FEBS Letters 584 (2010) 3153–3157

FEBS Letters

journal homepage: www.FEBSLetters.org

Processing of the dynamin Msp1p in *S. pombe* reveals an evolutionary switch between its orthologs Mgm1p in *S. cerevisiae* and OPA1 in mammals

Ingrid Leroy [1], Farnoosh Khosrobakhsh [1], Alan Diot, Marlène Daloyau, Laetitia Arnauné-Pelloquin, Cindy Cavelier, Laurent J. Emorine, Pascale Belenguer *

Université de Toulouse, UPS CNRS UMR5241, Laboratoire Métabolisme Plasticité Mitochondries, F-31062, France

ARTICLE INFO

Article history:
Received 5 March 2010
Revised 18 May 2010
Accepted 28 May 2010
Available online 1 June 2010

Edited by Vladimir Skulachev

Keywords:
Mitochondrial dynamics
Mitochondrial proteases
Mitochondria
Msp1p
Mgm1p
OPA1

ABSTRACT

Mitochondrial fusion depends on the evolutionary conserved dynamin, OPA1/Mgm1p/Msp1p, whose activity is controlled by proteolytic processing. Since processing diverges between Mgm1p (*Saccharomyces cerevisiae*) and OPA1 (mammals), we explored this process in another model, Msp1p in *Schizosaccharomyces pombe*. Generation of the short isoform of Msp1p neither results from the maturation of the long isoform nor correlates with mitochondrial ATP levels. Msp1p is processed by rhomboid and a protease of the matrix ATPase associated with various cellular activities (m-AAA) family. The former is involved in the generation of short Msp1p and the latter in the stability of long Msp1p. These results reveal that Msp1p processing may represent an evolutionary switch between Mgm1p and OPA1.

1. Introduction

Mitochondrial dynamics, an adaptative process to cells needs, results from a regulated balance between antagonist fusion and fission forces acting on mitochondrial membranes [1]. It specifies the morphology of the organelle that ranges from isolated bead-like to filamentous structures. The core machinery governing mitochondrial dynamics is best understood in *Saccharomyces cerevisiae* where it comprises three main proteins belonging to the dynamin family that are conserved throughout evolution [2]. Dnm1p and Fzo1p, respectively, mediate fission and fusion of the mitochondrial outer membrane and Mgm1p acts on fusion of the mitochondrial inner membrane. The importance of this later protein in mitochondrial functions and cell survival has been highlighted by the discovery that OPA1, the mammalian counterpart of Mgm1p, is mutated in autosomal dominant optic atrophy (ADOA), leading to retinal ganglion cell death, optic nerve atrophy, and eventually blindness [3].

OPA1 is involved in mitochondrial fusion, maintenance of cristae structure and apoptosis but the extend to which each of these processes contributes to the etiology of ADOA is currently unknown. Understanding the functions and dysfunctions of OPA1 is complicated by the existence of eight alternatively spliced mRNAs, each of them leading to several isoforms generated by proteolysis of the precursor protein [4].

Upon import into mitochondria, the first maturation step of OPA1 is the removal of the mitochondrial import signal by the mitochondrial processing peptidase (MPP), generating long isoforms of the protein (l-OPA1) [5]. Limited proteolysis of l-OPA1 then gives rise to short isoforms (s-OPA1), but the nature of the proteases implicated remains controversial [4,6]. It has been initially proposed that l-OPA1 is processed into s-OPA1 by the rhomboid protease PARL [7], but PARL implication was not subsequently confirmed [8–11]. Matrix ATPase associated with various cellular activities (m-AAA) proteases were shown to be involved in generation of s-OPA1 while the subunit composition of the complex, i.e., paraplegin and/or AFG3L2, remains unclear, probably reflecting the capacity of these proteases to act as homo- or hetero-oligomers [8,10,11]. Furthermore, some studies reported an OPA1 processing by the inter-membrane space ATPase associated with various cellular activities (i-AAA) protease YME1L [4,9,11].

Abbreviations: MPP, mitochondrial processing peptidase; m-AAA, matrix ATPase associated with various cellular activities; i-AAA, inter-membrane space ATPase associated with various cellular activities

* Corresponding author. Address: Belenguer Pascale, MPM-UMR5241, Université Paul Sabatier Bât4R3B1, 118 route de Narbonne, 31062 Toulouse cedex 04, France. Fax: +33 561 55 88 94.
E-mail address: pascale.belenguer@cict.fr (P. Belenguer).

[1] The authors contributed equally to this work.

0014-5793/$36.00 © 2010 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2010.05.060

In *S. cerevisiae*, the Mgm1p precursor (p-Mgm1p) is translated from a single mRNA and further processed into two isoforms in the mitochondria following the "alternative topogenesis" model [12,13]. p-Mgm1p is either cleaved by MPP and liberated from the import machinery as a long isoform (l-Mgm1p) or processed by both MPP and Pcp1p, the yeast counterpart of PARL, to generate a short isoform (s-Mgm1p) [12,14,15]. Experiments with the heterologous expression of mammalian OPA1 in *S. cerevisiae* did not enlighten the implication of the rhomboid protease in the dynamin processing since its cleavage was either compromised or unaffected by the deletion of *PCP1* [8,10]. Moreover, no implication of m-AAA proteases in Mgm1p processing has been shown, although yeast m-AAA proteases Yta10p and Yta12p can efficiently cleave OPA1 [8].

Since contradictory results have been obtained concerning OPA1 processing in mammalian cells and since it may differ from that of Mgm1p in *S. cerevisiae*, we explored the maturation process of Msp1p in *Schizosaccharomyces pombe*, another yeast model that diverged from *S. cerevisiae* and which mitochondria are more closely related to those of human in several aspects [16]. Unlike *S. cerevisiae*, *S. pombe* are "petite negative" and, as mammalian cells, die when they lose their mtDNA, although nuclear mutations may yield viable rho zero *S. pombe* strains [17,18]. Also, mitochondria associate to microtubules in *S. pombe* and mammals rather than to actin and intermediate filaments in *S. cerevisiae*. Furthermore, since cytokinesis occurs by fission in *S. pombe* and higher eukaryotes, inheritance of mitochondria and of mitochondrial nucleoids among daughter cells might be more similar in these cells as compared to the budding yeast *S. cerevisiae*.

Similarly to Mgm1p but contrarily to OPA1, we show that generation of s-Msp1p does not result from l-Msp1p maturation. Moreover, we do not find any correlation between s-Msp1p generation and mitochondrial ATP levels as shown for the two other orthologous dynamins [13,19]. Finally, Msp1p is processed by both rhomboid and m-AAA proteases, the former being involved in the generation of the s-Msp1p and the later in the stability of l-Msp1p. Together, our results reveal that the processing of Msp1p may represent an evolutionary switch between Mgm1p and OPA1.

Table 1
S. pombe strains constructed or used in this study.

Description	Phenotype
WT	*h⁻ ade6-M210 ura4-D18 leu1-32 arg11⁺::mCherry*
Δ*rh1*	*h⁻ ade6-M210 ura4-D18 leu1-32 ΔSPBC13E7.11::kanMX6 arg11⁺::mCherry*
Δ*rh2*	*h⁺ ade6-M216 ura4-D18 leu1-32 ΔSPBP4H10.10::natMX6 arg11⁺::mCherry*
Δ*m-AAA*	*h⁻ ade6-M210 ura4-D18 leu1-32 ΔSPBC543.09::kanMX6 arg11⁺::mCherry*
Δ*yme1*	*h⁻ ade6-M210 ura4-D18 leu1-32 ΔSPCC965.04c::hphMX6 arg11⁺::mCherry*
Δ*atp1*	*h⁺ leu1-32 ura4-D18 atp1::kanMX6*
Δ*atp2*	*h⁺ leu1-32 ura4-D18 atp2::kanMX6*
Δ*atp3*	*h⁺ ade 6-M216 ura 4-D18 leu 1-32 atp3::kanMX6*
Δ*anc1*	*h⁺ leu 1-32 anc1::leu2*

2. Materials and methods

2.1. Yeast strains and cultures

S. pombe strains deleted for the various proteases were obtained by standard techniques for fission yeast molecular genetics [20,21]. Gene deletions were verified by PCR and Southern's blotting. Strains disrupted for *atp1⁺* (Δ*atp1*), *atp2⁺* (Δ*atp2*), *atp3⁺* (Δ*atp3*) and *anc1⁺* (Δ*anc1*) were gifts from N. Bonnefoy (IGM, Gif-sur-Yvette, France) and V. Trézeguet (IBGC, Bordeaux, France), respectively. Strains carrying pREP41-Msp1HA plasmid have been described [22].

Deleted and control wild-type strains (Table 1) were exponentially grown (0.5–1 × 10^7 cells/ml) at 25 °C in glucose rich medium (YES) provided by Formedium (Norfolk, UK) or in home-made respiratory medium containing 1% yeast extract, 2% bactopeptone, 0.1% glucose, 3% glycerol and 3% ethanol. Cells carrying plasmids with the *nmt1⁺* promoter were grown in glucose minimal medium (EMM, Formedium) complemented with uracil and adenine (both at 225 mg/L) and in the presence of 4 μM thiamine to repress Msp1 expression, which was induced by three washes in H_2O and further growth at 25 °C in the same medium lacking thiamine during 24 h.

2.2. Preparation and analysis of total protein extracts

Protein extraction and Western blot analysis were done as described [22]. Immunodetection was performed with the chemiluminescence detection kit from NEN, using antibodies as follows: anti-HA (1/5000, Boehringer), purified anti-Msp1p (1/100 [23]), anti-Actin (1/1 000, Chemicon), anti-HSP60 (1/400, Sigma), anti-rabbit IgG-HRP and anti-mouse IgG-HRP (1/10 000, AbCam).

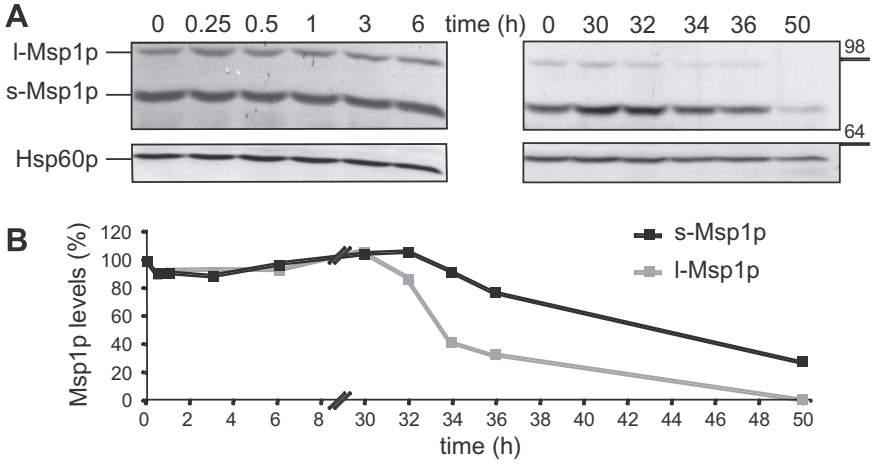

Fig. 1. Generation of s-Msp1p does not result from l-Msp1p maturation. (A) Cells were cultured in YES ($t = 0$). Cycloheximide (200 μg/ml) was then added to the ongoing culture and aliquots were taken at the indicated period of time for analysis by Western blot with anti-Msp1p and anti-HSP60 antibodies. Panels are representative of three independent experiments. MW (kDa) of protein standards are indicated on the right. (B) Protein expression levels were measured by densitometric analysis and normalized to those of the loading control Hsp60p. Results represent the percentage of l-Msp1p and s-Msp1p levels relative to those before cycloheximide addition.

Quantification of protein expression levels was done using the Image J software. Quantitative experiments were analyzed using Student's *t* test. All *P* value were determined by 2-sided test.

2.3. Cytological observation

S. pombe cells expressing a genome mCherry-tagged *arg11*$^{+}$ gene were fixed in 3.7% formaldehyde for 10 min and observed using Nikon Eclipse 80*i* microscope.

3. Results

3.1. Biosynthesis of l-Msp1p and s-Msp1p; relationships with mitochondrial ATP levels and growth on fermentable or respiratory media

To analyze the biosynthetic process of Msp1p, we used cycloheximide to inhibit protein synthesis that was evidenced by a slowing down of cell growth [24]. l-Msp1p and s-Msp1p were very stable, with steady cellular amounts for at least 30 h (Fig. 1). No disappearance of l-Msp1p at the profit of s-Msp1p was observed during the time course, showing that no precursor to product relationships exist between the long and short isoforms.

The influence of mitochondrial ATP levels on Msp1p cleavage was evaluated by analyzing the ratio of Msp1p isoforms in strains with individual deletion of the genes coding for the alpha, beta or gamma subunits of the ATP synthase (Δ*atp1*, Δ*atp2* and Δ*atp3*, respectively), or for the ADP/ATP translocase Anc1p (Δ*anc1*). All these strains presented a default in mitochondrial ATP synthesis which features slow or no growth on fermentable and non-fermentable carbon sources, respectively (Fig. 2A). Lowered ATP levels, also suggested by accumulation of Msp1p and Hsp60p precursors, did not induce any variation of total Msp1p amounts (Fig. 2B) nor of the relative levels of long and short isoforms (Fig. 2C). This suggested that s-Msp1p generation does not correlate with mitochondrial ATP levels.

The influence of the carbon source on Msp1p isoforms ratio was then analyzed (Fig. 3). In cells grown on fermentable (3% glucose) medium, l-Msp1p contributed to 23% of total Msp1p as compared to 45% in respiratory media (3% ethanol–glycerol).

3.2. Msp1p is processed by both Rhomboid 1 and Paraplegin

To specify which protease is implicated in Msp1p processing, we constructed *S. pombe* strains bearing deletions of mitochondrial proteases homologous to those implicated in Mgm1p or OPA1 processing.

In cells deleted for the gene coding for Rhomboid 1 (Δ*rh1*), the *S. pombe* orthologue of Pcp1p and PARL, the long to short Msp1p isoforms ratio increased significantly; l-Msp1p contributing to 67% of total Msp1p compared to 19% in a control wild-type yeast (WT) (Fig. 4A). When normalized to actin, l-Msp1p levels increased 3-fold in Δ*rh1* cells as compared to control cells (WT), with a

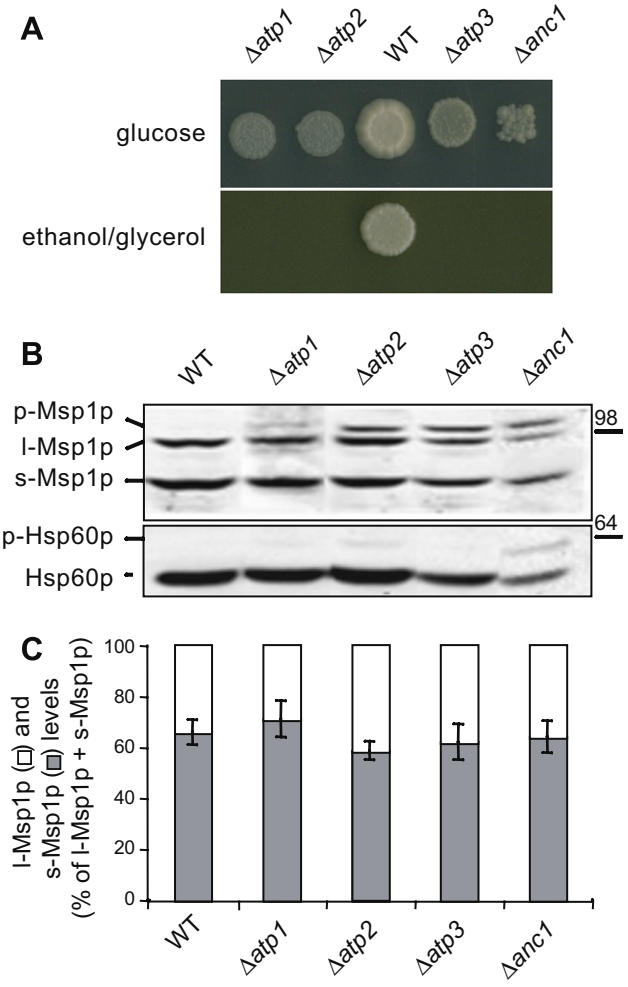

Fig. 2. s-Msp1p generation does not correlate with mitochondrial ATP levels. (A) Cells (4000), of either wild-type (WT) or of strains with a deletion of the genes coding for ATP synthase subunit alpha (Δ*atp1*), beta (Δ*atp2*) or gamma (Δ*atp3*) or for Anc1p (Δ*anc1*) were plated onto solid glucose or ethanol–glycerol containing media and grown for 7 days. (B) Western blot analysis of Msp1p and Hsp60p in WT, Δ*atp1*, Δ*atp2*, Δ*atp3* and Δ*anc1* strains cultured in YES. MW (kDa) of protein standards are indicated on the right. (C) Levels of l-Msp1p (white bars) and s-Msp1p (grey bars) were measured by densitometric scanning. Results, expressed as the percentage of each isoform to l-Msp1p plus s-Msp1p, are the means ± S.D. of three independent experiments. No significant differences between each mutant and the control wild-type strain were observed using Student's *t* test ($P > 0.05$).

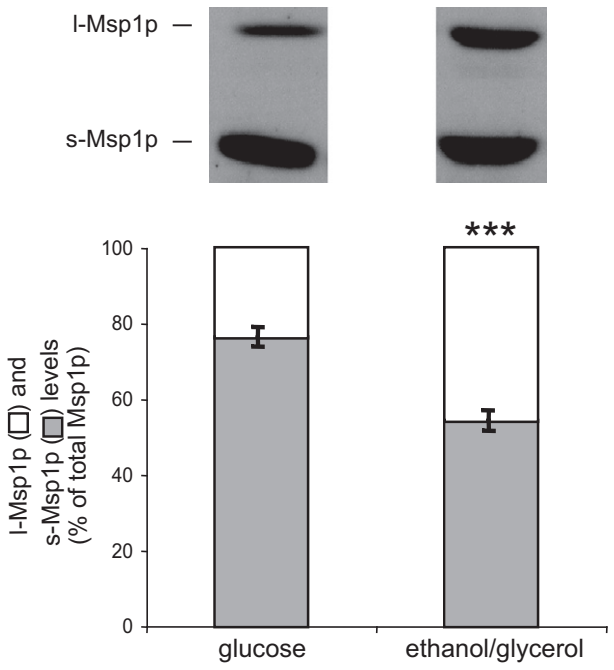

Fig. 3. Variation of l-Msp1p expression levels in adaptation to carbon sources. Msp1p expression levels were analyzed by Western blot using anti-Msp1p antibodies in cells grown in glucose-containing YES medium and shifted to ethanol–glycerol medium for 6 h. Levels of l-Msp1p (white bars) and s-Msp1p (grey bars) were measured by densitometric scanning. Results, expressed as the percentage of each isoform to total Msp1p, are the means ± S.D. of three independent experiments (***$P < 0.001$ as compared to glucose).

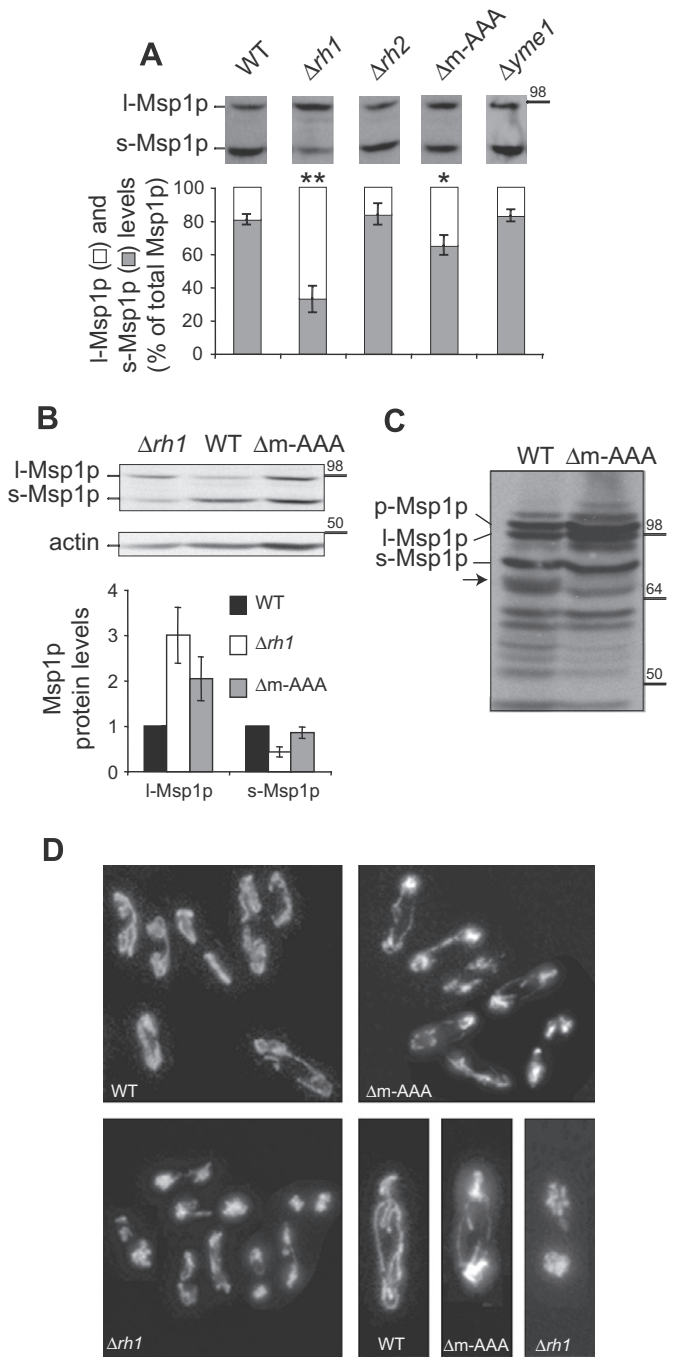

Fig. 4. Msp1p is cleaved by both Rhomboid 1 and the m-AAA protease. (A) Msp1p expression levels were analyzed by Western blot (upper panel) using anti-Msp1p antibody in control wild-type cells (WT) or in cells bearing a deletion of the gene coding for the mitochondrial proteases Rhomboid 1 (Δ*rh1*), Rhomboid 2 (Δ*rh2*), m-AAA protease (Δm-AAA) and Yme1p (Δ*yme1*) grown in YES medium. Quantification (lower panel) of l-Msp1p (white bars) and s-Msp1p (grey bars) levels was obtained by densitometric analysis. Results, expressed as the percentage of each isoform to total Msp1p, are the means ± S.D. of three independent experiments ($^{*}P < 0.05$ and $^{**}P < 0.01$ as compare to WT). (B) Msp1p and actin expression levels in WT, Δ*rh1* or Δm-AAA strains grown in YES medium were analyzed by Western blot (upper panel) using corresponding antibodies. Expression levels of l-Msp1p and s-Msp1p were estimated by densitometric analysis and normalized to actin loading control. Results (lower panel) represent the quantity of each isoform (arbitrary units) and are the means ± S.D. of three independent experiments. (C) Total protein extracts from WT or Δm-AAA cells over-expressing HA-tagged Msp1p were analyzed by Western blotting using anti-HA antibody. Arrow indicated a 77 kDa-migrating product that accumulated in the WT strain but not in Δm-AAA strain. MW (kDa) of protein standards are indicated on the right. (D) WT, Δ*rh1* or Δm-AAA cells expressing the mitochondrial mCherry-tagged Arg11p were grown on YES medium, fixed and observed by fluorescence microscopy.

concomitant 2-fold decrease in s-Msp1p amounts (Fig. 4B). Thus, Rhomboid 1 is used in *S. pombe* to generate a short isoform of the dynamin. Interestingly, alteration of the relative levels of Msp1p isoforms in Δ*rh1* cells was concomitant with modification of mitochondrial morphology which appeared more fragmented (Fig. 4D). Neither a second Rhomboid protease, present only in *S. pombe* as compared to *S. cerevisiae* and mammals, nor the i-AAA peptidase Yme1p were implicated in the formation of s-Msp1p since deletion of the corresponding genes (Δ*rh2* and Δ*yme1*, respectively) did not modify the ratio or quantities of s-Msp1p and l-Msp1p (Fig. 4A). A modified ratio of Msp1p isoforms was observed in a strain where the only putative *S. pombe* m-AAA peptidase, homologous to Yta10p/Yta12p in budding yeast and to Paraplegin and AFG3L2 in human, was deleted (Δm-AAA, Fig. 4A). This was due to a 2-fold increase of l-Msp1p levels in Δm-AAA cells *versus* wild-type strain (WT), with no significant modification of s-Msp1p amounts (Fig. 4B). This suggested that the m-AAA peptidase is not implicated in s-Msp1p generation but rather in controlling the levels of l-Msp1p. In Δm-AAA as in control cells (WT), over-production of HA-tagged Msp1p, which was previously shown to be correctly localized and functional [22,25], resulted in accumulation of p-, l- and s-Msp1p and of several degradation products (Fig. 4C), one of which that migrated at 77 kDa (arrow), being however absent in the protease-deleted strain. This suggests that the m-AAA protease might cleave Msp1p at a specific site for a quality/quantity control survey regulating l-Msp1p to s-Msp1p ratio and/or Msp1p cellular levels. Again, alteration of the relative levels of Msp1p isoforms in Δm-AAA cells was accompanied by a modified morphology of mitochondria which became more aggregated (Fig. 4D).

4. Discussion

Mitochondrial fusion depends on the evolutionary conserved dynamin, OPA1/Mgm1p/Msp1p whose activity is controlled by proteolytic processing generating long and short isoforms of the protein. These are not functionally equivalent since both isoforms within a controlled ratio are required to mediate mitochondrial fusion in mammals and yeasts, and to maintain mtDNA in the later [4,12,25]. Furthermore *S. cerevisiae* l-Mgm1p and s-Mgm1p share distinct biochemical properties and localization [26,27]. Since long and short forms of the dynamin do not have identical functions, it is of interest to characterize species divergent processing that may relate to function specialization. By studying biogenesis of l- and s-Msp1p in *S. pombe*, we demonstrated that the two isoforms of Msp1p are independently generated from the same precursor. In this respect, Msp1p behaves like *S. cerevisiae* Mgm1p but diverges from OPA1 in mammals. However, reduced ATP levels *in vivo* does not modify the formation of s-Msp1p as it does for Mgm1p and OPA1 [13,19]. Fission yeast rapidly adapted to a shift from fermentation to respiration by changing the ratio of l- to s-Msp1p while similar conditions left the ratio of Mgm1p isoforms unaffected in *S. cerevisiae* [28]. Since l-Msp1p is integral to the mitochondrial inner membrane whereas s-Msp1p is peripheral to it, this possibly reflects a specific role of l-Msp1p in the organization of the mitochondrial inner membrane and thus in the activity of respiratory complexes. Such specificity may correlate with different regulation of respiration in "petite negative" (*S. pombe* and mammals) *versus* "petite positive" (*S. cerevisiae*) cells.

In *S. pombe*, as in *S. cerevisiae*, generation of s-Msp1p implicates Rhomboid 1. Another protease might also be involved in *S. pombe* since s-Msp1p was still significantly present in Δ*rh1* (Fig. 4) and in Δ*rh1*/Δ*rh2* strains (not shown). During evolution, processing by Rhomboid might have almost disappeared for the mammalian dynamin, since generation of s-OPA1 by PARL only concerns a few

percents of total OPA1 [7]. Again as in *S. cerevisiae*, Yme1p is not involved in the generation of the short form of Msp1p whereas it is required to process OPA1 [12]. This may relate to the fact that OPA1 exons 4b and 5b that contain the cleavage sites of YME1 have no corresponding sequences in yeast [4,9]. Recent works have involved the metallopeptidase OMA1 in OPA1 cleavage induced by dissipation of mitochondrial membrane potential ($m\Delta\psi$) or apoptosis [29,30]. However, changes in Msp1p processing were observed neither upon $m\Delta\psi$ decrease nor after *Oma1*$^+$ (SPAP14E8.04) deletion (not shown).

The m-AAA protease is not involved in the generation of the short form of the dynamin in both fission and budding yeasts. m-AAA proteases have been implicated in two types of proteolysis: they can act either as processing peptidases, as they do for OPA1 in mammals [8,10] and cytochrome c peroxidase in yeast [31], or as quality-control enzymes degrading non-native polypeptides to peptides [32]. We showed that the m-AAA protease might cleave Msp1p at a specific site(s), generating a second short isoform of 77 kDa (s2-Msp1p) that is not accumulated in basal conditions possibly due to its rapid elimination by oligopeptidases. Processing by m-AAA protease could serve for controlling the abundance of l-Msp1p in mitochondria, but we cannot exclude that s2-Msp1p could accumulate under specific conditions.

Altogether, our results showed that Msp1p processing in fission yeast occurs by two distinct mechanisms, both found in Mgm1p and/or OPA1 maturation. Thus Msp1p cleavage represents a step in the evolutionary switch of proteases involved in the processing of the sole known intramitochondrial dynamin and may thus represent an interesting tool for studying the regulation of Msp1p and OPA1 processing and its involvement in the adaptation of mitochondria to metabolic or other stresses.

Acknowledgments

We thank Dr. N. Bonnefoy and Dr. V. Trézeguet for providing *S. pombe* strains and Dr. K. Sawin and Dr. R. Tsien for the mCherry vector. This work was supported by grants from the CNRS, Université Paul Sabatier, ANR, AFM, Rétina France and AMMi. AD was a recipient of a fellowship from ARC.

References

[1] Detmer, S.A. and Chan, D.C. (2007) Functions and dysfunctions of mitochondrial dynamics. Nat. Rev. Mol. Cell Biol. 8, 870–879.
[2] Hoppins, S., Lackner, L. and Nunnari, J. (2007) The machines that divide and fuse mitochondria. Annu. Rev. Biochem. 76, 751–780.
[3] Olichon, A. et al. (2006) Mitochondrial dynamics and disease, OPA1. Biochim. Biophys. Acta 1763, 500–509.
[4] Song, Z., Chen, H., Fiket, M., Alexander, C. and Chan, D.C. (2007) OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J. Cell Biol. 178, 749–755.
[5] Olichon, A. et al. (2002) The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. FEBS Lett. 523, 171–176.
[6] Landes, T. et al. (2010) OPA1 (dys)functions. Semin. Cell Dev. Biol., doi:10.1016/j.semcdb.2009.12.012.
[7] Cipolat, S. et al. (2006) Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. Cell 126, 163–175.
[8] Duvezin-Caubet, S. et al. (2007) OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. Mol. Biol. Cell 18, 3582–3590.
[9] Griparic, L., Kanazawa, T. and van der Bliek, A.M. (2007) Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. J. Cell Biol. 178, 757–764.
[10] Ishihara, N., Fujita, Y., Oka, T. and Mihara, K. (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J. 25, 2966–2977.
[11] Guillery, O. et al. (2008) Metalloprotease-mediated OPA1 processing is modulated by the mitochondrial membrane potential. Biol. Cell 100, 315–325.
[12] Herlan, M., Vogel, F., Bornhovd, C., Neupert, W. and Reichert, A.S. (2003) Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. J. Biol. Chem. 278, 27781–27788.
[13] Herlan, M., Bornhovd, C., Hell, K., Neupert, W. and Reichert, A.S. (2004) Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor. J. Cell Biol. 165, 167–173.
[14] McQuibban, G.A., Saurya, S. and Freeman, M. (2003) Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. Nature 423, 537–541.
[15] Sesaki, H., Southard, S.M., Hobbs, A.E. and Jensen, R.E. (2003) Cells lacking Pcp1p/Ugo2p, a rhomboid-like protease required for Mgm1p processing, lose mtDNA and mitochondrial structure in a Dnm1p-dependent manner, but remain competent for mitochondrial fusion. Biochem. Biophys. Res. Commun. 308, 276–283.
[16] Chiron, S., Gaisne, M., Guillou, E., Belenguer, P., Clark-Walker, G.D. and Bonnefoy, N. (2007) Methods in Molecular Biology. Mitochondria: Practical protocols 372, 91–106.
[17] Haffter, P. and Fox, T.D. (1992) Nuclear mutations in the petite-negative yeast *Schizosaccharomyces pombe* allow growth of cells lacking mitochondrial DNA. Genetics 131, 255–260.
[18] Massardo, D.R., Manna, F., Schafer, B., Wolf, K. and Del Giudice, L. (1994) Complete absence of mitochondrial DNA in the petite-negative yeast *Schizosaccharomyces pombe* leads to resistance towards the alkaloid lycorine. Curr. Genet. 25, 80–83.
[19] Baricault, L., Segui, B., Guegand, L., Olichon, A., Valette, A., Larminat, F. and Lenaers, G. (2007) OPA1 cleavage depends on decreased mitochondrial ATP level and bivalent metals. Exp. Cell Res. 313, 3800–3808.
[20] Bahler, J. et al. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast 14, 943–951.
[21] Hentges, P., Van Driessche, B., Tafforeau, L., Vandenhaute, J. and Carr, A.M. (2005) Three novel antibiotic marker cassettes for gene disruption and marker switching in *Schizosaccharomyces pombe*. Yeast 22, 1013–1019.
[22] Guillou, E., Bousquet, C., Daloyau, M., Emorine, L.J. and Belenguer, P. (2005) Msp1p is an intermembrane space dynamin-related protein that mediates mitochondrial fusion in a Dnm1p-dependent manner in *S. Pombe*. FEBS Lett. 579, 1109–1116.
[23] Pelloquin, L., Belenguer, P., Menon, Y. and Ducommun, B. (1998) Identification of a fission yeast dynamin-related protein involved in mitochondrial DNA maintenance. Biochem. Biophys. Res. Commun. 251, 720–726.
[24] Polanshek, M.M. (1977) Effects of heat shock and cycloheximide on growth and division of the fission yeast, *Schizosaccharomyces pombe*. With an Appendix. Estimation of division delay for *S. pombe* from cell plate index curves. J. Cell Sci. 23, 1–23.
[25] Diot, A., Guillou, E., Daloyau, M., Arnaune-Pelloquin, L., Emorine, L.J. and Belenguer, P. (2009) Transmembrane segments of the dynamin Msp1p uncouple its functions in the control of mitochondrial morphology and genome maintenance. J. Cell Sci. 122, 2632–2639.
[26] DeVay, R.M., Dominguez-Ramirez, L., Lackner, L.L., Hoppins, S., Stahlberg, H. and Nunnari, J. (2009) Coassembly of Mgm1 isoforms requires cardiolipin and mediates mitochondrial inner membrane fusion. J. Cell Biol. 186, 793–803.
[27] Zick, M., Duvezin-Caubet, S., Schafer, A., Vogel, F., Neupert, W. and Reichert, A.S. (2009) Distinct roles of the two isoforms of the dynamin-like GTPase Mgm1 in mitochondrial fusion. FEBS Lett. 583, 2237–2243.
[28] Sesaki, H., Dunn, C.D., Iijima, M., Shepard, K.A., Yaffe, M.P., Machamer, C.E. and Jensen, R.E. (2006) Ups1p, a conserved intermembrane space protein, regulates mitochondrial shape and alternative topogenesis of Mgm1p. J. Cell Biol. 173, 651–658.
[29] Ehses, S. et al. (2009) Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J. Cell Biol. 187, 1023–1036.
[30] Head, B., Griparic, L., Amiri, M., Gandre-Babbe, S. and van der Bliek, A.M. (2009) Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J. Cell Biol. 187, 959–966.
[31] Esser, K., Tursun, B., Ingenhoven, M., Michaelis, G. and Pratje, E. (2002) A novel 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.
[32] Koppen, M. and Langer, T. (2007) Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. Crit. Rev. Biochem. Mol. Biol. 42, 221–242.