Division versus Fusion: Dnm1p and Fzo1p Antagonistically Regulate Mitochondrial Shape

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Abstract. In yeast, mitochondrial division and fusion are highly regulated during growth, mating and sporulation, yet the mechanisms controlling these activities are unknown. Using a novel screen, we isolated mutants in which mitochondria lose their normal structure, and instead form a large network of interconnected tubules. These mutants, which appear defective in mitochondrial division, all carried mutations in DNM1, a dynamin-related protein that localizes to mitochondria. We also isolated mutants containing numerous mitochondrial fragments. These mutants were defective in FZO1, a gene previously shown to be required for mitochondrial fusion. Surprisingly, we found that in dnm1 fzo1 double mutants, normal mitochondrial shape is restored. Induction of Dnm1p expression in dnm1 fzo1 cells caused rapid fragmentation of mitochondria. We propose that dnm1 mutants are defective in the mitochondrial division, an activity antagonistic to fusion. Our results thus suggest that mitochondrial shape is normally controlled by a balance between division and fusion which requires Dnm1p and Fzo1p, respectively.

Key words: mitochondrial division • mitochondrial fusion • dynamin • GTPase • yeast

Mitochondria undergo regulated fusion and division in many cell types (Bereiter-Hahn and Voth, 1994; Kawano et al., 1995), which appear to play key roles in establishing and maintaining mitochondrial shape (Tyler, 1992). In the yeast S. cerevisiae, mitochondria are elongated, tubule-shaped organelles that are very dynamic during growth, mating, and sporulation. Mitochondria constitutively divide and fuse during cell growth (Nunnari et al., 1997), but change their number depending on growth conditions (Stevens, 1977). During mating, mitochondria fuse immediately after cell fusion, mixing their contents, including mitochondrial DNA (mtDNA) and matrix proteins (Nunnari et al., 1997; Oka moto et al., 1998). When diploids sporulate, mitochondria are dramatically reorganized moving into the four spores and surrounding each haploid nucleus (Miyakawa et al., 1984). The yeast homologue of fuzzy onions (Hales and Fuller, 1997), FZO1 was recently identified and shown to play an important role in mitochondrial fusion (Hermann et al., 1998; Rapaport et al., 1998). However, the mechanisms that control mitochondrial division are unknown.

Materials and Methods

Strain Construction

Strain YHS2, which expresses red-shifted GFP (F64L, T65C, and I167T) fused to the Cox4p presequence (residues 1-21) under the ADH1 promoter (ADH1-COX4-GFP), was constructed as follows. First, pHS1 was constructed by replacing wild-type GFP in pOK29, a HIS3-CEN plasmid which carries ADH1-COX4-GFP (Kerscher, O., unpublished information), with a NcoI-BamHI fragment carrying red-shifted GFP from pQBI25 (Quantum Biotechnologies). The EcoRV-BamHI fragment from pHS1 was inserted into pDH9, which carries 5′ and 3′ untranslated regions of MFA2 (a gift from S. Michaelis), forming pHS2. To integrate ADH1-COX4-GFP at chromosomal MFA2, a XhoI-SmaI fragment carrying 5′-MFA2-ADH1-COX4-GFP-3′-MFA2 from pH52 was transformed into strain SM1, which carries mfa2::URA3 (Michaelis and Herskowitz, 1988). Strain YHS2, which contains MATα mfa2::ADH1-COX4-GFP, was selected on 5-fluoro-orotic acid medium (Adams et al., 1997). Strain 1002 (MATα, his3, trp1, ura3, mfa2::ADH1-COX4-GFP) was constructed by crossing YHS2 to BY4731 (Brachmann et al., 1998).
Mutant Isolation

Y H2 was mutagenized with 3% ethanesulfonate to ~30% survival (A dams et al., 1997). Mutagenized cells were suspended at ~9 x 10^6 cells/mL on coverslips and observed using an inverted microscope and the H1G GFP 410-4 filter set (Chroma). Mutants were isolated using micropipettes (10 μm diameter; World Precision Instruments), transfected to a drop of SD on the same coverslip, and then to YPD plates. Micropipettes were handled by an Eppendorf micromanipulator 5171.

Crossovers to wild-type strain 1002 showed that all class I, II, and III mutations were recessive and caused by a defect in a single gene. Complementation tests revealed that all eight recessive class IV mutants were defective in the same gene. Crossovers between class IV mutants and TPR1 strain 194 (a gift from E. Schweizer) or dnm1Δ and allelic to recessive class IV mutants. All dominant and semidominant class IV mutations were visualized using pHS12, a plasmid which contains GFP with a NotI site at its NH2 terminus (Aiken, A., unpublished data), forming pDNM1-HA (pHS14). DNM1-GFP plasmid pHS20 was constructed as described above except that pAA3, a CEN-LU2 plasmid which contains GFP with a NotI site at its NH2 terminus (Aiken, A., unpublished data), was used instead of pAA3. To form pH515, DNM1-HA coding sequences were PCR amplified from pH514 with 50 bp of flanking sequences homologous to the GAL-1-URA3 promoter in pH314U (Nigro et al., 1992). The DNM1-HA fragment and linearized pH314U were cotransformed into yeast and pGAL1-DNM1-HA (pHS15) was formed by homologous recombination (Odenburg et al., 1997). pGAL1-DNM1-HA (pHS40) was constructed as described for pH515 except that pH520 was used instead of pH514.

Plasmid Construction

The DNM1 gene with a NotI site immediately preceding its termination codon was PCR amplified from yeast genomic DNA and subcloned into pAA3, a CEN-LU2 plasmid which contains the HA epitope with a NotI site at its NH2 terminus (Aiken, A., unpublished data), forming pDNM1-HA (pHS14). DNM1-GFP plasmid pHS20 was constructed as described above except that pAA3, a CEN-LU2 plasmid which contains GFP with a NotI site at its NH2 terminus (Aiken, A., unpublished data), was used instead of pAA3. To form pH515, DNM1-HA coding sequences were PCR amplified from pH514 with 50 bp of flanking sequences homologous to the GAL-1-URA3 promoter in pH314U (Nigro et al., 1992). The DNM1-HA fragment and linearized pH314U were cotransformed into yeast and pGAL1-DNM1-HA (pHS15) was formed by homologous recombination (Odenburg et al., 1997). pGAL1-DNM1-HA (pHS40) was constructed as described for pH515 except that pH520 was used instead of pH514.

Gene Disruption

Complete disruptions of the DNM1 and FZO1 genes were constructed by PCR-mediated gene replacement as described (Lorenz et al., 1995) into strains BY4733 and BY4744 (Brachmann et al., 1998). For dnm1Δ, we used HIS3 plasmid pRS303 (Sikorski and Hieter, 1989) and for fzo1Δ we used kanMX4 plasmid pS400 (Brachmann et al., 1998). MATa dnm1Δ fzo1Δ strain Y H527 and MATa dnm1Δ fzo1Δ strain Y H523 were constructed by crossing MATa dnm1Δ strain Y H519 to MATa fzo1Δ strain Y H522. Mitochondria in the disruption strains were visualized using pH512, a CEN-LU2 plasmid containing ADH1-COX4-GFP. pH512 was created by inserting the Xhol-NotI fragment from pH51 into pRS315 (Sikorski and Hieter, 1989).

Results and Discussion

We screened for yeast mutants defective in mitochondrial shape using a novel strategy in which mitochondria are visualized by the green fluorescent protein (GFP) and mutants were isolated by micromanipulation. GFP was fused to wild-type strain 1002 showed that all class I, II, and III mutations were recessive and caused by a defect in a single gene. Complementation tests revealed that all eight recessive class IV mutants were defective in the same gene. Crossovers between class IV mutants and TPR1 strain 194 (a gift from E. Schweizer) or dnm1Δ and allelic to recessive class IV mutants. All dominant and semidominant class IV mutations were visualized using pHS12, a plasmid which contains GFP with a NotI site at its NH2 terminus (Aiken, A., unpublished data), forming pDNM1-HA (pHS14). DNM1-GFP plasmid pHS20 was constructed as described above except that pAA3, a CEN-LU2 plasmid which contains GFP with a NotI site at its NH2 terminus (Aiken, A., unpublished data), was used instead of pAA3. To form pH515, DNM1-HA coding sequences were PCR amplified from pH514 with 50 bp of flanking sequences homologous to the GAL-1-URA3 promoter in pH314U (Nigro et al., 1992). The DNM1-HA fragment and linearized pH314U were cotransformed into yeast and pGAL1-DNM1-HA (pHS15) was formed by homologous recombination (Odenburg et al., 1997). pGAL1-DNM1-HA (pHS40) was constructed as described for pH515 except that pH520 was used instead of pH514.
to the presequence (residues 1–21) of mitochondrial cytochrome oxidase subunit IV (COX4; Pon and Schatz, 1991). When expressed in yeast, COX4-GFP targets the mitochondrial matrix, and mitochondria were visible by fluorescence microscopy. We integrated the COX4-GFP gene at the nonessential MFA2 locus (Michaelis and Herskowitz, 1988), which made fluorescence intensity uniform among cells and enabled efficient screening. After mutagenesis, individual cells with abnormal mitochondrial shape were hand-isolated using micropipettes (Fig. 1 A). This screening procedure allowed us to isolate individual mutant cells with interesting mitochondrial phenotypes from a large total population of cells.

Of ~72,000 cells screened, we isolated 20 mutants, which were classified into four categories (Fig. 1 B). Class I mutants (two isolates) contained one or two large, spherical mitochondria instead of the normal tubules seen in wild-type cells. Genetic crosses showed that both carried mdm10 mutants (Sogo and Yaffe, 1994). The single class II mutant contained one or two oblong mitochondria collapsed to one side of the cell and was found to be defective in SLM1. slm1 was previously identified as an mmm1 synthetic-lethal mutant (Burgess et al., 1994; Burgess et al., manuscript in preparation). Class III mutants (three isolates) contained numerous mitochondrial fragments and were shown to carry fzo1 mutations. FZO1 encodes a GTPase anchored in the mitochondrial outer membrane that is required for mitochondrial fusion (Hermann et al., 1998; Rapaport et al., 1998). Class IV mutants (14 isolates) exhibited a novel phenotype consisting of an interconnected network of mitochondrial tubules. In contrast to wild-type, which have 5-10 separate mitochondria per cell, class IV mutants appear to contain a single organelle. Because of their unique networked mitochondrial shape, these mutants were examined further.

Genetic crosses showed that our 14 class IV mutants comprised 8 recessive, 5 dominant and 1 semi-dominant mutations. Mapping studies showed that all 14 mutations were centromere linked (1.1 cM) and located on chromosome XII. We noted that DNM1 (Gammie et al., 1995), a gene related to dynamin GTPase (Obara et al., 1990), maps to chromosome XII near the centromere and is required for mitochondrial shape (Otsuga et al., 1998). Using a dnm1Δ strain and a plasmid containing DNM1 (kindly provided by J. Shaw), we found that all 14 class IV mutants carried dnm1 alleles. These results were unexpected since mitochondrial shape in our mutants was strikingly different from previously seen in dnm1 mutants, where mitochondria collapse to one side of the cell and form a single tubule (Otsuga et al., 1998).

![Figure 2. dnm1Δ fzo1Δ cells have normal-shaped mitochondria. Wild-type (BY4733) and isogenic deletion strains (dnm1Δ, fzo1Δ, and dnm1Δ fzo1Δ) expressing COX4-GFP (pHS12), were grown in YPGal to log phase and examined by fluorescence microscopy. Bars, 2 μm.](image-url)
A complete disruption of DNM1 coding sequences was constructed, and examined for mitochondrial shape (Fig. 1 C). ~90% of dnm1Δ cells showed a single highly branched mitochondrial network. ~10% of dnm1Δ cells displayed a single mitochondrial tubule localized to one side of the cell, similar to that seen earlier (Otsuga et al., 1998). The mitochondrial shape was not dramatically altered by growth conditions (not shown). Mitochondria in dnm1 mutants were efficiently segregated during cell division (Figs. 1 B and 2). Small daughter buds often contained a single mitochondrial tubule without branches, while larger buds had small networks. Most mitochondria were continuous from mother cells to buds, and separate mitochondria were only seen after the two cells separated. Our results strongly suggest that dnm1 mutants are defective in mitochondrial division. We speculate that mitochondria in dnm1 mutants may be divided indirectly, perhaps by cytokinesis. The yeast cell division machinery is clearly robust enough for the job, since nuclei are efficiently severed by cytokinesis in S. pombe cut mutants (Uzawa et al., 1990).

In yeast, mitochondria are very dynamic, fusing or dividing on average every two minutes (Nunnari et al., 1997). Thus there appears to be an equilibrium between fusion and division. Supporting this idea, when FZO1, a gene required for mitochondrial fusion, is defective, mitochondria fragment due to continued fission of the organelle (Hermann et al., 1998; Rapaport et al., 1998). We hypothesized that if mitochondrial division were blocked, cells would have fewer (larger) organelles. Our working model, based on the morphology of dnm1 mutants, is that Dnm1p is required for mitochondrial division. To test this hypothesis, we constructed double mutants containing both dnm1Δ and fzo1Δ by genetic crosses. We found that normal mitochondrial shape was restored (Fig. 2). ~85% of double mutants contained multiply-branched, tubular mitochondria very similar to those seen in wild-type cells (Table I). This was in marked contrast to dnm1Δ mutants, which usually had a single organelle, and fzo1Δ mutants, with numerous mitochondrial fragments (Fig. 2; Hermann et al., 1998; Rapaport et al., 1998). Mitochondria in dnm1Δ fzo1Δ cells were not always completely normal; the tubules tended to be longer and more curved than in wild-type cells, and occasionally formed bundles. Nonetheless, our observations suggest that excess mitochondrial division in fzo1Δ cells is suppressed by inactivating DNM1, and that excess mitochondrial fusion in dnm1Δ cells is rescued by fzo1Δ. We propose that division, which requires Dnm1p, and fusion, controlled by Fzo1p, have antagonistic effects on mitochondrial shape and number. Our results also suggest that mitochondrial tubule formation occurs by a mechanism independent of fusion and division.

Interestingly, mitochondrial shape and number in dnm1Δ fzo1Δ cells was dependent upon the order of gene disruption. When cells were first disrupted for FZO1 and subsequently for DNM1 (Table I, fzo1Δ→dnm1Δ, ~40% of cells carried mitochondrial fragments similar to those seen in fzo1Δ single mutants. In contrast, when cells were first disrupted for DNM1 and then for FZO1 (Table I, dnm1Δ→fzo1Δ), ~30% of cells displayed a mitochondrial network like that seen in dnm1Δ cells. Our results indicate that the mitochondrial networks found in dnm1Δ mutants

Table I. Mitochondrial Morphology in dnm1Δfzo1Δ Cells Created by Consecutive Gene Disruption or Genetic Cross

| Mitochondrial morphology | dnm1Δfzo1Δ Cells created by consecutive gene disruption* | dnm1Δfzo1Δ Cells created by cross† |
|--------------------------|---------------------------------------------------------|----------------------------------|
|                          | fzo1Δ→dnm1Δ | dnm1Δ→fzo1Δ |                                   |
| Tubules                  | 50.0 ± 7.5  | 60.1 ± 4.7  | 84.7 ± 3.4                       |
| Networks                 | 0.3 ± 0.7   | 30.6 ± 3.6  | 0.3 ± 0.6                        |
| Fragments                | 39.9 ± 5.2  | 0 ± 0       | 0 ± 0                            |
| Other‡                   | 9.8 ± 3.3   | 9.3 ± 3.1   | 14.9 ± 2.9                       |

*Double mutants were generated by disrupting FZO1 in dnm1Δ cells (dnm1Δ→fzo1Δ) or by disrupting DNM1 in fzo1Δ cells (fzo1Δ→dnm1Δ). Cells were grown in YPGal, stained with 1 μg/ml DIOC4, and examined for mitochondrial shape (tubules, networks, and fragments) by fluorescence microscopy.
†dnm1Δ fzo1Δ mutants were generated by crossing dnm1Δ to fzo1Δ cells (see Materials and Methods).
‡Other indicated disorganized mitochondrial aggregates. The results were presented as the mean ± SD. At least 300 cells were scored in each experiment.

Figure 3. Expression of Dnm1p causes fragmentation of mitochondria in dnm1Δ fzo1Δ cells. dnm1Δ fzo1Δ cells carrying pCOX4-GFP (pHS12) and pGAL1-DNM1-HA (pHS15) were pregrown in raffinose medium, centrifuged and resuspended to an OD600 of 0.2 in galactose medium (SGS) to induce Dnm1p-HA expression. Cells were examined for mitochondrial shape (n = 100) or used to prepare total protein at the indicated timepoints. (A) Mitochondrial shape was classified into the following four groups: tubules (○), partially fragmented tubules (□), fragments (△), and other (○). (B) Total protein was extracted as described (Yaffe and Schatz, 1984) and subjected to Western blot analysis using antibodies to the HA epitope (Field et al., 1988), or hexokinase (D. Davis, A., unpublished data) followed by chemiluminescence (Pierce). Relative amounts of Dnm1p-HA (+) were quantitated and plotted in A.
When fzo1 crossing a nation and growth of a mitochrondial networks or fragments (Table I). During germi-
ruption (of the double mutant cells formed by consecutive gene dis-
clear why tubules form in the absence of division and fusion. It is not
chondrial tubules were adjacent to small fragments. Our
contained partially fragmented tubules, and many mito-
fragmented mitochondria increased. By 5 h,
cells with tubular mitochondria decreased, and those with
shape changed dramatically (Fig. 3 A). The number of
tubular mitochondria typical of
was detected (Fig. 3 B) and
were grown in the absence of galactose, no Dnm1p-HA
mutant persist in the absence of fusion activity, and fragments
formed in the fzo1Δ mutant persist in the absence of fission activity. We also found tubular mitochondria in many
of the double mutant cells formed by consecutive gene dis-
DNm1p was fused to the HA epitope (Dnm1p-HA) (Field et al., 1988) and ex-
pressed under the galactose-inducible GAL1 promoter (Nigro et al., 1992). Our pGAL1-DNM1-HA rescued the
dnm1Δ phenotype on galactose medium (not shown). When dnm1Δ fzo1Δ cells containing pGAL1-DNM1-HA were
in the absence of galactose, no Dnm1p-HA was detected (Fig. 3 B) and ~70% of cells displayed the
tubular mitochondria typical of dnm1Δ fzo1Δ mutants (Fig. 3 A). Upon transfer to galactose medium, Dnm1p-HA levels gradually increased, while the level of hexokinase, a control protein, remained constant (Fig. 3 B). Concomi-
tant with the accumulation of Dnm1p-HA, mitochondrial shape changed dramatically (Fig. 3 A). The number of
cells with tubular mitochondria decreased, and those with fragmented mitochondria increased. By 5 h, ~65% of the
cells contained completely fragmented mitochondria. At intermediate times (2 h) after inducing Dnm1p-HA, cells
contained partially fragmented tubules, and many mitochrondial tubules were adjacent to small fragments. Our
results clearly show that the division of mitochondria in
dnm1Δ fzo1Δ cells coincides with the expression of Dnm1p.
Further supporting a role for Dnm1p in mitochondrial fission, we found that the Dnm1 protein was preferentially
localized to sites of mitochondrial division. We con-
structed a fusion between Dnm1p and the green fluores-
cent protein (GFP). Consistent with previous results (Ot-
suga et al., 1998), we found that much of Dnm1p-GFP was
associated with mitochondria in punctate structures (Fig. 4 A). In cells that were constitutively expressing Dnm1p-
GFp, it was difficult to determine the precise location of Dnm1p because of the complex morphology of the mitochrondia and the large number of Dnm1p-GFP dots. To simplify our analyses, we induced the expression of Dnm1p-GFP in the dnm1Δ fzo1Δ mutant and examined
cells at early times after induction. We found a tight corre-
lation between the appearance of Dnm1p-GFP and frag-
mentation of mitochondria. Two representative cells are
shown in Fig. 4, B and C; both cells contained two Dnm1p-
GFp dots, one of which was located at the end of a tubule, the other appeared to be reside near a constricted region of the mitochondrion. After analysis of additional cells, we
found that Dnm1p-GFP was localized to ends of mitochrondial fragments much more frequently (~60%) than
predicted if Dnm1p-GFP was randomly distributed on mitochondria (~11%). These results suggest that Dnm1p acts at the site of mitochondrial fission. We note that
Dnm1p-GFP is not exclusively found at the ends of mitochrondia. We surmise that Dnm1p on the sides of the tu-
bules may mark future sites of division, or represent
Dnm1p-containing complexes that have diffused away from the end of the tubule. More definitive experiments (e.g., time-lapse videomicroscopy) to determine the role of Dnm1p in fission are in progress.

The relatively normal mitochondria seen in dnm1Δ fzo1Δ mutants could be explained by a restoration of fu-

![Figure 4. A Dnm1p-GFP preferentially localizes to site of mitochondrial division. (A) dnm1Δ strain YHS19 was transformed with pHS20 expressing Dnm1p-GFP. Cells were grown in SGal medium, labeled with 0.1 μM MitoTracker Red CMX Ros (Molecular Probes) and then examined under the fluorescence microscope. Merged images taken in the red (MitoTracker) and green (GFP) channels are shown. (B and C) dnm1Δ fzo1Δ diploid cells carrying pGAL1-DNM1-GFP were pregrown in YPGlycerol/ethanol medium, centrifuged and grown in galactose medium (SGS) for 1–2 h. Cells were then stained with MitoTracker and examined for mitochondrial localization of Dnm1p-GFP. Two representative cells are shown. Bar, 3 μm.](image-url)
sion activity; for example, if Dnm1p were an inhibitor of mitochondrial fusion. To test this possibility, we monitored mitochondrial fusion during mating (Nunnari et al., 1997; Okamoto et al., 1998). Mitochondria were visualized in one parent, by the galactose-induced expression of a matrix-targeted GFP (CS1-GFP) on plasmid pCLbGFP (Okamoto et al., 1998). MATa cells containing pCLbGFP were pregrown in galactose medium to induce CS1-GFP expression, then transferred to glucose to inhibit further synthesis. MATa cells were then mixed with MATα cells, which did not carry pCLbGFP, and allowed to mate on glucose medium. Mitochondria were visualized in zygotes using MitoTracker. If mitochondrial fusion occurred, GFP and MitoTracker fluorescence would completely overlap, because the matrix-localized CS1-GFP from the MATa mitochondria diffused into the mitochondrial matrix of the MATα cell. If no fusion occurred, GFP-labeled mitochondria would be seen in only one half of the zygote.

Zygotes formed by two wild-type cells, or two dnm1Δ mutants, exhibited efficient mitochondrial fusion, with GFP fluorescence and MitoTracker overlapping in all mitochondria (Fig. 4 A). In contrast, fusion was defective in fzo1Δ mutants, consistent with previous observations (Hermann et al., 1998). Mitochondrial fragments tended to aggregate in fzo1Δ cells and individual fragments were difficult to distinguish. Nonetheless, in matings between two fzo1Δ cells, MitoTracker showed clusters of fragmented mitochondria in the zygote and diploid bud, but we detected GFP fluorescence in only half of the mitochondrial clusters. Like fzo1Δ mutants, dnm1Δ fzo1Δ double mutants failed to fuse their mitochondria. Although mitochondria in dnm1Δ fzo1Δ/dnm1Δ fzo1Δ diploid cells had normal shape, only half of the organelles contained GFP. Our results indicate that dnm1Δ fzo1Δ cells are defective in mitochondrial fusion.

To eliminate the possibility that low, basal levels of fu-
sion occur in dnm1Δ fzo1Δ cells, we used a more sensitive fusion assay using the matrix markers, CS1-GFP and mitochondrial DNA (mtDNA; Okamoto et al., 1998). 4,6-diamidino-2-phenylindole (DAPI) stained mtDNA was a more stable probe compared with MitoTracker, allowing us to examine cells for longer times following the initial mating event. MATα cells, which lacked mtDNA and carried pCLbGFP were mated to MATα cells, which contained mtDNA, but not the plasmid (Fig. 5 B). The DAPI and GFP fluorescence overlapped in all the mitochondrial tubules in wild-type zygotes (52 zygotes examined). When 500 dnm1Δ fzo1Δ zygotes were examined, we found no overlap between DAPI and GFP. The fusion activity in dnm1Δ fzo1Δ mutants is therefore at least 500-fold less than that in wild-type cells. Even after the zygotes were allowed to grow and divide, we found no fusion in the mutant cells (Fig. 5 B). When 100 dnm1Δ fzo1Δ/dnm1Δ fzo1Δ diploid cells were examined 24 h after mating, none contained an overlap between GFP and DAPI, whereas a complete GFP and DAPI overlap was seen in 43 wild-type diploids. dnm1Δ fzo1Δ cells clearly lack significant mitochondrial fusion activity. Our results above also suggest that dnm1Δ fzo1Δ cells lack fission activity. We therefore propose that in cells lacking Fzo1p and Dnm1p, mitochondrial tubule formation occurs by a mechanism independent of fusion and division, such as growth from the ends of preexisting organelles.

Dynamin has been proposed to work as a mechanochemical enzyme that ‘pinches off’ plasma membrane invaginations, forming intracellular vesicles (Takei et al., 1995). Supporting this idea, dynamin self-assembles into spiral-like structures (Hinshaw and Schmid, 1995) which can sever artificial membranes in vitro (Sweitzer and Hinshaw, 1998). It is also possible that dynamin plays a regulatory, instead of an enzymatic, function in membrane scission (Sever et al., 1999). Future studies are clearly needed to determine the precise mechanism that Dnm1p plays in mitochondrial division.

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