Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor

Mark Herlan, Carsten Bornhövd, Kai Hell, Walter Neupert, and Andreas S. Reichert
Adolf-Butenandt-Institut für Physiologische Chemie, Ludwig-Maximilians-Universität München, 81377 München, Germany

Mitochondrial morphology and inheritance of mitochondrial DNA in yeast depend on the dynamin-like GTPase Mgm1. It is present in two isoforms in the intermembrane space of mitochondria both of which are required for Mgm1 function. Limited proteolysis of the large isoform by the mitochondrial rhomboid protease Pcp1/Rbd1 generates the short isoform of Mgm1 but how this is regulated is unclear. We show that near its NH2 terminus Mgm1 contains two conserved hydrophobic segments of which the more COOH-terminal one is cleaved by Pcp1. Changing the hydrophobicity of the NH2-terminal segment modulated the ratio of the isoforms and led to fragmentation of mitochondria. Formation of the short isoform of Mgm1 and mitochondrial morphology further depend on a functional protein import motor and on the ATP level in the matrix. Our data show that a novel pathway, to which we refer as alternative topogenesis, represents a key regulatory mechanism ensuring the balanced formation of both Mgm1 isoforms. Through this process the mitochondrial ATP level might control mitochondrial morphology.

Introduction

Mitochondria in various eukaryotes from yeast to human form a tubular network, which depends on the balance of fusion and fission processes (Shaw and Nunnari, 2002). This dynamic nature of mitochondrial morphology is essential for the inheritance of mitochondrial DNA (mtDNA), apoptosis, and defense against oxidative damage and aging (for review see Westermann, 2003). One protein essential for mitochondrial morphology and inheritance of mtDNA in Saccharomyces cerevisiae is the dynamin-like GTPase Mgm1 (Guan et al., 1993; Wong et al., 2000). Its human orthologue, OPA1, is associated with optic atrophy type I in humans (Alexander et al., 2000; Delettre et al., 2000). Mgm1 was shown to be crucial for fusion of mitochondria (Sesaki et al., 2003b; Wong et al., 2003). Mgm1 is present in two isoforms in the intermembrane space of mitochondria, both of which are required for function (Herlan et al., 2003). The short isoform of Mgm1 (s-Mgm1) is generated by limited proteolysis of the large isoform of Mgm1 (l-Mgm1) by the mitochondrial rhomboid protease Pcp1 (Herlan et al., 2003; McQuibban et al., 2003; Sesaki et al., 2003a). However, it is largely unknown how the balanced formation of both isoforms is regulated. Pcp1 is also required for the processing of cytochrome c peroxidase (Ccp1; Esser et al., 2002) and is essential for wild-type mitochondrial morphology (Dimmer et al., 2002). Rhomboids form a conserved protein family of intramembrane serine proteases, which cleave substrate proteins within single transmembrane segments (Urban and Freeman, 2003). Here, we provide evidence for the pathway of Mgm1 biogenesis, which we termed alternative topogenesis.

Results and discussion

Mgm1 contains two conserved hydrophobic segments of which the more COOH-terminal one is cleaved by Pcp1

Two different cleavage sites for Pcp1 within Mgm1 have been proposed. One was suggested to reside in the predicted transmembrane segment between amino acid residues 94 and 111 (McQuibban et al., 2003), another one between residues 160 and 161 representing the start of s-Mgm1 as determined by NH2-terminal sequencing (Herlan et al., 2003). The latter cleavage site is part of a so far unrecognized

Abbreviations used in this paper: Ccp1, cytochrome c peroxidase; DHFR, dihydrofolate reductase; l-Mgm1, large isoform of Mgm1; mtDNA, mitochondrial DNA; s-Mgm1, short isoform of Mgm1.

Supplemental Material can be found at: /content/suppl/2004/04/19/jcb.200403022.DC1.html

The online version of this article contains supplemental material.

Address correspondence to Andreas S. Reichert, Adolf-Butenandt-Institut für Physiologische Chemie, Ludwig-Maximilians-Universität München, Butenandstr. 5, 81377 München, Germany. Tel.: 49-89-2180-77100. Fax: 49-89-2180-77093. email: Andreas.Reichert@bio.med.uni-muenchen.de

Key words: mitochondrial fusion; protein import; mitochondrial diseases; rhomboid protease; dynamin-like protein
second hydrophobic segment comprising residues 156–169 (Fig. 1 A). This region contains helix-breaking residues like glycine and proline, which were reported to be important for cleavage by rhomboid proteases (Urban and Freeman, 2003). The organization of two consecutive hydrophobic segments close to the NH$_2$ terminus is conserved in Mgm1 orthologues from yeast to human (Fig. 1 A). To investigate where cleavage occurs, we deleted either segment and expressed these variants in a Δmgm1 background (Fig. 1 B). Deletion of the second hydrophobic segment (Fig. 1 B, Δ2) or of both segments (Fig. 1 B, Δ1&Δ2) prevented formation of s-Mgm1, which is consistent with earlier results (Herlan et al., 2003). Deletion of the first transmembrane segment (Fig. 1 B, Δ1), however, led to exclusive formation of s-Mgm1.

Figure 1. Balanced formation of both isoforms of Mgm1 is modulated by two consecutive hydrophobic segments. (A) Hydrophobicity plots of the NH$_2$-termini of Mgm1 and its orthologues according to Kyte and Doolittle (1982). Numbers below indicate amino acid position after which cleavage by mitochondrial processing peptidase (MPP) or mitochondrial rhomboid protease (Pcp1, C13E7.11, 1D784, PARL) occurs. Putative rhomboid proteases and predicted cleavage regions are indicated by question mark. S.c., Saccharomyces cerevisiae; S.p., Schizosaccharomyces pombe; C.e., Caenorhabditis elegans; H.s., Homo sapiens; MTS, mitochondrial targeting sequence. For OPA1 splice variant 8 was analyzed. (B) Immunoblotting with antibodies against Mgm1 of total yeast cell extracts from Δmgm1 strains (or Δpcp1Δmgm1 strain, respectively) expressing indicated Mgm1 version. Bands corresponding to l-Mgm1 and s-Mgm1 are indicated. Mgm1 versions: WT, wild-type; Δ1, lacking first hydrophobic segment (residues 91–111); Δ2, lacking second hydrophobic segment (residues 154–167); Δ1&Δ2, lacking both hydrophobic segments; G100D, G100K, respective point mutations; VVL, three residues (GGM) at position 100–102 were replaced by VVL. (C) Mitochondrial morphology of indicated strains was scored for at least 150 cells in three experiments. The amount of cells containing a mitochondrial tubular network is expressed as percentage of the control strain expressing Mgm1. SD is indicated by the errors bars. (D) Representative fluorescence (left) and phase contrast (right) images of indicated strains expressing mitochondrially targeted GFP. Bar, 5 μm.
Therefore, the cleavage site for Pcp1 resides in the second hydrophobic segment of Mgm1.

The hydrophobicity of the first hydrophobic segment determines the ratio of l-Mgm1 to s-Mgm1 and affects mitochondrial morphology

Our results suggest that the balanced formation of Mgm1 isoforms is influenced by the first hydrophobic segment of Mgm1. We altered the hydrophobicity of this stretch by site-directed mutagenesis and expressed these variants in a Δmgm1 background. When its hydrophobicity was increased (Fig. 1 B, VVL), formation of s-Mgm1 was strongly inhibited, which is consistent with another study in which s-Mgm1 could not be detected using the same variant of Mgm1 (McQuibban et al., 2003). In contrast, we observed low levels of s-Mgm1 with this variant in a Δmgm1, but not in a Δpcp1Δmgm1, background (Fig. 1 B). Thus, Pcp1 dependent cleavage is still possible. However, introducing a charged residue resulted in the conversion of most (Fig. 1 B, G100K) if not all (Fig. 1 B, G100D) of Mgm1 to s-Mgm1. All variants of Mgm1 were correctly targeted to the intermembrane space. The membrane association of the two isoforms, as judged from salt and carbonate extraction experiments, was not altered in the variants as compared with wild-type Mgm1 (unpublished data). We conclude that the hydrophobicity of the first hydrophobic segment determines the relative proportion of the two isoforms of Mgm1. The absence of either isoform of Mgm1 results in fragmentation of mitochondria and loss of mtDNA (Herlan et al., 2003). Consistently, extensive fragmentation of mitochondria was observed when the ratio of both isoforms strongly deviated from 1:1 (Fig. 1, B–D).
A functional import motor is crucial for formation of s-Mgm1 and mitochondrial morphology

The first transmembrane segment of Mgm1 may act as a stop transfer signal during import of Mgm1 into mitochondria. Cleavage of the targeting signal by the mitochondrial processing peptidase leads to l-Mgm1, which is anchored to the inner membrane via this segment (Herlan et al., 2003). To check whether the balance between both isoforms is established already at the level of import of the precursor protein, we investigated whether down-regulation of essential components of the import motor of the inner mitochondrial membrane shifts the ratio of the two Mgm1 isoforms. Tim44 and Tim14 are such components. Together with Ssc1, the mitochondrial Hsp70 in yeast, and its nucleotide exchange factor Mge1, they mediate the ATP-driven import of preproteins into the mitochondrial matrix and the inner membrane (Neupert and Brunner, 2002; Mokranjac et al., 2003). Indeed, down-regulation of Tim44 and of Tim14 resulted in a substantial reduction in the formation of s-Mgm1 (Fig. 2 A), which is paralleled by increased fragmentation of mitochondria (Fig. 2, B and E). To rule out that reduced levels of the rhomboid protease Pcp1 caused decreased proteolysis of Mgm1, we determined the processing efficiency of Ccp1, the only other known substrate of Pcp1 (Esser et al., 2002). Upon down-regulation of Pcp1, accumulation of the intermediate form of Ccp1 and decreased levels of s-Mgm1 occur simultaneously showing that processing of Ccp1 and of Mgm1 are affected to a similar extent (Fig. 2 A; Fig. S1 A, available at http://www.jcb.org/cgi/content/full/jcb.200403022/DC1). Down-regulation of Tim14 or Tim44 resulted in reduced Ccp1 levels at late time points but as no intermediate was observed Ccp1 processing was not impaired (Fig. 2 A). In this case, Pcp1 is not limiting for the formation of s-Mgm1. We conclude that Tim14 and Tim44 are necessary for the formation of s-Mgm1. Tim17 is an essential subunit of the TIM23 preprotein conducting channel of the inner membrane (Neupert and Brunner, 2002). Down-regulation of Tim17 only had a mild effect on the formation of either isoform of Mgm1. Reduced import of both Mgm1 isoforms and potentially of other components essential for wild-type mitochondrial morphology are most likely the reason for the effects on mitochondrial morphology upon down-regulation of Tim17 (Fig. 2, B and E). Tim22 is essential for import of proteins with internal signal sequences such as the ADP/ATP carrier (Sirrenberg et al., 1996). Mgm1 is synthesized as a precursor with an NH₂-terminal targeting sequence and therefore unlikely to be a substrate for Tim22. Indeed, down-regulation of Tim22 neither affected Mgm1 biogenesis nor mitochondrial morphology. No component essential for wild-type

Figure 3. Mitochondrial morphology and formation of s-Mgm1 is ATP dependent. (A–C; left) Radiolabeled Mgm11–228–DHFR precursors were imported into isolated yeast mitochondria and treated with 50 μg/ml trypsin after import. p, precursor; l, l-Mgm1(Δ21–228–DHFR; L, 20% of radiolabeled precursor used per import reaction. (Right) The relative amount of s-Mgm11–228–DHFR as percentage of total Mgm11–228–DHFR was determined by densitometric quantification. (A) Indicated variants (compare with Fig. 1) of radiolabeled Mgm11–228–DHFR precursors were imported into yeast mitochondria. For VVL and Δ2 background intensity at the size corresponding to s-Mgm11–228–DHFR was quantified. (B) Mitochondria were depleted from ATP before import where indicated. (C) Mitochondria isolated from wild-type or scc1–3 temperature-sensitive mutant were preincubated at the indicated temperature for 15 min before import. (A–C) Statistically highly significant deviations (P < 0.01) compared (A) to wild-type (n = 8), (B) to import without ATP depletion (n = 6), and (C) to import after preincubation at 24°C (n = 6) according to Wilcoxon test are indicated by **. (D) Analysis of the M28–82 strain (atp6) containing a mutation, which was mapped to the mitochondrially encoded ATP6 gene. Wild-type and mutant strain were grown on nonfermentable carbon source at 30°C and used for immunoblotting of total yeast cell extracts with antibodies against Mgm1 and Ccp1. (E) Mitochondrial morphology for cells analyzed in D (at least 150 cells in four experiments). SD is indicated by error bars. (F) Representative fluorescence (left) and phase contrast (right) images of the M28–82 strain stained with rhodamine B hexyl ester. Bar, 5 μm.
mitochondrial morphology seems to require the Tim22 import pathway into the inner membrane. Moreover, the reduction of s-Mgm1 levels is not a general consequence of down-regulating an essential mitochondrial protein.

We checked whether in temperature-sensitive mutants of Ssc1 similar effects are observed. Ssc1 is an essential part of the import motor (Gambill et al., 1993; Neupert and Brunner, 2002). Already at the permissive temperature (24°C) l-Mgm1 dominated slightly over s-Mgm1 in the ssc1-2 and the ssc1-3 mutants but not in the isogenic wild-type strain (Fig. 2, C). Upon shift to the nonpermissive temperature (37°C) the amount of l-Mgm1 increased progressively with time compared with s-Mgm1 in the ssc1 mutants (Fig. 2 C). This increase was well correlated with the loss of wild-type mitochondrial morphology (Fig. 2, D and E). Ccp1 processing was not altered indicating that the effect was not due to reduced activity of Pcp1 (Fig. 2 C). Interestingly, these ssc1 mutants were observed previously to exhibit altered mitochondrial morphology at 37°C (Kawai et al., 2001). We conclude that after the NH2-terminal transmembrane segment has entered the TIM23 translocase in the inner membrane, a functional import motor is necessary to drive further translocation until the second hydrophobic segment reaches the inner membrane and subsequently is cleaved by Pcp1.

Formation of s-Mgm1 but not of l-Mgm1 is ATP dependent

To further investigate topogenesis of Mgm1 isoforms in vitro, radiolabeled variants of Mgm11-228-dihydrofolate reductase (DHFR) precursors were imported into isolated yeast mitochondria and subsequently treated with trypsin. After import, bands corresponding to l- and s-Mgm11-228–DHFR were observed (Fig. 3 A). Consistent with the results obtained in vivo (Fig. 1 B) formation of s-Mgm11-228–DHFR was increased with variants in which the first hydrophobic segment was more hydrophilic (Fig. 3 A, G100D, G100K). No formation of s-Mgm11-228–DHFR was observed when it was more hydrophobic (Fig. 3 A, VVL) or when the second hydrophobic segment was absent (Fig. 3 A, Δ2). We imported Mgm11-228–DHFR into isolated mitochondria with and without prior depletion of matrix ATP. Upon ATP depletion, generation of s-Mgm11-228–DHFR was strongly reduced (Fig. 3 B). Finally, formation of s-Mgm11-228–DHFR was strongly affected when isolated mitochondria derived from the ssc1-3 mutant were preincubated at the nonpermissive temperature before import experiments (Fig. 3 C). Therefore, the formation of s-Mgm11-228–DHFR but not of l-Mgm11-228–DHFR is ATP dependent, which most likely results from the ATP dependency of Ssc1. We suggest that the cleavage site for Pcp1 only becomes accessible and cleaved in the inner membrane when sufficient matrix ATP is present.

Reduced ATP levels in vivo lead to a decreased formation of s-Mgm1 and to fragmentation of mitochondria

We investigated whether under growth conditions leading to reduced levels of matrix ATP an increase of the ratio of l-Mgm1 to s-Mgm1 can be observed. We analyzed the M28-82 strain containing a mutation, which was mapped to the mitochondrially encoded ATP6 gene and leads to reduced ATP synthesis and to slow growth on nonfermentable carbon sources (Foury and Tzagoloff, 1976). The ratio of the Mgm1 isoforms was found to be indeed shifted towards l-Mgm1, and mitochondrial morphology was strongly affected (Fig. 3, D–F). Therefore, mitochondrial morphology seems to be altered under energetically unfavorable conditions.

Model of alternative topogenesis

Our data support a novel mechanism that regulates the balanced formation of both Mgm1 isoforms (Fig. 4). The mitochondrial membrane potential (Fig. 4, ΔΨ) is sufficient to import the presequence of Mgm1 (residues 1–80) even at low levels of matrix ATP. The immediately following first hydrophobic segment can act as a stop-transfer sequence as shown previously for other preproteins (Neupert and Brunner, 2002). The efficiency of the stop transfer depends on the hydrophobicity of this segment. Processing by the mitochondrial processing peptidase and lateral insertion into the inner membrane lead to l-Mgm1. At high levels of matrix ATP the mitochondrial import motor “pulls in” part of the preprotein further and the second hydrophobic segment reaches the inner membrane. Pcp1 cleavage within this segment generates s-Mgm1. In this way, lateral insertion of the first hydrophobic segment into the inner membrane yielding l-Mgm1 and further ATP driven import with subsequent processing yielding s-Mgm1 are competing processes. This novel pathway of alternative topogenesis of Mgm1 during import into mitochondria is a key regulatory mechanism, which is crucial for the balanced formation of both isoforms. The process of alternative topogenesis implies that once its topology is established l-Mgm1 cannot be cleaved by Pcp1 because the cleavage site does not reach the protease in the inner membrane. Therefore, it is unlikely that the activity of

---

**Figure 4. Model of alternative topogenesis of Mgm1.** The TIM23 translocase containing all essential subunits such as Tim23, Tim17, Tim50, Tim14, Tim44, and Ssc1 is shown in transparent gray color. The first and second hydrophobic segments in Mgm1 are indicated by gray and dark gray boxes, respectively. Numbers describe the order of the topogenesis pathway for the generation of l-Mgm1 (1 and 2a) and s-Mgm1 (1, 2b, 3b, and 4b). Processing by Pcp1 only occurs when the cleavage site in the second segment reaches the inner membrane, which is dependent on matrix ATP and a functional import motor. IMS, intermembrane space; IM, inner membrane; ΔΨ, membrane potential; MPP, mitochondrial processing peptidase; pMgm1, precursor protein of Mgm1; l-Mgm1 and s-Mgm1, large and short isoform of Mgm1, respectively.
Pcp1 is a physiologically important regulator of Mgm1 bio-
genesis. Consistent with this and in contrast to data by Mc-
Quibban et al. (2003), Pcp1 has not been found to be rate
limiting for Mgm1 processing in our experiments (except
when Pcp1 was down-regulated) at any growth stage includ-
ing stationary cells (Fig. S1). Both isoforms are required for
Mgm1 function (Herlan et al., 2003) and a strong shift in
the ratio between both isoforms of Mgm1 is sufficient to
alter mitochondrial morphology. We speculate that the
ATP level in mitochondria, through alternative topogenesis,
may play a role in controlling mitochondrial morphology.
This would provide a molecular link between the bioener-
ggetic state of mitochondria and their morphology. We hy-
pothesize that mitochondrial damage such as the acquisition
of mutations in mtDNA by oxidative stress would lead to re-
duced ATP levels in the matrix. Such damaged mitochon-
dria may be prevented from fusing with intact mitochondria
because formation of s-Mgm1 is impaired. Alternative topo-
genesis would serve as a mechanism that counterselects
against bioenergetically disordered mitochondria and ex-
clude them from the mitochondrial network and from in-
heriting the damaged mtDNA. A similar mechanism may
apply to the human orthologue of Mgm1, OPA1, which is
associated with the neurodegenerative disorder autosomal
dominant optic atrophy type I (Alexander et al., 2000;
Delettre et al., 2000). Therefore, alternative topogenesis of
Mgm1/OPA1 may have major implications in the patho-
genesis of mitochondrial diseases.

Materials and methods

Plasmids and strains

For expression of the Mgm1 variants VVL, G100D, G100K, and
G100A, the respective Sac1–Netl-fragments were exchanged for those in pRS315 con-
taining Mgm1 or Mgm1 ΔΩ (Herlan et al., 2003) and verified by DNA se-
quencing. For import in vitro Mgm1-harboring plasmid pT126
were transformed into pGEM4 (Promega) containing mouse DHFR. The Δmgm1Δmgm1 strain was from the homozygous dip-
loid strain, and a Δmgm1Δmgm1/Δmgm1 strain was from the homozygous dip-
loid deletion library (Research Genetics). Mitochondria for in vitro import
were amplified from S. cerevisiae D273–10B (Sirenenberg et al., 1996). ssc1
mutants were described in Gambill et al. (1993). The GAL10–PCP1 strain
was transformed by obtaining a PCR product with homologous regions for
PCP1 containing the HIS3 marker and the GAL10 promoter from pT126
into W303α (Lalontaine and Tollervey, 1996). Strains containing Tim17, Tim12,
Tim44 (W334 background), and Tim14 (YPH499 background) un-
der control of the GAL10 promoter (Sirenenberg et al., 1996; Milisavl et al.,
2001; Mokranjac et al., 2003) were shifted from lactate medium contain-
ing 0.5% galactose and 0.1% glucose (W334 background) or 0.1% galac-
tose (YPH499 background) to lactate medium containing 0.1% glucose
(W334 background) or 0.1% galactose (YPH499 background) to lactate medium containing 0.1% glucose.
The GAL10-PCP1 strain was shifted from YPGal to YPD. Samples were 
removed from the culture (OD600 = 0.2–0.8) and total cell extracts were pre-
apared as described previously (Herlan et al., 2003). The M28-82 strain was obtained from A. Tzagoloff (Columbia University, New York, NY; Fourny and Tzagoloff, 1976).

Fluorescence microscopy

Strains were transformed with plasmid pVT100U-mtGFP expressing mi-
tochondria targeted GFP (Westermann and Neupert, 2000) and analyzed
by standard fluorescence microscopy on an Axioplan 2 (Carl Zeiss Micro-
Imaging, Inc.) with a NA 1.3 oil immersion objective (100×; model Plan
Neofluar; Carl Zeiss MicroImaging, Inc.) and a CCD camera 1.3.1.0 (Diag-
nostic Instruments) at RT using Metawiew 3.6a software (Universal Imaging
Corp.). The M28-82 mutant was stained with 0.1-μM rhodamine B Bex-
eyl (Molecular Probes). Classification of the morphology phenotypes was performed without knowledge of strain identity at the time of analysis.

In vitro import

In vitro import of radiolabeled precursor proteins was performed as de-
scribed previously (Herlan et al., 2003). Matrix ATP was depleted by prein-
cubation with 40 U/ml apyrase and 10 μg/ml oligomycin for 20 min at
25°C and subsequent addition of 5 μM atractyloside for 5 min at 4°C. After
import mitochondria were treated with 50 μg/ml tetrapsin for 25 min at 4°C
to remove proteins bound to the surface of mitochondria. Efficiency of ATP
depletion and loss of Sc1 function at 37°C in mitochondria isolated from
the ssc1-3 strain were controlled by importing radiolabeled precursor of
psu9-30-DHFR, which is imported in an ATP- and Sc1-dependent manner
(Gambill et al., 1993).

Hydropathicity analysis

Hydropathicity plots were calculated according to Kyte and Doolittle (1982; window size, 15) using ProtScale software (Swiss Institute of Bioin-

Online supplemental material

Evidence that Pcp1 is not rate limiting for the processing of Mgm1 in sta-
tionary cells is provided in Fig. S1. Online supplemental material is avail-
able at http://www.jcb.org/cgi/content/full/jcb.200403022/DC1.

We thank C. Kothoff for excellent technical assistance, A. Tzagoloff for help-
ful discussions and providing the M28-82 strain, D. Mokranjac (Adolf-
Butenandt-Institut für Physiologische Chemie) for providing the GAL10-
Tim14 strain, and B. Westermann for critically reading the manuscript.

This work was supported by Deutsche Forschungsgemeinschaft, SFB
594, B8, Deutsches HumangenomprojektlNationales Genomforschungs-
netzwerk (MITOP Project), and Fonds der Chemischen Industrie.

Submitted: 3 March 2004
Accepted: 23 March 2004

References

Rodriguez, U. Kellner, B. Leo-Kottler, G. Auburger, et al. 2000. OPA1, en-
coding a dynamin-related GTPase, is mutated in autosomal dominant optic
OPA1, encoding a mitochondrial dynamin-related protein, is mutated in
Dimmer, K.S., S. Fritz, F. Fuchs, M. Messerschmitt, N. Weinbach, W. Neupert,
and B. Westermann. 2002. Genetic basis of mitochondrial function and
two-step mechanism for removal of a mitochondrial signal sequence involves
the mAAA complex and the putative rhomboid protease pcp1. J. Mol. Biol.
323:835–843.
Fourny, F., and A. Tzagoloff. 1976. Localization on mitochondrial DNA of muta-
tions leading to a loss of rumatycin-sensitive adenine triphosphatase. Eur.
Gambill, B.D., W. Voos, P.J. Kang, B. Miao, T. Langer, E.A. Craig, and N. Pfan-
drial structure and genome maintenance in yeast requires the dynamin-like
Herlan, M., F. Vogel, C. Bornhövd, W. Neupert, and A.S. Reichert. 2003. Pro-
cessing of Mgm1 by the rhomboid-type protease Pcp1 is required for main-
tenance of mitochondrial morphology and of mitochondrial DNA. J. Biol.
Chem. 278:27781–27788.
Hsp70 functions causes aggregation of mitochondria in yeast cells. J. Cell
Lafontaine, D., and D. Tollervey. 1996. Two-step mechanism for removal of a mito-
chondrial signal sequence involves the mAAA complex and the putative rhomboid protease pcp1. J. Mol. Biol.
24:3469–3471.
McQuibban, G.A., S. Saurya, and M. Freeman. 2003. Mitochondrial membrane re-


