecY (Kihara et al. 1995). Significantly, in vitro degradation of  $\sigma^{32}$  by purified FtsH is the first direct proof of ATP- and divalent metal-dependent protease activity of a member of the FtsH-subfamily (Tomoyasu et al. 1995).

Yet another protease seems to be responsible for the degradation of the intermembrane space (IMS)-proteins, as neither of the above mentioned ATP-dependent proteases is responsible for degradation of cytochrome *c* (Pearce and Sherman 1995b). Putative candidates are cytochrome *c* hydrolyzing proteases associated with sub-mitochondrial particles (Novikova et al. 1981; Zubatov et al. 1984), an ATP-dependent proteolytic activity in the inter-membrane space (Sitte et al. 1995) and, in mammalian cells, an ATP-dependent protease activity that requires ATP outside the inner membrane (Rapoport et al. 1982). Furthermore, the presence of an additional protease associated with the inner membrane is suggested by the fact that degradation of CoxV in a cytochrome *c*-less mutant is not suppressed by disrupting any of the genes for the currently known ATP-dependent proteases (Pearce and Sherman 1995a). In fact, it is not known at present which protease(s) is(are) responsible for the turnover of nuclear-encoded subunits of mitochondrial inner membrane complexes (except for F<sub>1</sub> $\beta$ , see above).

---

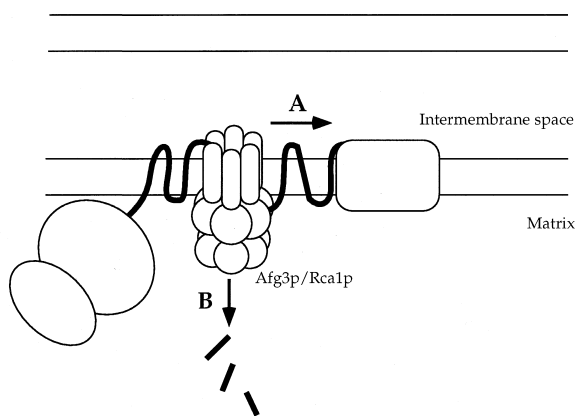
### Dual roles of ATP-dependent proteases in mitochondrial biogenesis?

As described in the introductory sections, an important function of membrane-bound ATP-dependent proteases is the removal of polypeptides that may adversely influence the structure or function of the membrane. However, the defects arising in mitochondria or bacteria as a result of loss of an ATP-dependent protease may not (only) be caused by the loss of protease activity. Indeed, as will be discussed below, phenotypes associated with mutations of membrane-bound ATP-dependent proteases are sometimes more readily explained by assuming a dual function for these proteins. Independent of the protease activity, the ATPase domain of these proteins may have a chaperone-like activity.

#### Afg3p and Rca1p

Afg3p/Yta10p and Rca1p/Yta12p were both found to be essential for respiratory growth (Tauer et al. 1994; Tzagoloff et al. 1994) and the assembly of inner membrane complexes (Tzagoloff et al. 1994; Paul and Tzagoloff 1995). As argued above, these phenotypes may simply be explained by the accumulation of polypeptides that are det-





**Fig. 2** Quality control of mitochondrial translation products. A complex of Afg3p and Rca1p may function both as a protease and a chaperone (see text). Newly synthesized mitochondrial translation products are either assembled into a complex of the inner membrane (A) or broken down (B). It is not known if these processes occur co-translationally, as shown in the picture, or only post-translationally, or both. If assembly occurs co-translationally, the Afg3p/Rca1p complex may assist in the attainment of the correct membrane topology by multiple membrane-spanning proteins, as has been suggested for FtsH, the *E. coli* homologue of Afg3p and Rca1p (see text). Degradation of complete mitochondrial translation products in an Afg3p-dependent manner indicates that proteolysis can occur post-translationally (Guélin et al. 1996). Thus, Afg3p/Rca1p may also have a role in the removal of denatured or otherwise damaged proteins from the membrane

perimental to the integrity of the inner membrane. However, inactivation of the proteolytic activity of Afg3p by a point mutation in the protease active site does not affect respiratory growth (Guélin et al. 1996). Complex assembly apparently proceeds normally in this mutant, although degradation of mitochondrial translation products was reduced to a similar extent as in an *AFG3*-deletion mutant. These observations raise the intriguing possibility that at least some ATP-dependent proteases are dual-function proteins, with both chaperone and degradation activities. In one model (Fig. 2), the ATPase domains of Afg3p and Rca1p interact with substrate (membrane proteins) either for degradation by the C-terminal protease domain, or for folding/assembly/integration purposes. Through such a dual function, these ATP-dependent proteases could be part of a quality control system for mitochondrial translation products (Grivell 1995; Nakai et al. 1995).

A similar model may hold for the *E. coli* homologue FtsH, although the question is still unresolved whether this protein is directly involved in the maturation of membrane proteins. Mutations in FtsH affect the membrane translocation of secretory proteins and the topology of membrane proteins in *E. coli* (Tomoyasu et al. 1993b; Akiyama et al. 1994a, b) and cause a different pattern of membrane-associated proteins in *L. lactis* (Nilsson et al. 1994). One possibility is that all these phenotypes result from the stabilization of various host proteins, like the heat-shock transcription factor  $\sigma^{32}$  (Herman et al. 1995). However, some observations are still difficult to explain by such a model. For example, although FtsH is involved in the degradation of unassembled SecY, and the accumulation of SecY in-

hibits protein translocation (Kihara et al. 1995), the negative effects of *ftsH*<sup>-</sup> mutations on the translocation and stop-transfer of trans-membrane segments of the SecY-PhoA fusion proteins are not caused by SecY or SecY-PhoA accumulation (Kihara et al. 1995). Kihara et al. proposed that FtsH is involved in both the degradation of unassembled SecY and its assembly/integration into the membrane. One explanation offered for the effect of FtsH on stop-transfer is that FtsH is necessary for opening of the Sec-channel to allow lateral diffusion of trans-membrane segments (Akiyama et al. 1994a, b). Alternatively, FtsH may bind to the PhoA moiety of the SecY-PhoA fusion protein, a function that could be shared with HtpG, a Hsp90-type chaperone (Shirai et al. 1996).

### Yme1p

A combination of chaperone and protease function has also been proposed for Yme1p/Osd1p (Nakai et al. 1995). Mutation of *YME1* yields cells that are respiratory deficient at high temperature (Thorsness et al. 1993) and have reduced activity levels of respiratory chain complexes (Nakai et al. 1995). Moreover, such mutants show additional defects such as intolerance of deletions in mitochondrial DNA (mtDNA) (leading to the absence of mitochondrial translation products), leakiness of mitochondria resulting in mtDNA escaping to the nucleus, and cold-sensitive growth on rich glucose medium. All these phenotypes are thought to result from a morphologically altered inner membrane (Thorsness et al. 1993). Whether this can be fully attributed to the accumulation of membrane proteins that are degraded by Yme1p or indeed involves another function of Yme1p, is still a matter of speculation (Nakai et al. 1995; Weber et al. 1996).

A very interesting but puzzling finding is that an altered form of Ynt1p (Yta2p), a probable regulatory subunit of the 26s protease and like Yme1p a member of the AAA family (Confalonieri and Duguet 1995), can suppress all *yme1*-associated phenotypes (Campbell et al. 1994). As it seems unlikely that the 26s proteasome, a 1500–2000-kDa protein complex (Fischer et al. 1994), can pass the mitochondrial outer membrane, the altered form of Ynt1p probably acts independently from the proteasome. At least some other AAA-type subunits of the 26s proteasome can be part of different subcomplexes and can bind certain proteins as free subunits both in vivo and in vitro (Demartino et al. 1996; Rubin et al. 1996; vom Baur et al. 1996). Significantly, Ynt1p does not have a C-terminal protease domain like Yme1p, suggesting that the Ynt1 mutant protein replaces a non-protease function of Yme1p. However, it can be argued that Ynt1p binds Yme1p substrates and subsequently presents them to the proteasome, or another protease, for degradation.

A chaperone-activity of mitochondrial ATP-dependent proteases is perhaps not so surprising in view of the close functional link between ATP-dependent proteases and chaperones (Squires and Squires 1992; Craig et al. 1994; Horwich 1995). Two *E. coli* proteins, ClpA and ClpX, that

can combine with ClpP subunits to form an ATP-dependent protease (Gottesman and Maurizi 1992; Gottesman et al. 1993), can function independently as chaperones (Wickner et al. 1994; Levchenko et al. 1995; Wawrzynow et al. 1995). Yeast mitochondrial Hsp78p, another member of the Clp-family, can partly substitute for mt-Hsp70 (Schmitt et al. 1995) and/or stabilize mutant forms of the latter (Moczko et al. 1995). Comparison with Clp proteins is significant for two reasons: (1) there is significant homology between members of the Clp-family and the conserved domain of the AAA-family, to which the FtsH subfamily belongs (Dubiel et al. 1992; Gottesman et al. 1993), and (2) there are functional similarities between the *E. coli* Clp system and the eukaryotic 26S proteasome, where members of the AAA-family may function like ClpA and ClpX proteases, namely: selection of substrate and presentation to a protease 'core' for degradation (Rechsteiner et al. 1993; Kessel et al. 1995) and/or unfolding of the substrate to allow entry into the interior of the protease (through a 'reverse chaperone' activity) (Peters 1994; Goldberg 1995; Wenzel and Baumeister 1995). A chaperone-like function for two other mitochondrial members of the AAA-family has also been proposed: Bcs1p, involved in the biogenesis of complex III (Nobrega et al. 1992) and Msp1p, an outer-membrane protein whose over-production results in the mislocalization of an outer-membrane reporter protein to the inner membrane (Nakai et al. 1993). Finally, chaperone activity has been proposed for NSF, a AAA-protein involved in vesicle fusion (Morgan and Burgoyne 1995).

In spite of a potential chaperone activity of ATP-dependent proteases, conventional chaperones like Hsp70 or DnaK appear to be necessary for these proteases to degrade at least some of their substrates (Wagner et al. 1994 and references therein). These chaperones may retain the accessibility of denatured proteins by preventing their aggregation. Alternatively, prior binding of a chaperone to a substrate protein may be necessary for recognition by the protease, thus providing an additional layer of control on proteolysis. In yeast mitochondria, proteolysis by Pim1p requires prior binding of the substrate to chaperones Hsp70 and Mdj1p (Wagner et al. 1994). Accordingly, degradation of the only mitochondrially encoded non-membrane protein, the small ribosomal subunit protein Var1p, depends on functional Hsp70. This is not true, however, for the membrane protein Atp6p (Herrmann et al. 1994), which is a target of Afg3p (Guélin et al. 1996). In the latter case, either another chaperone is involved or the protease is by itself capable of recognizing its substrate.

The quality control model of mitochondrial translation products (Fig. 2) raises several questions that demand imaginative experimental approaches. One of the main questions is by what mechanism the 'choice' between assembly and degradation is made. Another is what the proposed chaperone function of the FtsH-subfamily proteins is in molecular terms. It must be noted in this respect that while 'assembly' or 'folding' are commonly used when referring to the proposed (second) function of these proteins, ClpA and ClpX promote disaggregation or monomeriza-

tion (Wickner et al. 1994; Levchenko et al. 1995; Wawrzynow et al. 1995).

---

### Concluding remarks

Our knowledge concerning the turnover of mitochondrial proteins has benefited immensely from the power of the molecular genetics of *S. cerevisiae*. Indeed, the isolation of genes for proteases and the manipulation of individual components is essential to unravel the complicated, entangled processes of assembly, turnover, enzymic activity and membrane physiology. We now know that mitochondria have inherited their ATP-dependent proteases from their endosymbiotic predecessors, as is the case for many other basic components of gene expression. Especially exciting is the recent characterization of a family of ATP-dependent proteases that specifically act on membrane constituents, and the possibility that these are also involved in the building of membrane complexes. Of equal interest is the characterization of the major ATP-dependent protease of the mitochondrial matrix.

The mechanisms of quality control of the inner membrane and its constituents remain to a large extent to be uncovered. One level which is still difficult to access is the higher-order organization of the inner membrane. Are there superstructures of different complexes? Are there sub-regions of the membrane specialized in assembly and/or turnover? With the different factors identified, multiple approaches can be used to resolve these questions.

Looking forward, we can anticipate the gradual uncovering of the division of substrates among the different proteases, and the identification of new proteases and of complexes with protease and/or chaperone activities. With the current acceleration of yeast research due to the recent release of the sequence of the complete yeast genome, new strides forward are expected in the near future. Our understanding of the underlying causes of clinically important phenomena, like the apparent high turnover rates of mitochondrial protein in tumor cells (Luciaková and Kuzela 1992) and the intra-mitochondrial degradation failure of subunit *c* of  $F_1F_0$ -ATPase in Batten disease (Ezaki et al. 1995), should increase concomitantly.

**Acknowledgements** We thank Thomas Langer (Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München) for communicating results prior to publication, and Hans van der Spek for critical reading of the manuscript. This work was supported by grants to L. A. Grivell from the Netherlands Organization for the Advancement of Science (NWO) and from the EU under contract no. CHRX-CT94 0520 of the Human Capital and Mobility Programme.

---

### References

- Akiyama Y, Ogura T, Ito K (1994a) Involvement of FtsH in protein assembly into and through the membrane. I. Mutations that reduce retention efficiency of a cytoplasmic reporter. *J Biol Chem* 269:5218-5224

- Akiyama Y, Shirai Y, Ito K (1994b) Involvement of FtsH in protein assembly into and through the membrane. II. Dominant mutations affecting FtsH functions. *J Biol Chem* 269:5225–5229
- Akiyama Y, Yoshihisa T, Ito K (1995) FtsH, a membrane-bound ATPase, forms a complex in the cytoplasmic membrane of *Escherichia coli*. *J Biol Chem* 270:23485–23490
- Alberti KGMM, Bartley W (1969) The localization of proteolytic activity in rat liver mitochondria and its relation to mitochondrial swelling and aging. *Biochem J* 111:763–776
- Amerik AY, Petukhova GV, Grigorenko VG, Lykov IP, Yarovoi SV, Lipkin VM, Gorbalenya AE (1994) Cloning and sequence analysis of cDNA for a human homolog of eubacterial ATP-dependent Lon proteases. *FEBS Lett* 340:25–28
- Aoki Y (1978) Crystallization and characterization of a new protease in mitochondria of bone marrow cells. *J Biol Chem* 253:2026–2032
- Arlt H, Tauer R, Feldmann H, Neupert W, Langer T (1996) The YTA10/12-complex, an AAA protease with chaperone-like activity in the inner membrane of mitochondria. *Cell* 85:875–885
- Bakalkin GY, Kalnov SL, Galkin AV, Zubatov AS, Luzikov VN (1978) The lability of the products of mitochondrial protein synthesis in *Saccharomyces cerevisiae*. A novel method for protein half-life determination. *Biochem J* 170:569–576
- Baranowska H, Szczésniak B, Ejchart A, Kruszewska A, Claisse M (1983) Recombinational analysis of *oxi2* mutants and preliminary analysis of their translation products in *S. cerevisiae*. *Curr Genet* 7:225–23383
- Baur E vom, Zechel C, Heery D, Heine MJS, Garnier JM, Vivat V, Ledouarin B, Gronemeyer H, Chambon P, Losson R (1996) Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J* 15:110–124
- Beer DG, Hjelle JJ, Petersen DR, Malkinson AM (1982) Calcium-activated proteolytic activity in rat liver mitochondria. *Biochem Biophys Res Commun* 109:1276–1283
- Behrens M, Michaelis G, Pratje E (1991) Mitochondrial inner membrane protease 1 of *Saccharomyces cerevisiae* shows sequence similarity to the *Escherichia coli* leader peptidase. *Mol Gen Genet* 228:167–176
- Beppu M, Inoue M, Ishikawa T, Kikugawa K (1994) Presence of membrane-bound proteinases that preferentially degrade oxidatively damaged erythrocyte membrane proteins as secondary antioxidant defense. *Biochim Biophys Acta* 1196:81–87
- Berden JA, Schoppink PJ, Grivell LA (1988) A model for the assembly of the ubiquinol:cytochrome *c* oxidoreductase in yeast. In: Palmieri F, Quagliariello E (eds) *Molecular basis of biomembrane transport*. Elsevier Science Publishers, Amsterdam, pp 195–208
- Bertrand H, Werner S (1977) Deficiency of subunit 2 of cytochrome oxidase in the mi-3 cytoplasmic mutant of *Neurospora crassa*. *Eur J Biochem* 79:599–606
- Black-Schaefer CL, McCourt JD, Poyton RO, McKee EE (1991) Mitochondrial gene expression in *Saccharomyces cerevisiae*. Proteolysis of nascent chains in isolated yeast mitochondria optimized for protein synthesis. *Biochem J* 274:199–205
- Böhni PC, Daum G, Schatz G (1983) Import of proteins into mitochondria. Partial purification of a matrix-located protease involved in cleavage of mitochondrial precursor polypeptides. *J Biol Chem* 258:4937–4943
- Bottorff DA, Parmaksizoglu S, Lemire EG, Coffin JW, Bertrand H, Nargang FE (1994) Mutations in the structural gene for cytochrome *c* result in deficiency of both cytochromes *aa<sub>3</sub>* and *c* in *Neurospora crassa*. *Curr Genet* 26:329–335
- Cabral F, Schatz G (1978) Identification of cytochrome *c* oxidase subunits in nuclear yeast mutants lacking the functional enzyme. *J Biol Chem* 253:4396–4401
- Calder KM, McEwen JE (1991) Deletion of the *COX7* gene in *Saccharomyces cerevisiae* reveals a role for cytochrome *c* oxidase subunit VII in assembly of remaining subunits. *Mol Microbiol* 5:1769–1777
- Campbell CL, Tanaka N, White KH, Thorsness PE (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
- Charette M, Henderson GW, Markovitz A (1981) ATP hydrolysis-dependent activity of the Lon (CapR) protein of *E. coli* K12. *Proc Natl Acad Sci USA* 78:4728–4732
- Chevillotte-Brivet P, Salou G, Meunier-Lemesle D (1987) Missense exonic mitochondrial mutation in cytochrome *b* gene of *Saccharomyces cerevisiae*, resulting in core protein deficiency in complex III of the respiratory chain. *Curr Genet* 12:111–117
- Chrzanowska-Lightowlers ZMA, Turnbull DM, Bindoff LA, Lightowlers RN (1993) An antisense oligodeoxynucleotide approach to investigate the function of the nuclear-encoded subunits of human cytochrome *c* oxidase. *Biochem Biophys Res Commun* 196:328–335
- Chung CH, Goldberg AL (1981) The product of the *lon* (*capR*) gene in *Escherichia coli* is the ATP-dependent protease, protease La. *Proc Natl Acad Sci USA* 78:4931–4935
- Conboy JG, Fenton WA, Rosenberg LE (1982) Processing of pre-ornithine transcarbamylase requires a zinc-dependent protease localized to the mitochondrial matrix. *Biochem Biophys Res Commun* 105:1–7
- Confalonieri F, Duguet M (1995) A 200-amino acid ATPase module in search of a basic function. *BioEssays* 17:639–650
- Constantino P, Attardi G (1977) Metabolic properties of the products of mitochondrial protein synthesis in HeLa cells. *J Biol Chem* 252:1702–1711
- Cote C, Boulet D, Poirier J (1990) Expression of the mammalian mitochondrial genome. Role for membrane potential in the production of mature translation products. *J Biol Chem* 265:7532–7538
- Craig EA, Weissman JS, Horwich AL (1994) Heat-shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. *Cell* 78:365–372
- Crivellone MD (1994) Characterization of *CBP4*, a new gene essential for the expression of ubiquinol-cytochrome *c* reductase in *Saccharomyces cerevisiae*. *J Biol Chem* 269:21284–21292
- Crivellone MD, Wu MA, Tzagoloff A (1988) Assembly of the mitochondrial membrane system. Analysis of structural mutants of the yeast coenzyme QH<sub>2</sub>-cytochrome *c* reductase complex. *J Biol Chem* 263:14323–14333
- Davies KJA, Lin SW, Pacifici RE (1987) Protein damage and degradation by oxygen radicals. *J Biol Chem* 262:9914–9920
- De Haan M, Van Loon AP, Kreike J, Vaessen RT, Grivell LA (1984) The biosynthesis of the ubiquinol-cytochrome *c* reductase complex in yeast. DNA sequence analysis of the nuclear gene coding for the 14-kDa subunit. *Eur J Biochem* 138:169–177
- Dean RT, Pollak JK (1985) Endogenous free radical generation may influence proteolysis in mitochondria. *Biochem Biophys Res Commun* 126:1082–1089
- Demartino GN, Proske RJ, Moomaw CR, Strong AA, Song XL, Hisamatsu H, Tanaka K, Slaughter CA (1996) Identification, purification, and characterization of a PA700-dependent activator of the proteasome. *J Biol Chem* 271:3112–3118
- Desautels M (1986) Mitochondrial proteolysis. In: Fiskum G (ed) *Mitochondrial physiology and pathology*. Van Nostrand Reinhold, New York, pp 40–65
- Desautels M, Goldberg AL (1982a) Demonstration of an ATP-dependent, vanadate-sensitive endoprotease in the matrix of rat liver mitochondria. *J Biol Chem* 257:11673–11679
- Desautels M, Goldberg AL (1982b) Liver mitochondria contain an ATP-dependent, vanadate-sensitive pathway for the degradation of proteins. *Proc Natl Acad Sci USA* 79:1869–1873
- Desautels M, Goldberg AL (1985) The ATP-dependent breakdown of proteins in mammalian mitochondria. *Biochem Soc Trans* 13:290–293
- Dowhan W, Bibus CR, Schatz G (1985) The cytoplasmically-made subunit IV is necessary for assembly of cytochrome *c* oxidase in yeast. *EMBO J* 4:179–184
- Duarte M, Sousa R, Videira A (1995) Inactivation of genes encoding subunits of the peripheral and membrane arms of *Neurospora* mitochondrial complex I and effects on enzyme assembly. *Genetics* 139:1211–1221

- Dubiel W, Ferrell K, Pratt G, Rechsteiner M (1992) Subunit 4 of the 26S protease is a member of a novel eukaryotic ATPase family. *J Biol Chem* 267:22699–22702
- Duque-Magalhaes (1979) On a neutral proteolytic system in rat liver mitochondria. *FEBS Lett* 105:317–320
- Ezaki J, Wolfe LS, Higuti T, Ishidoh K, Kominami E (1995) Specific delay of degradation of mitochondrial subunit c in late infantile neuronal ceroid lipofuscinosis (Batten disease). *J Neurochem* 64:733–741
- Fecke W, Sled VD, Ohnishi T, Weiss H (1994) Disruption of the gene encoding the NADH-binding subunit of NADH:ubiquinone oxidoreductase in *Neurospora crassa*. Formation of a partially assembled enzyme without FMN and the iron-sulphur cluster N-3. *Eur J Biochem* 220:551–558
- Fischer M, Hilt W, Richterruoff B, Gonen H, Ciechanover A, Wolf DH (1994) The 26S proteasome of the yeast *Saccharomyces cerevisiae*. *FEBS Lett* 355:69–75
- Fröhlich KU, Fries HW, Peters JM, Mecke D (1995) The ATPase activity of purified CDC48p from *Saccharomyces cerevisiae* shows complex dependence on ATP-, ADP-, and NADH-concentrations and is completely inhibited by NEM. *Biochim Biophys Acta* 1253:25–32
- Galkin AV, Makhlis TA, Zubatov AS, Luzikov VN (1975) Lack of positive correlation between cell respiration and cytochrome content in galactose-grown *Saccharomyces cerevisiae*. *FEBS Lett* 55:42–45
- Galkin AV, Tsoi TV, Luzikov VN (1979a) Abnormalities in mitochondrial respiratory chain assembly and their proteolytic elimination. *FEBS Lett* 103:111–113
- Galkin AV, Tsoi TV, Luzikov VN (1979b) Regulation of mitochondrial biogenesis: further evidence for proteinase involvement. *FEBS Lett* 105:373–375
- Galkin AV, Tsoi TV, Luzikov VN (1980) Regulation of mitochondrial biogenesis. Occurrence of non-functioning components of the mitochondrial respiratory chain in *Saccharomyces cerevisiae* grown in the presence of proteinase inhibitors: evidence for proteolytic control over assembly of the respiratory chain. *Biochem J* 190:145–156
- Gatti DL, Tzagoloff A (1990) Structure and function of the mitochondrial *bc<sub>1</sub>* complex. Properties of the complex in temperature-sensitive *cor1* mutants. *J Biol Chem* 265:21468–21475
- Giraud MF, Velours J (1994) ATP synthase of yeast mitochondria. Isolation of the F<sub>1</sub> δ subunit, sequence and disruption of the structural gene. *Eur J Biochem* 222:851–859
- Goldberg AL (1992) The mechanism and functions of ATP-dependent proteases in bacterial and animal cells. *Eur J Biochem* 203:9–23
- Goldberg AL (1995) Functions of the proteasome: the lysis at the end of the tunnel. *Science* 268:522–523
- Gottesman S, Maurizi MR (1992) Regulation by proteolysis: energy dependent proteases and their targets. *Microbiol Rev* 56:592–621
- Gottesman S, Clark WP, de Crecy-Lagard V, Maurizi MR (1993) ClpX, an alternative subunit for the ATP-dependent Clp protease of *Escherichia coli*. *J Biol Chem* 268:22618–22626
- Grivell LA (1995) Nucleo-mitochondrial interactions in mitochondrial gene expression. *Crit Rev Biochem Mol Biol* 30:121–164
- Guélin E, Rep M, Grivell LA (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
- Guélin E, Rep M, Grivell LA (1996) Afg3p, a mitochondrial ATP-dependent metalloprotease, is involved in 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
- Haas R, Heinrich PC (1978) The localization of an intracellular membrane-bound proteinase from rat liver. *Eur J Biochem* 91:171–178
- Haas R, Heinrich PC, Sasse D (1979) Proteolytic enzymes of rat liver mitochondria. Evidence for a mast cell origin. *FEBS Lett* 103:168–171
- Hall RE, Hare JF (1990) Respiratory chain-linked NADH dehydrogenase. Mechanisms of assembly. *J Biol Chem* 265:16484–16490
- Hare JF (1978) A novel proteinase associated with mitochondrial membranes. *Biochem Biophys Res Commun* 83:1206–1215
- Hare JF (1990) Mechanisms of membrane protein turnover. *Biochim Biophys Acta* 1031:71–90
- Hare JF, Hodges R (1982a) Turnover of mitochondrial inner membrane proteins in hepatoma monolayer cultures. *J Biol Chem* 257:3575–3580
- Hare JF, Hodges R (1982b) Turnover of mitochondrial matrix polypeptides in hepatoma monolayer cultures. *J Biol Chem* 257:12950–12953
- Hatefi Y (1985) The mitochondrial electron transport and oxidative phosphorylation system. *Annu Rev Biochem* 54:1015–1069
- Hawlitchek G, Schneider H, Schmidt B, Tropschug M, Hartl F-U, Neupert W (1988) Mitochondrial protein import: identification of processing peptidase and of PEP, a processing enhancing protein. *Cell* 53:795–806
- Hayashi J-I, Tanaka M, Sato W, Ozawa T, Yonekawa H, Kagawa Y, Ohta S (1990) Effects of ethidium bromide treatment of mouse cells on expression and assembly of nuclear-encoded subunits of complexes involved in oxidative phosphorylation. *Biochem Biophys Res Commun* 167:216–221
- Herman C, Ogura T, Tomoyasu T, Hiraga S, Akiyama Y, Ito K, Thomas R, D'Ari R, Bouloc P (1993) Cell growth and λ phage development controlled by the same essential *Escherichia coli* gene, *ftsH/hflB*. *Proc Natl Acad Sci USA* 90:10861–10865
- Herman C, Thevenet D, Dari R, Bouloc P (1995) Degradation of σ<sup>32</sup>, the heat-shock regulator in *Escherichia coli* is governed by HflB. *Proc Natl Acad Sci USA* 92:3516–3520
- Herrmann JM, Stuart RA, Craig EA, Neupert W (1994) Mitochondrial heat-shock protein 70, a molecular chaperone for proteins encoded by mitochondrial DNA. *J Cell Biol* 127:893–902
- Hofhaus G, Attardi G (1993) Lack of assembly of mitochondrial DNA-encoded subunits of respiratory NADH dehydrogenase and loss of enzyme activity in a human cell mutant lacking the mitochondrial ND4 gene product. *EMBO J* 12:3043–3048
- Holland DR, Hausrath AC, Juers D, Matthews BW (1995) Structural analysis of zinc substitutions in the active site of thermolysin. *Protein Sci* 4:1955–1965
- Horwich AL (1995) Molecular chaperones – resurrection or destruction? *Curr Biol* 5:455–458
- Isaya G, Miklos D, Rollins RA (1994) *MIP1*, a new yeast gene homologous to the rat mitochondrial intermediate peptidase gene, is required for oxidative metabolism in *Saccharomyces cerevisiae*. *Mol Cell Biol* 14:5603–5616
- Jean-Francois MJB, Hadikusumo RG, Watkins LC, Lukins HB, Linnane AW, Marzuki S (1986a) Correlation of defined lesions in the N, N'-dicyclohexylcarbodiimide-binding proteolipid with defects in the function and assembly of yeast mitochondrial H<sup>+</sup>-ATPase and other respiratory enzyme complexes. *Biochim Biophys Acta* 852:133–143
- Jean-Francois MJB, Lukins HB, Marzuki S (1986b) Post-transcriptional defects in the synthesis of the mitochondrial H<sup>+</sup>-ATPase subunit 6 in yeast mutants with lesions in the subunit 9 gene. *Biochim Biophys Acta* 868:178–182
- Jensen RE, Yaffe MP (1988) Import of proteins into yeast mitochondria: the nuclear *MAS2* gene encodes a component of the processing protease that is homologous to the *MAS1*-encoded subunit. *EMBO J* 7:3863–3871
- Kalnov SL, Novikova LA, Zubatov AS, Luzikov VN (1979a) Participation of a mitochondrial proteinase in the breakdown of mitochondrial translation products in yeast. *FEBS Lett* 101:355–358
- Kalnov SL, Novikova LA, Zubatov AS, Luzikov VN (1979b) Proteolysis of the products of mitochondrial protein synthesis in yeast mitochondria and sub-mitochondrial particles. *Biochem J* 182:195–202
- Kalousek F, Isaya G, Rosenberg LE (1992) Rat liver mitochondrial intermediate peptidase (MIP): purification and initial characterization. *EMBO J* 11:2803–2809
- Kaput J, Goltz S, Blobel G (1982) Nucleotide sequence of the yeast nuclear gene for cytochrome *c* peroxidase precursor. Functional implications of the presequence for protein transport into mitochondria. *J Biol Chem* 257:15054–15058

- Kessel M, Maurizi MR, Kim B, Kocsis E, Trus BL, Singh SK, Steven AC (1995) Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26S proteasome. *J Mol Biol* 250:587–594
- Kihara A, Akiyama Y, Ito K (1995) FtsH is required for proteolytic elimination of uncomplexed forms of SecY, an essential protein translocase subunit. *Proc Natl Acad Sci USA* 92:4532–4536
- Kimura T (1969) Effects of hypophysectomy and ACTH administration on the level of adrenal cholesterol side-chain desmolase. *Endocrinology* 85:492–499
- Klausner RD (1989) Architectural editing: determining the fate of newly synthesized membrane proteins. *New Biologist* 1:3–8
- Krummeck G, Rödel G (1990) Yeast SCO1 protein is required for a post-translational step in the accumulation of mitochondrial cytochrome *c* oxidase subunits I and II. *Curr Genet* 18:13–15
- Kumamoto T, Ito A, Omura T (1986) Characterization of a mitochondrial matrix protease catalyzing the processing of adrenodoxin precursor. *J Biochem* 100:247–254
- Kutejová E, Durcová G, Surovková E, 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
- Langer T, Pajic A, Wagner I, Neupert W (1995) Proteolytic breakdown of membrane-associated polypeptides in mitochondria of *Saccharomyces cerevisiae*. *Methods Enzymol* 260:495–503
- Levchenko I, Luo L, Baker TA (1995) Disassembly of the mu transposase tetramer by the ClpX chaperone. *Genes Dev* 9:2399–2408
- Luciaková K, Kuzela S (1992) Increased steady state levels of several mitochondrial and nuclear gene transcripts in rat hepatoma with a low content of mitochondria. *Eur J Biochem* 205: 1187–1193
- Luzikov VN (1985) Mitochondrial biogenesis and breakdown. Plenum Press, New York
- Luzikov VN (1986) Proteolytic control over topogenesis of membrane proteins. *FEBS Lett* 200:259–264
- Luzikov VN, Makhliis TA, Galkin AV (1976) A probable role of cell proteinases in the biogenesis of mitochondria in yeast. *FEBS Lett* 69:108–110
- Luzikov VN, Novikova LA, Tikhonov AN, Zubatov AS (1983) Correlation between the rate of proteolysis of mitochondrial translation products and fluidity of the mitochondrial inner membrane in *Saccharomyces cerevisiae* yeast. Alteration of the rate of proteolysis under glucose repression. *Biochem J* 214:785–794
- Luzikov VN, Novikova LA, Zubatov AS, Tikhonov AN (1984) Physical state of the mitochondrial inner membrane as a factor controlling the proteolysis of mitochondrial translation products in yeast. *Biochim Biophys Acta* 775:22–30
- Manoil C, Traxler B (1995) Membrane protein assembly: genetic, evolutionary and medical perspectives. *Annu Rev Genet* 29: 131–150
- Marver HS, Collins A, Tschudy DP, Rechcigl M (1966)  $\delta$ -aminolevulinic acid synthetase. II. Induction in rat liver. *J Biol Chem* 241:4323–4329
- McAda PC, Douglas MG (1982) A neutral metallo endoprotease involved in the processing of an  $F_1$ -ATPase subunit precursor in mitochondria. *J Biol Chem* 257:3177–3182
- McEwen JE, Ko C, Kloekner-Gruissem B, Poyton RO (1986) Nuclear functions required for cytochrome *c* oxidase biogenesis in *Saccharomyces cerevisiae*. Characterization of mutants in 34 complementation groups. *J Biol Chem* 261:11872–11879
- Miura S, Mori M, Amaya Y, Tatibana M (1982) A mitochondrial protease that cleaves the precursor of ornithine carbamoyltransferase. *Eur J Biochem* 122:641–647
- Moczko M, Schonfisch B, Voos W, Pfanner N, Rassow J (1995) The mitochondrial ClpB homolog Hsp78 cooperates with matrix Hsp70 in the maintenance of mitochondrial function. *J Mol Biol* 254:538–543
- Morgan A, Burgoyne RD (1995) Is NSF a fusion protein? *Trends Cell Biol* 5:335–339
- Nakai M, Endo T, Hase T, Matsubara H (1993) Intra-mitochondrial protein sorting. Isolation and characterization of the yeast *MSP1* gene which belongs to a novel family of putative ATPases. *J Biol Chem* 268:24262–24269
- Nakai T, Mera Y, Yasuhara T, Ohashi A (1994) Divalent metal ion-dependent mitochondrial degradation of unassembled subunits 2 and 3 of cytochrome *c* oxidase. *J Biochem* 116:752–758
- Nakai T, Yasuhara T, Fujiki Y, 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
- Nicoletti M, Guerri C, Grisolia S (1977) Turnover of carbamyl-phosphate synthase, of other mitochondrial enzymes and of rat tissues. *Eur J Biochem* 75:583–592
- Nijtmans LGJ, Spelbrink JN, Van Galen MJM, Zwaan M, Klement P, Van den Bogert C (1995) Expression and fate of the nuclearly encoded subunits of cytochrome *c* oxidase in cultured human cells depleted of mitochondrial gene products. *Biochim Biophys Acta* 1265:117–126
- Nilsson D, Lauridsen AA, Tomoyasu T, Ogura T (1994) A *Lactococcus lactis* gene encodes a membrane protein with putative ATPase activity that is homologous to the essential *Escherichia coli* *ftsH* gene product. *Microbiology* 140:2601–2610
- Nobrega FG, Nobrega MP, Tzagoloff A (1992) *BCS1*, a novel gene required for the expression of functional Rieske iron-sulfur protein in *Saccharomyces cerevisiae*. *EMBO J* 11:3821–3829
- Norais N, Prome D, Velours J (1991) ATP synthase of yeast mitochondria. Characterization of subunit d and sequence analysis of the structural gene *ATP7*. *J Biol Chem* 266:16541–16549
- Norais N, Vaillier J, Velours J (1994) Over-expression of the yeast ATP synthase subunit d in *Escherichia coli*: use of polyclonal antibodies directed against recombinant subunit d. *Biochem Biophys Res Commun* 200:877–883
- Novikova LA, Zubatov AS, Luzikov VN (1981) Multiplicity of mitochondrial proteinases in yeast. *FEBS Lett* 135:245–248
- Nunnari J, Fox TD, Walter P (1993) A mitochondrial protease with two catalytic subunits of non-overlapping specificities. *Science* 262:1997–2004
- Pacifici RE, Davies KJA (1990) Protein degradation as an index of oxidative stress. *Methods Enzymol* 186:485–502
- Pajic A, Tauer R, Feldmann H, Neupert W, Langer T (1994) Yta10p is required for the ATP-dependent degradation of polypeptides in the inner membrane of mitochondria. *FEBS Lett* 353:201–206
- Paul M-F, Tzagoloff A (1995) Mutations in *RCA1* and *AFG3* inhibit  $F_1$ -ATPase assembly in *Saccharomyces cerevisiae*. *FEBS Lett* 373:66–70
- Paul M-F, Velours J, Arselin de Chateaubodeau G, Aigle M, Guerin B (1989) The role of subunit 4, a nuclear-encoded protein of the  $F_0$  sector of yeast mitochondrial ATP synthetase, in the assembly of the whole complex. *Eur J Biochem* 185:163–171
- Pearce DA, Sherman F (1995a) Degradation of cytochrome oxidase subunits in mutants of yeast lacking cytochrome *c* and suppression of the degradation by mutation of *yme1*. *J Biol Chem* 270:20879–20882
- Pearce DA, Sherman F (1995b) Diminished degradation of yeast cytochrome *c* by interaction with its physiological partners. *Proc Natl Acad Sci USA* 92:3735–3739
- Pearce DA, Sherman F (1995c) Enhanced stability of a thermodynamically stable mutant form of yeast iso-1-cytochrome *c*. *Mol Gen Genet* 249:155–161
- Peters J-M (1994) Proteasomes: protein degradation machines of the cell. *Trends Biochem Sci* 19:377–382
- Peters J-M, Walsh MJ, Franke WW (1990) An abundant and ubiquitous homo-oligomeric ring-shaped ATPase particle related to the putative vesicle fusion proteins Sec18p and NSF. *EMBO J* 9:1757–1767
- Peters J-M, Cejka Z, Harris JR, Kleinschmidt JA, Baumeister W (1993) Structural features of the 26S proteasome complex. *J Mol Biol* 234:932–937
- Pollock RA, Hartl F-U, Cheng MY, Ostermann A, Horwich A, Neupert W (1988) The processing peptidase of yeast mitochondria: the two co-operating components MPP and PEP are structurally related. *EMBO J* 7:3493–3500
- Purvis JL, Canick JA, Mason JI, Estabrook RW, McCarthy JL (1973) Lifetime of adrenal cytochrome p-450 as influenced by ACTH. *Ann NY Acad Sci* 212:319–343

- Rago JP di, Macadre C, Lazowska J, Slonimski PP (1993) The C-terminal domain of yeast cytochrome *b* is essential for a correct assembly of the mitochondrial cytochrome *bc*<sub>1</sub> complex. *FEBS Lett* 328:153–158
- Rapoport S, Dubiel W, Muller M (1982) Characteristics of an ATP-dependent proteolytic system of rat liver mitochondria. *FEBS Lett* 147:93–96
- Rechsteiner M, Hoffman L, Dubiel W (1993) The multi-catalytic and 26S proteases. *J Biol Chem* 268:6065–6068
- Rubin DM, Coux O, Wefes I, Hengartner C, Young RA, Goldberg AL, Finley D (1996) Identification of the *gal4* suppressor Sug1 as a subunit of the yeast 26S proteasome. *Nature* 379:655–657
- Rubio V, Grisolia S (1977) Prominent role of lysosomes in the proteolysis of rat liver mitochondria at neutral pH. *FEBS Lett* 75:281–284
- Sagbini M, Broomfield PLE, Scheffler IE (1994) Studies on the assembly of complex II in yeast mitochondria using chimeric human/yeast genes for the iron-sulphur protein subunit. *Biochemistry* 33:159–165
- Schmitt M, Neupert W, Langer T (1995) Hsp78, a Clp homologue within mitochondria, can substitute for chaperone functions of mt-Hsp70. *EMBO J* 14:3434–3444
- Schnall R, Mannhaupt G, Stucka R, Tauer R, Ehnle S, Schwarzlose C, Vetter I, Feldmann H (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
- Schoppink PJ, Hemrika W, Reynen JM, Grivell LA, Berden JA (1988) Yeast ubiquinol: cytochrome *c* oxidoreductase is still active after inactivation of the gene encoding the 17-kDa subunit VI. *Eur J Biochem* 173:115–122
- Schoppink PJ, Berden JA, Grivell LA (1989) Inactivation of the gene encoding the 14-kDa subunit VII of yeast ubiquinol: cytochrome *c* oxidoreductase and analysis of the resulting mutant. *Eur J Biochem* 181:475–483
- Schulze M, Rödel G (1989) Accumulation of the cytochrome *c* oxidase subunits I and II in yeast requires a mitochondrial membrane-associated protein, encoded by the nuclear *SCO1* gene. *Mol Gen Genet* 216:37–43
- Sebald W, Weiss H, Jackl G (1972) Inhibition of the assembly of cytochrome oxidase in *Neurospora crassa* by chloramphenicol. *Eur J Biochem* 30:413–417
- Sen K, Beattie DS (1985) Decreased amounts of core proteins I and II and the iron-sulfur protein in mitochondria from yeast lacking cytochrome *b* but containing cytochrome *c*<sub>1</sub>. *Arch Biochem Biophys* 242:393–401
- Shirai Y, Akiyama Y, Ito K (1996) Suppression of *ftsH* mutant phenotypes by overproduction of molecular chaperones. *J Bacteriol* 178:1141–1145
- Sitte N, Drung I and Dubiel W (1995) Identification of a novel ATP-dependent proteolytic activity in mitochondrial intermembrane space. *Biochem Mol Biol Int* 36:871–881
- Squires C, Squires CL (1992) The Clp proteins: proteolysis regulators or molecular chaperones. *J Bacteriol* 174:1081–1085
- Subramanian M, Katyare SS, Fatterpaker P, Sreenivasan A (1975) A protease in rat liver mitochondria: its physiological significance. *Ind J Biochem Biophys* 12:307–310
- Suzuki CK, Suda K, Wang N, Schatz G (1994) Requirement for the yeast gene *LON* in intra-mitochondrial proteolysis and maintenance of respiration. *Science* 264:273–276
- Swick RW, Rexroth AK, Stange JL (1968) The metabolism of mitochondrial proteins. III. The dynamic state of rat liver mitochondria. *J Biol Chem* 243:3581–3587
- Takehige K, Baba M, Tsuboi S, Noda T, Ohsumi Y (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 119:301–311
- Tanaka K, Waxman L, Goldberg AL (1983) ATP serves two distinct roles in protein degradation in reticulocytes, one requiring and one independent of ubiquitin. *J Cell Biol* 96:1580–1585
- Tauer R, Mannhaupt G, Schnall R, Pajic A, Langer T, Feldmann H (1994) Yta10p, a member of a novel ATPase family in yeast, is essential for mitochondrial function. *FEBS Lett* 353:197–200
- Thorsness PE, Fox TD (1993) Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. *Genetics* 134:21–28
- Thorsness PE, White KH, Fox TD (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
- Tomoyasu T, Yamanaka K, Murata K, Suzuki T, Bouloc P, Kato A, Niki H, Hiraga S, Ogura T (1993a) Topology and subcellular localization of FtsH protein in *Escherichia coli*. *J Bacteriol* 175:1352–1357
- Tomoyasu T, Yuki T, Morimura S, Mori H, Yamanaka K, Niki H, Hiraga S, Ogura T (1993b) The *Escherichia coli* FtsH protein is a prokaryotic member of a protein family of putative ATPases involved in membrane functions, cell-cycle control, and gene expression. *J Bacteriol* 175:1344–1351
- Tomoyasu T, Gamer J, Bukau B, Kanemori M, Mori H, Rutman AJ, Oppenheim AB, Yura T, Yamanaka K, Niki H, Hiraga S, Ogura T (1995) *Escherichia coli* FtsH is a membrane-bound, ATP-dependent protease which degrades the heat-shock transcription factor  $\sigma^{32}$ . *EMBO J* 14:2551–2560
- Tzagoloff A, Yue J, Jang J, Paul MF (1994) A new member of a family of ATPases is essential for assembly of the mitochondrial respiratory chain and ATP synthetase complexes in *Saccharomyces cerevisiae*. *J Biol Chem* 269:26144–26151
- Van Dyck L, Pearce DA, 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
- Voellmy WV, Goldberg AL (1981) ATP-stimulated endoprotease is associated with the cell membrane of *E. coli*. *Nature* 290:419–421
- Wagner I, Arlt H, van Dyck L, Langer T, Neupert W (1994) Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. *EMBO J* 13:5135–5145
- Wang N, Gottesman S, Willingham MC, Gottesman MM, Maurizi MR (1993) A human mitochondrial ATP-dependent protease that is highly homologous to bacterial Lon protease. *Proc Natl Acad Sci USA* 90:11247–11251
- Watabe S, Kimura T (1985a) Adrenal cortex mitochondrial enzyme with ATP-dependent protease and protein-dependent ATPase activities. *J Biol Chem* 260:14498–14504
- Watabe S, Kimura T (1985b) ATP-dependent protease in bovine adrenal cortex. Tissue specificity, subcellular localization, and partial characterization. *J Biol Chem* 260:5511–5517
- Watabe S, Hara T, Kohno H, Hiroi T, Yago N, Nakazawa T (1993) In vitro degradation of mitochondrial proteins by ATP-dependent protease in bovine adrenal cortex. *J Biochem* 113:672–676
- Watabe S, Kohno H, Kouyama H, Hiroi T, Yago N, Nakazawa T (1994) Purification and characterization of a substrate protein for mitochondrial ATP-dependent protease in bovine adrenal cortex. *J Biochem* 115:648–654
- Watabe S, Hasegawa H, Takimoto K, Yamamoto Y, Takahashi SY (1995) Possible function of SP-22, a substrate of mitochondrial ATP-dependent protease, as a radical scavenger. *Biochem Biophys Res Commun* 213:1010–1016
- Wawrzynow A, Wojtkowiak D, Marszalek J, Banecki B, Jonsen M, Graves B, Georgopoulos C, Zylicz M (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
- Waxman L, Fagan JM, Tanaka K, Goldberg AL (1985) A soluble ATP-dependent system for protein degradation from murine erythroleukemia cells. Evidence for a protease which requires ATP hydrolysis but not ubiquitin. *J Biol Chem* 260:11994–12000
- Weber ER, Hanekamp T, Thorsness PE (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
- Weiss-Brummer B, Guba R, Haid A, Schweyen RJ (1979) Fine structure of *OXI1*, the mitochondrial gene coding for subunit II of yeast cytochrome *c* oxidase. *Curr Genet* 1:75–83

- Wenzel T, Baumeister W (1995) Conformational constraints in protein degradation by the 20S proteasome. *Nature Struct Biol* 2:199–204
- Wheeldon LW, Dianoux AC, Bof M, Vignais PV (1974) Stable and labile products of mitochondrial protein synthesis in vitro. *Eur J Biochem* 46:189–199
- Whiteheart SW, Rossnagel K, Buhrow SA, Brunner M, Jaenicke R, Rothman JE (1994) N-ethylmaleimide-sensitive fusion protein: a trimeric ATPase whose hydrolysis of ATP is required for membrane fusion. *J Cell Biol* 126:945–954
- Wickner S, Gottesman S, Skowyra D, Hoskins J, Mckenney K, Maurizi MR (1994) A molecular chaperone, ClpA, functions like DnaK and DnaJ. *Proc Natl Acad Sci USA* 91:12218–12222
- Wu M, Tzagoloff A (1989) Identification and characterization of a new gene (*CBP3*) required for the expression of yeast coenzyme QH<sub>2</sub>-cytochrome *c* reductase. *J Biol Chem* 264:11122–11130
- Yang M, Trumpower BL (1994) Deletion of *QCR6*, the gene encoding subunit six of the mitochondrial cytochrome *bc*<sub>1</sub> complex, blocks maturation of cytochrome *c*<sub>1</sub>, and causes temperature-sensitive petite growth in *Saccharomyces cerevisiae*. *J Biol Chem* 269:1270–1275
- Yang M, Jensen RE, Yaffe MP, Oppliger W, Schatz G (1988) Import of proteins into yeast mitochondria: the purified matrix-processing protease contains two subunits which are encoded by the nuclear *MAS1* and *MAS2* genes. *EMBO J* 7:3857–3862
- Yasuhara T, Ohashi A (1987) New chelator-sensitive proteases in the matrix of yeast mitochondria. *Biochim Biophys Acta* 144:277–283
- Yasuhara T, Mera Y, Nakai T, Ohashi A (1994) ATP-dependent proteolysis in yeast mitochondria. *J Biochem* 115:1166–1171
- Zubatov AS, Mikhailova AE, Luzikov VN (1984) Detection, isolation and some properties of membrane proteinases from yeast mitochondria. *Biochim Biophys Acta* 787:188–195