Msp1p is an intermembrane space dynamin-related protein that mediates mitochondrial fusion in a Dnm1p-dependent manner in S. pombe

Emmanuelle Guillou, Céline Bousquet, Marlène Daloyau, Laurent J. Emorine, Pascale Belenguer*

Laboratoire de Biologie Cellulaire et Moléculaire du Contrôle de la Prolifération, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 04, France

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Abstract Mitochondrial morphology is controlled by large GTPases, such as Msp1p, whose action on mitochondrial membranes is not yet understood. The sub-mitochondrial localization of Msp1p, the subject of ongoing controversies, was found to be within the intermembrane space. Overexpression of Msp1p led to aggregation of the mitochondrial network, while its downregulation resulted in fragmentation of this network. Mutations affecting the integrity of the Msp1p GTPase function had a dominant phenotype and induced mitochondrial fragmentation followed by mitochondrial DNA loss and cell death. These effects were not observed in cells deleted for Dnm1p, an actor in mitochondrial fission, suggesting that Msp1p is involved in the fusion of mitochondria.

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1. Introduction

GTPases of the dynamin family are structurally similar, with GTP-binding, middle, and GTPase effector domains, but functionally divergent [1–3]. The prototypic conventional dynamins, Dyn-1, -2 and -3, play a prominent role in clathrin-dependent endocytosis and Golgi trafficking while related members are involved in pathogen resistance, plant cell plate formation or chloroplast biogenesis. Interestingly, two of these dynamin-like GTPases have been reported to modulate mitochondrial network morphology by acting on fusion and fission processes of the inner and outer membranes (IM and OM) of the organelle [4].

In the budding yeast Saccharomyces cerevisiae, the cytosolic dynamin Dnm1p (known as DRP1, Dlp1p or DVLp1 or Dynp in other species) is [5,6], together with Net2p/Gag3p/Mdv1p/Fis2p, recruited to punctuate structures of the OM by Fis1p/Mdv2p where it mediates mitochondrial division [7–10]. As for conventional dynamins, the mammalian DRP1/Dlp1 may self-assemble on the OM into multimeric rings or spirals to promote mitochondrial constriction and division [11]. In S. cerevisiae where fission is blocked by inactivation of Dnm1p, mitochondria form a net of highly interconnected tubules [12].

A second mitochondrial dynamin, Mgm1p, involved in maintenance of the mitochondrial genome and organelle morphology, was first identified in S. cerevisiae [13,14]. We have characterized its counterpart in the fission yeast Schizosaccharomyces pombe, Msp1p [15,16], and in humans, OPA1, and found that mutations in the OPA1 gene are associated to type-1 autosomal dominant optic atrophy (ADOA-1, MIM165500) [17]. Loss of Mgm1p or OPA1 function leads to fragmentation of the mitochondrial tubules [18–20], probably due to a decreased capacity of mitochondria to fuse [21,22]. This defect of fusion is also apparent in S. cerevisiae zygotes formed by mating MGM1 mutants, which are unable to mix their mitochondrial contents [21,22].

In S. cerevisiae, Mgm1p is engaged in a complex comprising Fzo1p and Ugo1p to promote mitochondrial fusion [23]. Fzo1p, and its homologues fuzzy onions in Drosophila or mitofusines (Mfn1 and Mfn2) in mammals, is a GTPase that spans the OM twice, with both its N-terminal GTPase domain and C-terminal tail facing the cytosol [24]. Ugo1p has a single membrane spanning domain. It possibly coordinates dynamics of the IM and OM by physically linking Fzo1p to Mgm1p through interactions with Fzo1p via its cytosolic N-terminus and with Mgm1p via its intermembrane space (IMS) C-terminus [23]. When any of the corresponding gene is deleted in yeast, mitochondria fragment and lose their mitochondrial DNA (mtDNA).

Interestingly, the fragmented mitochondrial phenotype in FZO1, MGM1 or UGO1 mutants, and the interconnected one in DNM1 deleted cells (Δdnm1), can be alleviated when DNM1 is inactivated together with either of them [12,18,21,22,25,26]. Similarly, simultaneous inactivation of Dnm1p in these mutants suppresses the loss of mtDNA. A regulated balance between fission and fusion thus seems essential to control morphogenesis of mitochondria and to maintain mtDNA.

Whereas deletion of MGM1 and of msp1+ induces mtDNA loss in the corresponding yeasts, Δmsp1 S. pombe cells die but Δmgm1 S. cerevisiae cells are viable on a fermentable carbon source. Unlike S. cerevisiae, fission yeasts are indeed “petite-negative” and as mammalian cells die when they lose their mtDNA. Mitochondria also differ between these highly divergent yeasts with respect to their association to cytoskeleton elements, which are microtubules in S. pombe and mammals.
S. cerevisiae as compared to the budding yeast. Directed among daughter cells might be more similar in these cells in S. pombe and genome in the "petite-negative" yeast S. pombe. The role of Msp1p in maintaining mitochondrial morphology and genome in the "petite-negative" yeast S. pombe. Mutations affecting the GTPase activity of Msp1p, and deletion of the Msp1 gene, induce fragmentation of the mitochondrial network. This primary defect consequently triggers the loss of the mitochondrial genome and ultimately leads to cell death. These effects are suppressed when the gene encoding Dnn1p, an essential actor in mitochondrial fission, is inactivated, suggesting that Msp1p is involved, either directly or indirectly, in mitochondrial fusion.

2. Materials and methods

2.1. Plasmid constructions

Plasmids for expression of Myc-tagged versions of the tobacco etch virus (TEV) protease in the IMS or in the MA consisted of the protease coding region N-terminally fused to the mitochondrial import sequences of S. cerevisiae COY2 and ATP9, respectively (a gift from J. Shaw, University of Utah, Salt Lake City, UT). For expression in S. pombe, the corresponding inserts were subcloned into pREP42, yielding TEVMS-Myc and TEVMAM-Myc. Msp1-TEV-HA was constructed by annealing complementary oligonucleotides coding for a TEV protease cleavage site (CTAGTGAAGACTATATCTTCA, CTA-GATTGAGATGTATAGATGCTTCA) and insertion into the unique NheI site of the Msp1 coding region containing 3XHA epitope repetitions at the C-terminus (Msp1-HA). This construct was then cloned into the pREP41 vector. pADH1 and pREP4 plasmids expressing mitochondrial-targeted GFP (Mito-GFP) were constructed by subcloning the N-terminal targeting sequence of S. cerevisiae COXIV fused to GFP from the vector provided by M. Yaffe (University of California, San Diego, La Jolla, CA). Plasmids carrying either wild-type Msp1p or Msp1p deleted of its mitochondrial targeting sequence expressed under the control of the moderate nmt1 promoter have been described elsewhere [16]. The substitution K276A was introduced by QuickChange site-directed mutagenesis (Stratagene). Deletion of the 25 or 50 C-terminal residues was performed by PCR. Modified fragments were sequenced before re-introduction into pREP41-msp1.

2.2. Yeast strains and cultures

Complete disruption of the dnm1+ gene (SPBC12C2.08) was obtained by homologous recombination using a DNA fragment encompassing the kanaMX6 gene [36] flanked by the 5‘UTR and 3‘UTR sequences of dnm1+. Gene replacement was checked by PCR and Southern blotting. Fission yeast growth media (YES, EMM, ME) were from Bio101 Inc. (La Jolla, CA). S. pombe strains carrying the indicated plasmids were transfected by electroporation using the Biorad Gene-Pulser [37]. Transformants were selected by growth on medium lacking leucine or uracil. For cells carrying plasmids with the nmt1 promoter, repression was obtained by adding 4 μM thiamine and expression was induced by three washes in minimal medium and further growth for 24 h at 25 °C in the absence of thiamine [38,39]. Sporulation was obtained by transfer of diploids to ME medium.

2.3. Preparation and analysis of total protein extracts

Cells were harvested by centrifugation and resuspended at 4 °C in the presence of an equal volume of 500 μm glass beads in lysis buffer (50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 1 mM DTT, 1% Triton X100, 0.1% SDS, 0.5% DOC and 1% NP40) containing a cocktail of protease inhibitors (Roche). Samples were vortexed using the Fast-Prep (Bio101 Inc) until approximately 90% of the cells were disrupted. The soluble protein fraction was recovered by two centrifugations of 5 min at 20,000 × g. Protein samples (100 μg) were boiled for 3 min in Laemmli's sample buffer, electrophoresed on 7.5% SDS-PAGE and electrotransferred onto Protran membrane (Schleicher and Schuell). Immunodetection was performed with the chemiluminescence detection kit from NEN using antibodies as follows: anti-HA (1/5000, Boehringer), anti-Myc (1/5000, Invitrogen), anti-Msp1p (1/5000, [16], anti-Tat1 (1/1000, provided by S. Tournier, LBMCMP, University of Toulouse, France), anti-rabbit IgG-HRP and anti-mouse IgG-HRP (1/1000, New England Biolabs).

2.4. Cytological observations

S. pombe cells expressing a Mito-GFP were fixed in 3.7% formaldehyde for 10 min and observed using Leica DMS500B or TCS SP2 microscopes. 4,6-diamino-2-phenylindole (DAPI) staining was performed as described [40].

3. Results and discussion

3.1. Msp1p is an intermembrane space protein

Because of the reported differing localizations of the three homologous proteins Msp1p, Mgm1p and OPA1 [16,18,20–22,33–35], we wished to re-examine the sub mitochondrial compartmentalisation of Msp1p. We used a method recently applied to Mgm1p [21]. A TEV protease cleavage site was introduced into Msp1-TEA, a C-terminally HA-tagged form of Msp1. This version of Msp1p (Msp1-TEV-HA) maintained its function, as assessed by its ability to complement the deletion of the msp1+ gene (not shown). Using this construct, cleavage of Msp1p by the TEV protease was predicted to produce a 32 kDa C-terminal fragment containing the HA tag. An N-terminally Myc-tagged TEV protease was targeted either to the MA (TEVMAM-Myc) or to the IMS (TEVIMS-Myc), using the well-characterized mitochondrial targeting sequences of At9p or Cyb2p of S. cerevisiae, respectively. Expression from three constructs was under the control of the moderate thiamine-repressible promoter nmt1+. Msp1-TEV-HA was co-transfected into S. pombe together with either TEVMAM-Myc or TEVIMS-Myc. Immunoblot analyses were then performed using anti-HA or anti-Myc antibodies on total protein extracts from cells grown without thiamine. The expected 32 kDa peptide was observed when Msp1-TEV-HA was co-expressed with TEVMAM-Myc (Fig. 1, lane 5) but not with TEVMAM-Myc, or without the protease (Fig. 1, lanes 4 and 6). Furthermore, the 32 kDa product was absent under any of these conditions in strains expressing Msp1-TEV-HA lacking the TEV cleavage site (Fig. 1, lanes 1–3). These results indicate that the C-terminus of Msp1p was accessible only to the IMS-targeted protease.

Thus, it can now be accepted that the three homologues Mgm1p, Msp1p and OPA1p are localized to the IMS [18,20–22,33–35]. In our initial study, we used immunoelectron
microscopy to reveal the presence of Msp1p within the mitochondria in most cases close to the cristae, and we showed that Msp1p strongly bound to membranes [16]. We thus suggest that Msp1p is anchored to the IM by an N-terminal transmembrane segment, with its GTPase domain facing the IMS. While this topology globally agrees with that recently proposed for Mgm1p and OPA1, some discrepancies still persist concerning the relationship of these dynamins with mitochondrial membranes. Mgm1p was found to be either peripherally associated with, or integrated into, the OM and/or the IM [18,22,33,34], and OPA1 was described to co-sediment with both mitochondrial membranes and to interact more or less tightly with the IM [20,35,41]. Further studies are needed to clarify this point, keeping in mind that the existence of several isoforms of these dynamins could explain these controversial findings [18,33,35,41,42]. In particular, a short form of Mgm1p, deleted in the N-terminal region, was shown to be generated by the action of the mitochondrial rhomboid-type protease Pcp1p [34,43].

3.2. Mitochondrial morphology depends on Msp1p expression levels

As a member of the dynamin family, Msp1p was expected to act on membrane dynamics [3]. To study this possibility, we used an haploid Δmsp1 + disruptant strain (Δmsp1) rescued by moderate ectopic Msp1p overexpression [15]. Expression of the rescue plasmid was repressed by addition of thiamine and the level of ectopic Msp1p was analyzed at various time-points by Western blots of total protein extracts (Fig. 2A). At the beginning of the experiment the matrix peptidase-matured form of Msp1p (l-Msp1) [16] was detected, together with a shorter form (s-Msp1) probably generated by the mitochondrial rhomboid-like protease Pcp1p [34,43]. As repression of the nmt1 promoter took place, the level of both Msp1p species decreased and was barely detectable after 27 h of culture. Mitochondrial morphology was then examined by fluorescence microscopy using Mito-GFP (Fig. 2B). In Msp1p overexpressing cells, more than 85% of the cells had an aggregated filamentous mitochondrial network. After 12 h, expression of Msp1p was apparently decreased to endogenous levels since most cells contained wild-type mitochondria which appeared as long filamentous structures spanning the cell cortex [16]. By 27 h, when repression was almost complete, the mitochondrial network fragmented into clusters of small rounded mitochondria. This phenotype is reminiscent of the mitochondrial morphology defect observed in S. cerevisiae deleted for MGM1 [18]. Thus overexpression of the dynamin Msp1p led to aggregation of the mitochondrial network while its down-regulation induced fragmentation. This showed that Msp1p indeed participates in the control of mitochondrial membranes dynamics, consistent with the roles of Mgm1p and OPA1 [18–22].

3.3. Mitochondrial morphology requires integrity of the GTPase and coiled-coil domains of Msp1p

Msp1p shares a number of structural features with proteins of the dynamin family [1]. These include an N-terminal GTPase domain containing a dynamin signature and a C-terminal coiled-coil region which may correspond to a GTPase effector domain (Fig. 3A). To further characterize the function of Msp1p, we examined the effects of mutations expected to decrease Msp1p GTPase activity on mitochondrial morphology. Based on the analysis of the dynamins and other GTPases, mutation K276A in the G1 region of the GTPase

Fig. 1. Msp1p is an intermembrane space protein. Total protein extracts from cells expressing HA-tagged Msp1p (Msp1-HA) or the Msp1-HA with a TEV protease cleavage sequence (Msp1-TEV-HA), either alone (-, lanes 1 and 4) or in combination with a Myc-tagged TEV protease targeted to the IMS (TEVMA-Myc, lanes 2 and 5) or to the MA (TEVMA-Myc, lanes 3 and 6) were analyzed by SDS–PAGE and Western blotting with anti-HA or anti-Myc antibodies. The black and white arrowheads indicate the precursor and the mitochondrialy matured forms of the TEV protease, respectively. The apparent molecular weights (65 and 62 kDa for TEVMA-Myc, and 47 and 42 kDa for TEVMA-Myc) agree with those predicted from the constructs used [57,58].

Fig. 2. Mitochondrial morphology depends on Msp1p expression levels. Haploid Δmsp1 cells expressing Msp1p under the control of the moderate thiamine-repressible nmt1 + promoter and Mito-GFP under the control of the constitutive adh1 + promoter were cultivated for 0, 12 and 27 h in minimal medium containing thiamine. (A) Total protein extracts were analyzed by SDS–PAGE and Western blotting using anti-Msp1p antibodies. l-Msp1: mitochondrial matrix peptidase-matured Msp1p, s-Msp1p: mitochondrial rhomboid protease-matured Msp1p. (B) Cells were analyzed by fluorescence microscopy. Typical mitochondrial phenotypes (top panels) were quantified by examination of 120 cells (lower graph).
domain is anticipated to inhibit nucleotide binding and hydrolysis, while deletion of the last 25 (Δ25) or 50 (Δ50) residues is predicted to alter GTPase stimulated activity by inhibition of self-assembly and/or inhibition of association with other partners [44–47].

We first analyzed the expression of the various forms of Msp1p by performing Western blots on total protein extracts (Fig. 3 B). In untransfected cells, or in cells transfected with a control vector expressing bacterial chloroamphenicol-acetyltransferase (CAT), endogenous dynamin was mostly represented by the Pcp1p-generated s-Msp1p form. In these cells, full length precursor (p-Msp1) and mitochondrial matrix peptidase-matured l-Msp1p were barely detected, but their levels greatly increased upon ectopic expression of the wild-type or mutant forms of Msp1p. s-Msp1p levels were comparably augmented, as evidenced in Msp1pΔ50 cells, where the ectopic s-Msp1p could be separated from the endogenous form. For Msp1p deleted from its mitochondrial import sequence (Msp1pΔMIS) to prevent mitochondrial localization [16], only the species corresponding to the mature product was overexpressed, and s-Msp1p remained at endogenous levels. All ectopic variants, expressed under the control of the moderate nmt1+ promoter, were overexpressed at comparable levels (Fig. 3B).

We next examined the capacity of Msp1p mutants to complement the deletion of the msp1+ gene, essential for viability (Fig. 3C). Each variant was transfected into a diploid strain containing a wild-type and a disrupted allele of msp1+ [15]. After sporulation the viability of haploid Δmsp1 cells was checked by their ability to grow on minimal medium lacking thiamine. (D) Haploid msp1+ strains harboring the same vectors as in (B) were co-transfected with a plasmid coding for Mito-GFP under the control of the nmt1+ promoter. Cells were then cultivated for 18 h in medium without thiamine and observed by fluorescence microscopy after formaldehyde fixation. Typical mitochondrial phenotypes (upper panels) were quantified by examination of 100 cells (lower graph).
Finally, the effects of these Msp1p mutants on mitochondrial morphology were examined by fluorescence microscopy of *msp1*+ strains expressing Mito-GFP (Fig. 3D). To avoid possible non-specific effects due to strong overexpression of the mutant proteins, the induction time of the nmt1+ promoter, which requires more than 20 h at 25 °C for full derepression, was reduced to 18 h. Under these conditions, the expression of either wild-type Msp1p, Msp1pAMIS or CAT had no effect on mitochondrial morphology. The majority of the cells contained a tubular mitochondrial network similar to that of untransfected cells. On the contrary, even slight overexpression of Msp1pK276A, Msp1pK50 or Msp1pA25 induced mitochondrial fragmentation; in about 60% of the cells the mitochondria appeared as small more or less clustered individual dots. This phenotype is reminiscent of that observed in Δmsp1 cells, and agrees with the loss of functionality of these mutants. Thus, mutations in critical residues of the GTPase or GED domains of Msp1p caused dominant negative effects when over-expressed in wild-type cells.

Altogether, these results lead to the conclusion that the GTPase activity of Msp1p is essential for its action on mitochondrial morphology, in agreement with the importance of the catalytic activity of the conventional dynamins for their ability to remodel cellular membranes [45,46,48]. Accordingly, mutations in the GTPase and GED domains of Mgm1p [21,22,33] and OPA1 [20,49] have been shown to impair their function and to induce mitochondrial fragmentation. Furthermore, OPA1 is frequently mutated in either of these two regions in patients affected by ADOA-1 (see http://lbbma.univ-angers.fr/eOPA1), underscoring the importance of these domains in the function of the dynamins and highlighting the impact of mitochondrial morphology on cellular function.

3.4. Integrity of the GTPase and coiled-coil domains of Msp1p is required to maintain mtDNA and cell viability

Because the complete loss of function of Msp1p, obtained by deletion of the *msp1*+ gene, resulted in the depletion of mtDNA and cell death [15], we wondered whether this would also be the case when only the GTPase activity of Msp1p was altered. Cultures of an *msp1* strain transfected with plasmids harboring the various Msp1p mutants or bacterial CAT under the control of the moderate nmt1+ promoter were maintained in exponential growth by daily dilution into fresh thiamine-free medium. Time-course measurements of the doubling times of these cultures showed that at day 5 the growth rate of strains expressing Msp1pK276A, Msp1pK50 and Msp1pA25 was greatly increased, while control cells expressing CAT, wild-type Msp1p or cytosolic Msp1pAMIS were not affected (Fig. 4A). This correlated with the behavior of the mitochondrial genome, which was followed concomitantly by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy of DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining. After 24 h of expression, a typical pattern of mitochondrial morphology was examined by fluorescence microscopy using DAPI staining.

Lethality of Msp1 loss of function in “petite-negative” *S. pombe* cells may be due to the influence of membrane dynamics on mtDNA maintenance. Alternatively it may result from effects on other essential mitochondrial functions such as ATP synthesis or membrane potential. In this regard, it could be informative to perform the above experiments in *S. pombe* strains with a ptp1-1 or ptp2-1 genetic background that suppresses p0-lethality [50] or vice-versa to test viability of MGM1 deletion in *S. cerevisiae* strains converted to “petite-negativity” [51].

It should be mentioned however that several yeasts genes involved either in mitochondrial membrane dynamics (*FZO1, UGO1* and *MGMI/msp1*+) or in mitochondrial movement and tethering (*MDM10, MDM12 and MMM1*) also affect nucleoid inheritance [43,52]. A mounting body of data indeed suggests the existence of a “nucleoid segregation apparatus” linked to cytoskeleton elements governing mitochondrial distribution and dynamics by Mmm1p which colocalizes with nucleoid foci and spans both the OM and the IM [53,54].

In the present work (see paragraph 3.3) as with the above genes, loss of mtDNA induced by inactivation of *msp1*+ occurred later than mitochondrial morphology modifications. Similarly, in our previous studies using conditional repression
of msp1<sup>+</sup> [15], about 8 generations were necessary to dilute the mitochondrial genome sufficiently before the doubling time increased dramatically. Furthermore, inactivation of either FZO1, UGO1 or MGM1 in Δdnm1 S. cerevisiae cells suppresses morphology defects together with mtDNA loss. This was also observed here in S. pombe cells doubly defective for Msp1p and Dnm1p function (see below). We thus suggest that Msp1p basically acts on mitochondrial dynamics and that the defects in mtDNA maintenance originated from the alteration of this primary function. However, while links between organelle morphology and mitochondrial genome inheritance are well illustrated, the underlying molecular mechanisms are only beginning to emerge.

3.5. Altered mitochondrial morphology and mtDNA maintenance upon inactivation of Msp1p depend on Dnm1p

To obtain further insight into the mechanisms by which Msp1p may modulate mitochondrial morphology, possibly by acting on fusion or fission processes, we deleted the gene coding for Dnm1p, a dynamin required for mitochondrial scission [12]. Mitochondrial morphology was then assessed by fluorescence microscopy using Mito-GFP. In dnm1<sup>+</sup> disrupted S. pombe (Δdnm1) the mitochondrial network appeared as highly interconnected tubules forming net-like structures (Fig. 5A). This phenotype is reminiscent of that observed in S. cerevisiae deleted from DNM1 and has been proposed to arise because mitochondrial tubules fuse and new tubules ends cannot be generated by fission [12]. In Δdnm1 cells, low-level overexpression of Msp1p had no effect (Fig. 5B), and the mitochondrial fragmentation induced by expressing Msp1p<sub>K276A</sub> or Msp1p<sub>Δ25</sub> was not observed (Fig. 5C and D). In the double-disrupted Δmsp1Δdnm1 strain, the mitochondria formed elongated tubules which resembled those seen in wild-type cells, though they appeared to be somewhat collapsed (Fig. 5E). Thus, deletion of DNM1 in Msp1 mutants restored the mitochondrial tubules, suggesting that Msp1p is involved in the fusion process, either directly as proposed for its homologue Mgm1p, or by inhibiting Dnm1-driven fission.

In the Δmsp1Δdnm1 strain, mtDNA depletion (Fig. 5F) and lethality (not shown) did not occur. Thus, abolishing Dnm1p-dependent fission blocked mitochondrial fragmentation and prevented the loss of the mitochondrial genome induced by inactivation of Msp1p. This further supports the conclusion that defects in mtDNA segregation that occur upon loss of Msp1p function originate from alterations in mitochondrial membrane dynamics. Interestingly, a DNM1-dependent loss of mtDNA followed fragmentation of the mitochondrial network in S. cerevisiae mutated for MGM1, FZO1 and UGO1, all of which are directly involved in the fusion process [12,18,21,22,25,26]. Thus, mitochondrial diseases such as those associated with mutations in OPA1 [17,55] or Mfn2 [56], the human counterpart of FZO1, may originate from an imbalance in the control of mitochondrial morphology and may be further exacerbated by mtDNA deficiency.

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Fig. 5. Altered mitochondrial morphology and mtDNA maintenance upon inactivation of Msp1 is dependent on Dnm1p. Δdnm1 strains (A–F) expressing plasmids encoding Msp1p (B), Msp1p<sub>Δ25</sub> (C), Msp1p<sub>K276A</sub> (D) under the control of the moderate nmt1<sup>+</sup> promoter, or bearing a Δmsp1 allele (E–F), were transfected with a plasmid harboring the gene encoding Mito-GFP under the control of the nmt1<sup>+</sup> promoter. Cells were then cultivated for 18 h in medium without thiamine and observed by fluorescence microscopy for GFP (A–E), or after DAPI staining (F) after formaldehyde fixation. For each condition more than 90% of the cells displayed the illustrated mitochondrial morphology.
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