

**UGO1 Encodes an Outer Membrane Protein Required for Mitochondrial Fusion**

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**Abstract.** Membrane fusion plays an important role in controlling the shape, number, and distribution of mitochondria. In the yeast *Saccharomyces cerevisiae*, the outer membrane protein Fzo1p has been shown to mediate mitochondrial fusion. Using a novel genetic screen, we have isolated new mutants defective in the fusion of their mitochondria. One of these mutants, *ugo1*, shows several similarities to *fzo1* mutants. *ugo1* cells contain numerous mitochondrial fragments instead of the few long, tubular organelles seen in wild-type cells. *ugo1* mutants lose mitochondrial DNA (mtDNA). In zygotes formed by mating two *ugo1* cells, mitochondria do not fuse and mix their matrix contents. Fragmentation of mitochondria and loss of mtDNA in *ugo1* mutants are rescued by disrupting *DNM1*, a gene required for mitochondrial division. We find that *UGO1* encodes a 58-kD protein located in the mitochondrial outer membrane. Ugo1p appears to contain a single transmembrane segment, with its NH₂ terminus facing the cytosol and its COOH terminus in the intermembrane space. Our results suggest that Ugo1p is a new outer membrane component of the mitochondrial fusion machinery.

**Key words:** mitochondria • organelle dynamics • membrane fusion • outer membrane • yeast

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**Introduction**

Mitochondrial fusion is a fundamental process required for establishing and maintaining the specialized shapes and numbers of mitochondria in many cell types (Tyler, 1992; Bereiter-Hahn and Voth, 1994). In the yeast *Saccharomyces cerevisiae*, mitochondrial fusion and its opposite activity, division, are highly regulated during growth, mating, and sporulation (Hermann and Shaw, 1998; Yaffe, 1999; Jensen et al., 2000). During vegetative growth, mitochondria constitutively fuse, divide (Nunnari et al., 1997), and change their number depending on growth conditions (Stevens, 1977). For instance, exponentially growing cells contain several branched, tubular mitochondria. When cells enter stationary phase, the mitochondrial tubules fragment into numerous small organelles (Hoffman and Avers, 1973; Stevens, 1977). When yeast cells mate, mitochondria fuse immediately after cell fusion, mixing their contents, including mitochondrial DNA (mtDNA) and proteins (Thomas and Wilkie, 1968; Dujon, 1981; Nunnari et al., 1997; Okamoto et al., 1998). When diploids go through meiosis and sporulation, mitochondria undergo several fusion and division events, eventually encircling each of the four haploid nuclei (Miyakawa et al., 1984).

Mitochondrial fusion in yeast requires the Fzo1 protein (Hermann et al., 1998; Rapaport et al., 1998). Fzo1p was identified as a homologue to the *Drosophila* fuzzy onions protein, which is required for mitochondrial fusion during fly spermatogenesis (Hales and Fuller, 1997). Fzo1p is a mitochondrial outer membrane protein with a cytosolic GTPase domain at its NH₂ terminus (Hermann et al., 1998; Rapaport et al., 1998). In *FZO1* disruption mutants, cells contain many small mitochondrial fragments instead of the few tubular mitochondria seen in wild-type cells (Hermann et al., 1998; Rapaport et al., 1998), and a defect in mitochondrial fusion in *fzo1* mutants has been directly demonstrated using a mating assay (Hermann et al., 1998). In addition to fusion, Fzo1p is also important for maintenance of mtDNA. *fzo1* mutants lack mtDNA, but the mechanism by which mtDNA is lost in *fzo1* cells is not understood (Hermann et al., 1998; Rapaport et al., 1998).

The fragmentation of mitochondria in *fzo1* mutants depends on mitochondrial division. Dnm1p is a dynamin-related GTPase (Gammie et al., 1995; Otsuga et al., 1998) and *dnm1* mutants are defective in mitochondrial division (Bleazard et al., 1999; Sesaki and Jensen, 1999). Cells disrupted for *DNM1* contain a single mitochondrion consisting of a network of interconnected tubules. In *dnm1 fzo1*
Materials and Methods

The fusion of mitochondria.

We show that one of these mutants, ugo1, identifies a new mitochondrial outer membrane protein required for mitochondrial fusion.

In this report, we have used a novel genetic screen to isolate new yeast mutants defective in mitochondrial fusion. We show that one of these mutants, ugo1, identifies a new mitochondrial outer membrane protein required for the fusion of mitochondria.

Strains, Media, and Genetic Methods

Yeast strains used in this study are listed in Table I. Yeast media, including YEPD (yeast-extract peptone [YEP] medium containing 2% glucose), YEPGE (YEP medium containing 2% glycerol and 2% ethanol), YEPGall (YEP medium containing 2% galactose), SD (synthetic medium containing 2% glucose), SRAf (synthetic medium containing 2% raffinose), SGalSuc (synthetic medium containing 2% galactose and 2% sucrose), 5FOAD (synthetic medium containing 2% galactose), SGal (synthetic medium containing 2% glucose), SRaf (synthetic medium containing 2% raffinose), SGalSuc (synthetic medium containing 2% galactose and 2% sucrose), 5FOA (synthetic medium containing 0.1% 5-fluoro-orotic acid [5FOA] and 2% glucose), and SGalSuc (synthetic medium containing 0.1% 5FOA, 2% glucose, and 2% ethanol) are as described (Boeke et al., 1984). Standard molecular genetic techniques were used (Adams et al., 1997).

Plasmid Construction

pHS29, a CEN-LEU2 plasmid containing DNM1-111 (Jensen et al., 2000), tagged with the triple influenza hemagglutinin (HA) epitope (Field et al., 1998) at the COOH terminus (Dnm1p-111-HA), was constructed as follows. The DNM1-111 gene, which contains two mutations in the GTPase domain of Dnm1p (Jensen et al., 2000), was PCR amplified from yeast genomic DNA (Hoffman and Winston, 1987) prepared from DNM1-111 strain, YHS15 (Jensen et al., 2000), using oligos 268 (5'-CCGGTCTGGAGAACAGGAAAGCAGCCTACTACGTCAATC-3') and 269 (5'-ATAAGAACATGGCAGCCGCCCAGATTTACTAATAGC-3'). PCR product was digested with XhoI and NotI, and subcloned into XhoI-NotI-digested pAA3 (Sesaki and Jensen, 1999). pHS30, a 2µ-URA3 plasmid expressing Dnm1p-111-HA, was constructed by cotransforming a PvuII fragment from pHS29 with PvuII- and XhoI-HindIII–digested pRS426 (Sikorski and Hieter, 1989) into yeast cells. Homologous recombination between the DNM1-111-HA fragment and the linearized plasmid (Oldenbourg et al., 1997) created plasmid pH50.

pHS51, a CEN-URA3 plasmid expressing red fluorescent protein (RFP) fused to the presequence of Cox4 under the control of the GAL1 promoter was constructed as follows. RFP was PCR amplified from plasmid ST10 (a gift from B. Glick, University of Chicago, Chicago, IL) using oligos 461 (5'-GGGGTCTGGAGAACAGGAAAGCAGCCTACTACGTCAATC-3') and 462 (5'-ATCTAGGGGCCCAGGCCCAGACCATG-3') and 463 (5'-ATCTAGAGTGACCTACGTCAATC-3') and 464 (5'-GGGGGATCCCTA-3'). The PCR fragment was digested with XbaI and NotI and subcloned into XbaI-NotI-digested pH12 (Sesaki and Jensen, 1999), forming pH12-RFP. COX4-RFP was PCR amplified from pH12-RFP using oligos 473 (5'-GGGCTCGAGATGATTTCTTTGAGCGAC-3') and 474 (5'-GGGGGATCCCTA-3'). The PCR product was subcloned into XhoI-NotI-digested pRS314 (Sikorski and Hieter, 1989).

pHS52, a CEN-URA3 plasmid expressing cyan fluorescent protein (CFP) fused to the presequence of Cox4 from the GAL1 promoter, was constructed by PCR amplifying CFP from pECFP (CLONTECH Laboratories, Inc.) using oligos 355 (5'-TGCTCTAGAATGGTACCGAC-3') and 356 (5'-CCGGATCCCTA-3'). The PCR fragment was digested with XbaI and BamHI, and subcloned downstream of the GAL1 promoter in XhoI-BamHI-digested pRS1616U (Nigro et al., 1992).

pHS1, a CEN-URA3 plasmid expressing red fluorescent protein (RFP) fused to the presequence of Cox4 under the control of the GAL1 promoter was constructed as follows. RFP was PCR amplified from plasmid ST10 (a gift from B. Glick, University of Chicago, Chicago, IL) using oligos 461 (5'-GGGGTCTGGAGAACAGGAAAGCAGCCTACTACGTCAATC-3') and 462 (5'-ATCTAGGGGCCCAGGCCCAGACCATG-3') and 463 (5'-ATCTAGAGTGACCTACGTCAATC-3') and 464 (5'-GGGGGATCCCTA-3'). The PCR fragment was digested with XbaI and NotI, and subcloned into XbaI-NotI-digested pAA3 (Sesaki and Jensen, 1999).
Mitochondrial Fusion Assay

Mitochondrial fusion during mating was observed as described (Nunnari et al., 1997; Okamoto et al., 1998), but with the following modifications. MAta strains that carry pGAL1-COX4-RFP (pHS51) were grown to log phase in SRaf medium overnight, pelleted by centrifugation, and resuspended in S medium with 2% galactose and 2% sucrose (SgalSuc) to an OD 600 of 0.2 for 3–5 h to induce COX4-RFP expression. MAta strains carrying pGAL-COX4-CFP were grown to log phase in SgalSuc medium overnight. Cells were collected, washed, and resuspended in 2 ml of YEFP medium at an OD 600 of 0.2. Two strains were mixed and collected by centrifugation. Cells were resuspended in 5 μl of YEFP medium and placed on a nitrocellulose membrane and excess solution was removed by placing the membrane on filter papers. The nitrocellulose membrane was then incubated on YEFP medium at 30°C for 3.5 h. Zygotes were examined by fluorescence microscopy.

Immunofluorescence

Cells were fixed in 4% paraformaldehyde, converted to spheroplasts, attached to poly-L-lysine coated coverslips, and permeabilized as described (Harlow and Lane, 1988). Samples were incubated with a 1:100 dilution of antibodies to the myc epitope (9E10; Covance) in PBS containing 1% BSA and 0.05% Tween 20, and with 1:100 dilution of antiserum to the β subunit of the F1-ATPase (a gift from M. Yaffe, University of California, San Diego, CA) for 1 h, washed three times in PBS containing 0.05% Tween 20, and then stained a 1:200 dilution of FITC-conjugated goat anti-mouse IgG (Boehringer) and a 1:500 dilution of rhodamine-conjugated goat anti-rabbit IgG (Boehringer) for 1 h. Samples were washed and mounted in 95% glycerol containing 0.1% p-phenylene diamine and observed.

Subcellular and Submitochondrial Fractionation

Yeast cells were grown to an OD 600 of ~2 in Sgal medium. Cells were converted to spheroplasts, homogenized, and separated into a mitochondrial pellet and a postmitochondrial supernatant by centrifugation at 9,600 g for 10 min as described (Daum et al., 1982). Separation of outer membrane and inner membrane vesicles on sucrose gradients was performed as described (Ryan et al., 1994). For protease digestion, mitochondria were resuspended at 1 mg/ml in 250 mM sucrose, 20 mM Hepes-HCl, pH 7.5, and treated with 200 μg/ml trypsin (Sigma-Aldrich) for 20 min on ice, followed by the addition of 2 mg/ml soybean trypsin inhibitor (Sigma-Aldrich). For analysis, proteins were separated on SDS-PAGE (Laemmli, 1970) and transferred to Immobilon filters (Millipore; Haid and Suisa, 1985). Filters were probed with antibodies to the myc epitope (9E10), the HA epitope (12CA5; Niman et al., 1983), Fβ ATPase, Tim23p (Emtage and Jensen, 1993), and OM45p (Yaffe et al., 1989), all at 1:10,000 dilution, or hexokinase (Kerscher et al., 2000) at 1:20,000 dilution. Immune complexes were visualized using 1:10,000 dilution of HRP-conjugated secondary antibodies (Amersham Pharmacia Biotech) followed by chemiluminescence (SuperSignal; Pierce Chemical Co.).

Fluorescence Microscopy

Cells were observed using an Axioskop microscope (ZEISS) with a 100× Plan-Neofluar objective. Fluorescence and differential interference contrast (DIC) images were captured with a MicroMax CCD camera (Princeton Instruments) using IP Lab software v3.2.0 (Signal Analytics Co.).

Results

Isolation of Mutants That Lose Mitochondrial DNA in a Dnm1p-dependent Manner

fzo1 mutants are defective in mitochondrial fusion and also lose mtDNA. The loss of mtDNA in fzo1 cells can be suppressed by inactivation of Dnm1p function (Bleazard et al., 1999; Sesaki and Jensen, 1999; Jensen et al., 2000). To identify new components required for fusion, we screened for mutants that maintain mtDNA when Dnm1p activity is absent, but lose mtDNA in the presence of fun-

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We controlled Dnm1p activity using the URA3 plasmid pHS50, which carries a dominant negative version of Dnm1p, Dnm1p-111 (Fig. 1 A; Sesaki and Jensen, 1999; Jensen et al., 2000). Cells that lose pHS50 contain functional Dnm1p. (B) Mutant isolation scheme. The parental strain (WT) maintains mtDNA on glucose-containing medium in the presence (SD-Ura) or absence (5FOAD) of the URA3-DNM1-111 plasmid and form red colonies due to the ade2 mutation (Reaume and Tatum, 1949). ugo mutants maintain mtDNA only in the presence of the pHS50, forming red colonies on SD-Ura, but become white on 5FOAD medium, which selects for loss of the URA3-DNM1-111 plasmid (Boeke et al., 1984). Growth on glycerol and ethanol medium confirms that ugo mutants contain mtDNA in the presence of the URA3-DNM1-111 plasmid (YPGE), but that ugo mutants are inviable on 5FOA medium containing glycerol and ethanol (5FOAGE). (C) Four different genes were identified in the ugo screen. Complementation tests showed one fzo1 mutant, five mgm1 mutants, and two new mutants, ugo1 and ugo2, were isolated. (D) ugo mutants contain highly fragmented mitochondria. Wild-type (W303), ugo1-1 (YHS64), and ugo2-1 (YHS65) were grown on 5FOAD medium to select for cells that lost the URA3-DNM1-111 plasmid, pHS50, and then transformed with pKC2, which expresses GFP fused to the COOH terminus of the mitochondrial outer membrane protein, OM45p (OM45-GFP; Cerveny et al., 2001). Cells were grown to log phase in SRaf medium and examined by fluorescence microscopy. Fluorescence (OM45-GFP) and DIC images are shown. Bar, 3 μm.

Figure 1. Isolation of ugo mutants. (A) Strain used to isolate ugo1 and ugo2. ade2 DNM1 strains that carry plasmid pHS50, which expresses the dominant negative Dnm1-111 protein, lack Dnm1p activity. Cells that lose pHS50 contain functional Dnm1p. (B) Mutant isolation scheme. The parental strain (WT) maintains mtDNA on glucose-containing medium in the presence (SD-Ura) or absence (5FOAD) of the URA3-DNM1-111 plasmid and form red colonies due to the ade2 mutation (Reaume and Tatum, 1949). ugo mutants maintain mtDNA only in the presence of the pHS50, forming red colonies on SD-Ura, but become white on 5FOAD medium, which selects for loss of the URA3-DNM1-111 plasmid (Boeke et al., 1984). Growth on glycerol and ethanol medium confirms that ugo mutants contain mtDNA in the presence of the URA3-DNM1-111 plasmid (YPGE), but that ugo mutants are inviable on 5FOA medium containing glycerol and ethanol (5FOAGE). (C) Four different genes were identified in the ugo screen. Complementation tests showed one fzo1 mutant, five mgm1 mutants, and two new mutants, ugo1 and ugo2, were isolated. (D) ugo mutants contain highly fragmented mitochondria. Wild-type (W303), ugo1-1 (YHS64), and ugo2-1 (YHS65) were grown on 5FOAD medium to select for cells that lost the URA3-DNM1-111 plasmid, pHS50.

Figure 2. ugo1Δ cells grow slowly on glucose-containing medium and are inviable on nonfermentable carbon sources. (A) 10 meiotic products from the ugo1::HIS3/UGO1 diploid strain, YHS91, were separated by micromanipulation and allowed to grow at 30°C for 5 d on YEPD. (B) 10 tetrads from the sporulated ugo1::HIS3/UGO1 diploid strain, YHS91, were patched onto YEPD medium and then replica-plated to SD medium lacking histidine (SD-His) and YEPGE. Cells were incubated at 30°C for 6 d.
Our genetic screen identified four different genes. In crosses to fzo1 cells, we found that one of our mutants carried an fzo1 allele (Fig. 1 C). Since our genetic screen was based on the behavior of fzo1 mutants, this result was expected. Furthermore, since mgm1 mutants lose mtDNA in a Dnm1p-dependent manner (Fekkes et al., 2000), we anticipated that we would find mgm1 mutants. Five of our mutants carried mgm1 alleles. The two remaining mutants formed two new complementation groups, which we have called ugo1 and ugo2 (ugo is Japanese for fusion). We examined mitochondrial shape in our ugo1 and ugo2 mutants (Fig. 1 D). Wild-type, ugo1-1, and ugo2-1 cells were transformed with a plasmid expressing green fluorescent protein (GFP) fused to the mitochondrial outer membrane protein, OM45 (Cerveny et al., 2001). In wild-type cells, mitochondria were seen as long, tubular structures with occasional branches. In contrast, in both ugo1-1 and ugo2-1 mutants, fragmented mitochondria were found, similar to those seen in fzo1 cells (Hermann et al., 1998; Rapaport et al., 1998; see below). In this report, we focus on the characterization of the ugo1 mutant and the description of ugo2 is the subject of another study.

**UGO1 Encodes a Novel 58-kD Protein**

Since ugo1 cells lose mtDNA in the presence of functional Dnm1p, we isolated UGO1 by screening a genomic library for clones that allow ugo1-1 to maintain mtDNA. ugo1-1 cells containing the URA3-DNM1-111 plasmid pH50 were transformed with a yeast genomic DNA library. We then selected for loss of pH50 and asked if cells could retain their mtDNA by replica-plating transformants to 5FOA medium with glycerol and ethanol as the sole car-

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**Figure 3.** ugo1Δ cells contain fragmented mitochondria and lack mtDNA. Wild-type (FY833), rho0 WT (YHS92), and ugo1Δ (YHS72) and fzo1Δ (YHS74) cells were transformed with OM45-GFP–expressing plasmid, pKC2 (Cerveny et al., 2001). Cells were grown to log phase in SRaf medium. Cells were stained using 1 μg/ml DAPI, and viewed by DIC and fluorescence (OM45-GFP or DAPI) microscopy. rho0 cells (YHS92) were generated by treating wild-type cells (FY833) with 25 μg/ml ethidium bromide as described (Fox et al., 1991). N, nuclear DNA staining. Bar, 3 μm.
mtDNA nucleoids were present in wild-type cells (Fig. 3). The DNA-specific dye, DAPI, DAPI staining showed that mtDNA is essential for growth on glycerol and ethanol, we asked if mitochondrial energy transfer protein signatures (Nelson et al., 1998) at residues 132–144 and 310–319. This motif consists of 10 loosely conserved amino acids and is found in many mitochondrial carrier proteins such as AAC2, CTP1, and DIC1 (Nelson et al., 1998). We also found that Ugo1p is ~22% identical to a Schizosaccharomyces pombe protein encoded by the SPAc1B2.02c gene.

Ugo1p Is Essential for Growth on Nonfermentable Carbon Sources and for Maintenance of mtDNA

To further investigate the function of Ugo1p, we created a null allele by replacing the UGO1 open reading frame with the yeast HIS3 gene (ugo1Δ). UGO1/ugo1Δ diploid cells were sporulated and the haploid segregants were allowed to grow on YEPD. We found that all four spores in each tetrad were viable, but that two spores formed small colonies (Fig. 2 A). The slower growing cells were shown to carry the ugo1Δ gene, whereas cells from larger-sized colonies contained UGO1. Ugo1p appears to be required for normal cell growth. As shown in Fig. 2 B, cells carrying the ugo1Δ disruption failed to grow on YEPGE. Since mtDNA is essential for growth on glycerol and ethanol, we asked if ugo1Δ cells lack mtDNA by staining them with the DNA-specific dye, DAPI. DAPI staining showed that mtDNA nucleoids were present in wild-type cells (Fig. 3).

In contrast, ugo1Δ cells contained little or no mtDNA and only faint staining of nuclear DNA was seen, similar to wild-type cells lacking mtDNA (rho0 WT). Our results indicate that UGO1 is required for growth on nonfermentable carbon sources and to maintain mtDNA.

ugo1Δ Cells Contain Fragmented Mitochondria

To further probe the function of UGO1, we examined mitochondria in wild-type, fzo1Δ, and ugo1Δ cells. Mitochondria were visualized using an outer membrane–targeted GFP fusion protein, OM45-GFP (Fig. 3). Although wild-type cells contained a few elongated mitochondrial tubules with occasional branches, ugo1Δ and fzo1Δ cells showed many small mitochondrial fragments. In most ugo1Δ cells (64%, n = 102) mitochondria were distributed uniformly at the cell periphery (Fig. 3, left panel of ugo1Δ image). In the remaining ugo1Δ cells, mitochondria were somewhat aggregated (Fig. 3, right panel of ugo1Δ image). Although the morphology of mitochondria in ugo1Δ cells was dramatically altered, mitochondrial transmission was not altered. Mitochondrial fragments were always seen in both mother and daughter cells. The altered mitochondrial shape in ugo1Δ cells was not due to lack of mtDNA. Wild-type cells which lack mtDNA showed normal tubular-shaped mitochondria (Fig. 3, rho0 WT).

Although the mitochondrial fragments seen in ugo1Δ cells are similar to those seen in fzo1Δ cells, we found noticeable differences between ugo1Δ and fzo1Δ mutants (Fig. 3). For example, ugo1Δ cells contained slightly larger fragments (0.47 μm in average diameter, n = 50) than fzo1Δ cells (0.40 μm, n = 50). Mitochondria in ugo1Δ cells showed markedly different sizes (ranging from 0.21 to 1.06 μm), whereas the organelles in fzo1Δ cells displayed relatively uniform sizes (ranging from 0.28 to 0.69 μm). We also noticed that mitochondria in ugo1Δ cells were aggregated...
Figure 5. *ugo1Δ* cells contain mitochondrial tubules and maintain mtDNA. Wild-type (FY833, WT), *ugo1Δ* (YHS72), *dnm1Δ* (YHS83), and *ugo1Δ dnm1Δ* (YHS85) cells expressing OM45-GFP (pKC2) were grown to log phase in SRaf medium, stained with 1 μg/ml DAPI, and viewed by DIC and fluorescence (OM45-GFP) microscopy. (B) Wild-type, *ugo1Δ*, *dnm1Δ*, and *ugo1Δ dnm1Δ* cells were grown to log phase in YEPD medium. Cells were collected and resuspended in YEPD medium to an OD<sub>600</sub> of 2. Cells were then diluted in 10-fold increments, and 10 μl of each dilution was spotted onto YEPD and YEPGE media and incubated at 30°C for 2 and 6 d, respectively. N, nuclear DNA staining. Bar, 3 μm.

Disruption of DNM1 Rescues Fragmentation of Mitochondria and Loss of mtDNA in *ugo1Δ* Cells

Mitochondrial fusion and division are normally balanced in cells, leading to the few tubular-shaped mitochondria seen in wild-type cells. The fragmentation of mitochondria in *fzo1Δ* results from continued division in the absence of fusion, and disruption of a gene required for division, *DNM1*, in *fzo1Δ* cells restores normal mitochondrial shape and number (Sesaki and Jensen, 1999). To test if *ugo1Δ* shows a similar interplay with *dnm1Δ* as that seen with *fzo1Δ* and *dnm1Δ*, we compared mitochondrial shape in either *ugo1Δ* mutants or *dnm1Δ* mutants to those in *ugo1Δ dnm1Δ* double mutants. In *dnm1Δ* mutants, a single mitochondrial morphology consisting of a network of interconnected tubules is seen, resulting from ongoing fusion in the absence of mitochondrial division (Fig. 5 A; Bleazard et al., 1999; Sesaki and Jensen, 1999). As noted previously, *ugo1Δ* cells contain many small mitochondrial fragments (Figs. 3 and 5). In contrast, in the majority of *ugo1Δ dnm1Δ* cells (90%, n = 100), mitochondria appeared as elongated tubules, similar to those in wild-type cells (Fig. 5 A) or in *fzo1Δ dnm1Δ* double mutants (Sesaki and Jensen, 1999). In ~56% of *ugo1Δ dnm1Δ* cells mitochondrial tubules were often collapsed to one side of the cell and appeared to be bundled (Fig. 5 A, left panel of *ugo1Δ dnm1Δ* images). In 34% of *ugo1Δ dnm1Δ* cells individual mitochondrial tubules were clearly separated from other tubules (Fig. 5 A, right panel of *ugo1Δ dnm1Δ* images). Only a small fraction of *ugo1Δ dnm1Δ* cells (~10%) showed mitochondria that appeared to be fragmented and aggregated. Thus, our results demonstrate that the fragmentation of mitochondria in *ugo1Δ* cells can be suppressed by *dnm1Δ* disruption. Ugo1p, like Fzo1p, appears to function in mitochondrial fusion, an activity antagonistic to the Dnm1p-mediated division of mitochondria.

Disruption of *DNM1* also rescued the loss of mtDNA in *ugo1Δ* cells. When wild-type cells, *ugo1Δ* mutants, *dnm1Δ* mutants, or *ugo1Δ dnm1Δ* double mutants were stained with DAPI, we found that mtDNA was absent from *ugo1Δ* cells (Fig. 5 A). However, similar amounts of mtDNA nucleoids were found in wild-type cells, *dnm1Δ* mutants, and *ugo1Δ dnm1Δ* mutants (Fig. 5 A). Furthermore, in con-
RFP and CFP should be seen in separate organelles. COX4-RFP and COX4-CFP proteins. If no fusion occurs, completely overlap due to the diffusion of the matrix containing medium. If mitochondrial fusion occurred in the resulting zygotes, RFP and CFP fluorescence should contain only RFP or CFP. We found that mitochondria and the loss of mtDNA of ugo1Δ cells, fusion was defective in ugo1Δ mutants. ugo1Δ/ugo1Δ zygotes contained many mitochondrial fragments, but these organelles contained only RFP or CFP fluorescence. No organelles containing both fluorophores were seen. Although disruption of DNM1 suppresses the fragmentation of mitochondria and the loss of mtDNA of ugo1Δ mutants, ugo1Δ dnm1Δ double mutants still failed to fuse their mitochondria. Although ugo1Δ dnm1Δ cells displayed tubular mitochondrial shape, each mitochondrial tubule contained only RFP or CFP. We found that mitochondria in ugo1Δ/ugo1Δ or ugo1Δ dnm1Δ/ugo1Δ dnm1Δ zygotes were often closely positioned to each other near the middle of zygotes, but nonetheless did not fuse. Our results thus indicate that ugo1Δ and ugo1Δ dnm1Δ cells are defective in mitochondrial fusion and argue that Ugo1p plays a direct role in the fusion pathway.

Contribution of Ugo1p to Mitochondrial Fusion

MAT a cells were pregrown in galactose-containing medium overnight to induce COX4-RFP fusion protein. MATa cells containing matrix-targeted CFP under the control of the GAL1 promoter (pHS52) were grown to log phase in SGalSuc medium overnight to induce COX4-CFP. MATa and α cells were mated for 3.5 h on YEPD medium. The distribution of COX4-RFP and COX4-CFP in representative zygotes containing a medial diploid bud (asterisks) is shown. Zygotes formed by mating between wild-type cells (FY833 and FY834, WT), ugo1Δ mutants (YHS72 and YHS73), dnm1Δ mutants (YHS83 and YHS84), and ugo1Δ dnm1Δ mutants (YHS85 and YHS86) were examined. Bar, 3 μm.

Figure 6. Mitochondrial fusion is defective in ugo1Δ and ugo1Δ dnm1Δ cells. MATa cells containing matrix-targeted RFP under the control of the GAL1 promoter (pHS51) were grown to log phase in SRaf medium and then transferred to SGalSuc medium for 3–5 h to induce the expression of the COX4-RFP fusion protein. MATa cells containing matrix-targeted CFP under the control of the GAL1 promoter (pHS52) were grown to log phase in SGalSuc medium overnight to induce COX4-CFP. MATa and α cells were mated for 3.5 h on YEPD medium. The distribution of COX4-RFP and COX4-CFP in representative zygotes containing a medial diploid bud (asterisks) is shown. Zygotes formed by mating between wild-type cells (FY833 and FY834, WT), ugo1Δ mutants (YHS72 and YHS73), dnm1Δ mutants (YHS83 and YHS84), and ugo1Δ dnm1Δ mutants (YHS85 and YHS86) were examined. Bar, 3 μm.

ugo1Δ and ugo1Δ dnm1Δ Cells Are Defective in Mitochondrial Fusion

To ask if Ugo1p plays a direct role in fusion, we examined the ability of ugo1Δ cells to fuse their mitochondria after yeast cell mating (Nunnari et al., 1997; Okamoto et al., 1998). The mitochondria in MATa cells were labeled using a matrix-targeted RFP (pGAL1-COX4–RFP) expressed from pHS51. In MATa cells, mitochondria were visualized with a matrix-targeted CFP (pGAL1-COX4–CFP) carried on pHS52. Both plasmids express the fusion protein under control of the inducible GAL1 promoter. MATa and MATa cells were pregrown in galactose-containing medium to induce the expression of the fusion proteins and transferred to glucose medium to inhibit their further synthesis. Cells were mixed and allowed to mate on glucose-containing medium. If mitochondrial fusion occurred in the resulting zygotes, RFP and CFP fluorescence should completely overlap due to the diffusion of the matrix COX4-RFP and COX4-CFP proteins. If no fusion occurs, RFP and CFP should be seen in separate organelles.

ugo1Δ mutants, we found that ugo1Δ dnm1Δ cells were able to grow on a glycerol and ethanol-containing medium, indicating that the double mutant contained mtDNA (Fig. 5 B). We note that ugo1Δ dnm1Δ cells grew more slowly than wild-type and dnm1Δ cells on both glucose and glycerol/ethanol media. This growth appears to result from lack of Ugo1p, since ugo1Δ and ugo1Δ dnm1Δ cells grew more slowly than wild-type or dnm1Δ on glucose-containing medium (Figs. 2 A and 5 B).

We found that ugo1Δ and ugo1Δ dnm1Δ mutants are defective in fusion. In Fig. 6, representative examples of zygotes containing a medial diploid bud from each mating mixture are shown, but >50 zygotes for each mating mixture were actually examined. When two wild-type cells were mated, mitochondria in the zygote efficiently fused and a complete overlap of the RFP and CFP fluorescence was seen. Consistent with previous studies (Bleazard et al., 1999; Sesaki and Jensen, 1999), mitochondrial fusion also occurred in dnm1Δ/dnm1Δ zygotes. Interestingly, dnm1Δ/dnm1Δ zygotes often contained a single tubule emerging from the mitochondrial network of each parent. Fusion appeared to occur at a discrete point near the middle of the zygote. In contrast to wild-type and dnm1Δ cells, fusion was defective in ugo1Δ mutants. ugo1Δ/ugo1Δ zygotes contained many mitochondrial fragments, but these organelles contained only RFP or CFP fluorescence. No organelles containing both fluorophores were seen. Although disruption of DNM1 suppresses the fragmentation of mitochondria and the loss of mtDNA of ugo1Δ mutants, ugo1Δ dnm1Δ double mutants still failed to fuse their mitochondria. Although ugo1Δ dnm1Δ cells displayed tubular mitochondrial shape, each mitochondrial tubule contained only RFP or CFP. We found that mitochondria in ugo1Δ/ugo1Δ or ugo1Δ dnm1Δ/ugo1Δ dnm1Δ zygotes were often closely positioned to each other near the middle of zygotes, but nonetheless did not fuse. Our results thus indicate that ugo1Δ and ugo1Δ dnm1Δ cells are defective in mitochondrial fusion and argue that Ugo1p plays a direct role in the fusion pathway.
Ugo1p Is a Mitochondrial Outer Membrane Protein, with its NH₂ Terminus Facing the Cytosol and COOH Terminus in the Intermembrane Space

To localize Ugo1p in yeast cells, we constructed two epitope-tagged versions of Ugo1p, myc-Ugo1p, and Ugo1p-HA. myc-Ugo1p carries the myc epitope (Munro and Pelham, 1986) fused to the NH₂ terminus of the Ugo1 protein, and Ugo1p-HA contains the influenza HA epitope (Field et al., 1988) at its COOH terminus. Cells that expressed either myc-Ugo1p (Fig. 7 A) or Ugo1p-HA (data not shown) contained a single protein of ~65 kD. We found that both fusion proteins were functional and ugo1Δ cells expressing either myc-Ugo1p (Fig. 7 B) or Ugo1p-HA (data not shown) maintained mtDNA and normal mitochondrial shape.

Immunofluorescence studies showed that Ugo1p is a mitochondrial protein (Fig. 7 B). ugo1Δ cells expressing the myc-Ugo1p fusion protein were fixed, permeabilized, and then incubated with antibodies to the myc-epitope and the mitochondrial ATPase β subunit (F1β) protein. When immune complexes were visualized using fluorescence microscopy, we found that myc-Ugo1 protein colocalized with the mitochondrial F1β protein. Cell fractionation experiments also confirmed the mitochondrial localization of Ugo1p (Fig. 7 C). Cells expressing Ugo1p-HA were homogenized and separated into a mitochondrial fraction and a postmitochondrial supernatant. We found that Ugo1p cofractionated with the mitochondrial Tim23 protein, whereas little or no Ugo1p was found in the supernatant along with cytosolic hexokinase protein.

Ugo1p is an integral membrane protein located in the outer membrane. When mitochondria isolated from cells expressing Ugo1p-HA were treated with 1.5 M sodium chloride or 0.1 M sodium carbonate (Fig. 8 A), Ugo1p was not extracted from the mitochondria like the peripheral membrane protein, the β subunit of the F1-ATPase (F1β). Instead, Ugo1p remained in the membrane pellet with the integral membrane protein, Tim23p. To determine which mitochondrial membrane contains Ugo1p, we prepared membrane vesicles from myc-Ugo1p mitochondria and separated them into outer membrane and inner membrane fractions on sucrose gradients. As shown in Fig. 8 B, myc-Ugo1p cofractionated with the outer membrane marker, OMAp (YHS87) expressing Ugo1p-HA (pHS55) were grown in SGal medium. Cells were homogenized and separated into a mitochondrial pellet and a postmitochondrial supernatant by centrifugation. Cell-equivalent amounts of homogenate (H), mitochondrial pellet (M), and postmitochondrial supernatant (PMS) were analyzed by immune blotting using antibodies to the HA epitope (Ugo1p-HA), Tim23p, and hexokinase. Bar, 3 μm.

Figure 7. Ugo1p is a mitochondrial protein. (A) Expression of myc-Ugo1p. Wild-type cells (FY833) containing an empty vector, pRS314 (Control), and ugo1Δ cells (YHS88) containing the myc-Ugo1p plasmid (pHS57) were grown to log phase in SGal medium. Whole cell extracts were prepared (Yaffe and Schatz, 1984) and analyzed by immune blotting using antibodies to the myc epitope. (B) Ugo1p colocalizes with a mitochondrial protein. Wild-type cells containing pRS314 (Ugo1p) and ugo1Δ cells containing the myc-Ugo1p plasmid (pHS57) were grown to log phase in SGal medium. Cells were then fixed, spheroplasted, permeabilized (Harlow and Lane, 1988), and incubated with rabbit antibodies to the β subunit of F1-ATPase (anti-F1β) and mouse IgG to the myc epitope (anti-myc). Immune complexes were visualized by fluorescence microscopy using FITC-conjugated anti–mouse IgG and rhodamine-conjugated anti–rabbit IgG. (C) Ugo1p cofractionates with a mitochondrial marker, ugo1Δ cells (YHS87) expressing Ugo1p-HA (pHS55) were grown in SGal medium. Cells were homogenized and separated into a mitochondrial pellet and a postmitochondrial supernatant by centrifugation. Cell-equivalent amounts of homogenate (H), mitochondrial pellet (M), and postmitochondrial supernatant (PMS) were analyzed by immune blotting using antibodies to the HA epitope (Ugo1p-HA), Tim23p, and hexokinase. Bar, 3 μm.
membrane protein, with its NH\textsubscript{2} terminus facing the cytosol. Therefore, we conclude that Ugo1p is an outer membrane protein that mediates mitochondrial fusion. Ugo1p was identified using a genetic screen for mutants that maintain mitochondrial morphology defect in fzo1\textsuperscript{D} cells, like those seen in wild-type cells and in fzo1 dnm1 double mutants (Sesaki and Jensen, 1999). Our results suggest that a balance of fusion and division regulates mitochondrial shape and number. In the absence of mitochondrial fusion, mediated by Fzo1p and Ugo1p, ongoing division produces numerous small organelles. When division is defective, continuous fusion leads to the single interconnected mitochondrial network seen in dnm1 cells (Bleazard et al., 1999; Sesaki and Jensen, 1999).

We have found that ugo1\Delta cells, like fzo1 mutants (Hermann et al., 1998), are defective in mitochondrial fusion. Although ugo1\Delta dnm1\Delta double mutants contain tubular-shaped mitochondria, they do not fuse their mitochondria. Similarly, fzo1\Delta dnm1\Delta cells remain blocked for fusion (Bleazard et al., 1999; Sesaki and Jensen, 1999). Therefore, the normal-looking mitochondria found in ugo1 dnm1 and fzo1 dnm1 mutants (Sesaki and Jensen, 1999) suggest that some aspects of mitochondrial shape result from mechanisms independent of fusion and division. For example, tubular-shaped mitochondria may arise by directed growth of preexisting organelles along cytoskeletal filaments. Alternatively, internal mitochondrial proteins may provide a scaffold for tubulation of mitochondria. Regardless of how tubules are formed, we suggest that a balance of fusion and division regulates mitochondrial shape and number. In the absence of mitochondrial fusion, mediated by Fzo1p and Ugo1p, ongoing division produces numerous small organelles. When division is defective, continuous fusion leads to the single interconnected mitochondrial network seen in dnm1 cells (Bleazard et al., 1999; Sesaki and Jensen, 1999).

Discussion

We have identified a new outer membrane protein, Ugo1p, required for mitochondrial fusion. Ugo1p was identified using a genetic screen for mutants that maintain mtDNA in the absence of mitochondrial division, but lose mtDNA when division is active. Our screen was based on previous studies with fzo1 mutants, which are defective in an outer membrane protein that mediates mitochondrial fusion. fzo1 cells lose mtDNA, but the loss of mtDNA can be suppressed by disruption of DNM1, a gene required for mitochondrial division (Bleazard et al., 1999; Sesaki and Jensen, 1999; Jensen et al., 2000). Like fzo1 mutants, the loss of mtDNA in ugo1 mutants is suppressed by inactivation of Dnm1p function. ugo1 cells that carry the dominant negative DNM1-111 mutant or ugo1\Delta dnm1\Delta double mutants maintain mtDNA. Similar to fzo1 mutants, the mitochondrial morphology defect in ugo1\Delta cells is suppressed by DNM1 disruption. Both fzo1\Delta and ugo1\Delta mutants contain many small mitochondrial fragments, instead of the few long tubular-shaped mitochondria found in wild-type cells. ugo1\Delta dnm1\Delta cells contain mitochondrial tubules similar to those seen in wild-type cells and in fzo1 dnm1 double mutants (Sesaki and Jensen, 1999). Our results suggest that a balance of fusion and division regulates mitochondrial shape and number. In the absence of mitochondrial fusion, mediated by Fzo1p and Ugo1p, ongoing division produces numerous small organelles. When division is defective, continuous fusion leads to the single interconnected mitochondrial network seen in dnm1 cells (Bleazard et al., 1999; Sesaki and Jensen, 1999).

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ance between mitochondrial fusion and division regulates the length, number, and connection of mitochondrial tubules. For example, in ugo1 and fzo1 mutants where fusion is blocked, mitochondria form numerous tubular structures, but the length of each tubule is very short. In dnm1 mutants (Bleazard et al., 1999; Sesaki and Jensen, 1999), mdv1/gag3/net2 mutants (Fekkes et al., 2000; Tieu and Nunnari, 2000; Cerveny et al., 2001), or fis1 mutants (Mozdy et al., 2000) where division is defective, mitochondria form a single organelle consisting of interconnected tubules.

Our genetic screen identified five mgm1 mutants. mgm1 mutants lose mtDNA (Jones and Fangman, 1992; Guan et al., 1993) and mtDNA loss can be suppressed by inactivation of Dnm1p (Fekkes et al., 2000). Mgm1p is a mitochondrial GTPase, although its exact location in mitochondria is unclear (Shepard and Yaffe, 1999; Wong et al., 2000). In mgm1 mutants, mitochondria are fragmented like those in fzo1 and ugo1 cells, suggesting a role in mitochondrial fusion. Recently, mgm1 mutants have been shown to be defective in mitochondrial fusion (Wong et al., 2000). However, in contrast to fzo1 dnm1 (Bleazard et al., 1999; Sesaki and Jensen, 1999) and ugo1 dnm1 double mutants, mgm1 dnm1 double mutants are able to fuse their mitochondria, suggesting that Mgm1p plays an indirect role in mitochondrial fusion (Wong et al., 2000).

Ugo1p is embedded in the mitochondrial outer membrane with its COOH terminus of nearly 200 amino acids facing the IMS. Most other proteins involved in membrane fusion, such as SNAREs (Rothman and Warren, 1994; Pelham, 1999), the influenza HA protein (White et al., 1996), and Fzo1p (Hermann et al., 1998; Rapaport et al., 1998), contain few if any residues on the opposite side of the membrane to where fusion takes place. Mitochondria, in contrast to most other organelles, have two membranes. The mitochondrial inner membrane appears to fuse immediately after outer membrane fusion (Okamoto et al., 1998), suggesting a coupling between both fusion events. We speculate that the COOH terminus of Ugo1p may interact with inner membrane fusion machinery. We note that the COOH terminus of Ugo1p contains a mitochondrial energy transfer protein motif (Nelson et al., 1998) which is found in many inner membrane proteins. Studies to determine the role Ugo1p plays in mitochondrial inner and outer membrane fusion are in progress.

Although mitochondrial fusion occurs predominantly at the tips of mitochondrial tubules (Nunnari et al., 1997), our studies show that Ugo1p is present throughout the mitochondrial outer membrane. Similarly, Fzo1p shows a uniform distribution along the mitochondrial tube (Hermann et al., 1998). It is possible that mitochondrial fusion is activated only at sites of fusion. For example, the Fzo1p GTPase may act as a molecular switch that regulates mitochondrial fusion by activating the fusion machinery at the appropriate time. Alternatively, the fusion machinery may be transiently concentrated at fusion sites. It is also possible that mitochondria are competent to fuse anywhere along the tubule, but fusion is directed by controlling contact between organelles. For example, the cytoskeleton may play a crucial role in positioning mitochondria during their fusion.

Is Ugo1p part of a fusion machine? Gel filtration studies of detergent-solubilized mitochondria show that Fzo1p is found in an ~800-kD complex (Rapaport et al., 1998). Since both Fzo1p and Ugo1p are located in the mitochondrial outer membrane and play essential roles in mitochondrial fusion, it is possible that both proteins are part of the same complex. Both proteins appear to be defective in a late step in the fusion pathway. In matings between ugo1Δ mutants and mitochondria the two parent cells are closely paired in the neck of the zygote, but do not fuse. Similar connections between unfused mitochondria were seen in matings between fzo1Δ cells (Hermann et al., 1998). However, preliminary studies have shown that Fzo1p and Ugo1p do not coimmunoprecipitate (Sesaki, H., unpublished observations). Therefore, it is possible that Fzo1p and Ugo1p mediate distinct steps in mitochondrial fusion and do not physically interact. We note that whereas fzo1 and ugo1 mutants both contain fragmented mitochondria, the organelles tend to aggregate in ugo1 mutants, but mitochondria remain dispersed in fzo1 cells. Further studies are clearly needed to determine the role of Ugo1p and Fzo1p in mitochondrial fusion.

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References
Adams, A., D. Gottschling, C. Kaiser, and T. Stearns. 1997. Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, Plainview, NY. 177 pp.
Adams, A.E., and J.R. Pringle. 1991. Staining of actin with fluorochrome-conjugated phalloidin. Methods Enzymol. 194:729–731.
Bereiter-Hahn, J., and M. Voth. 1994. Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc. Res. Tech. 27:198–219.
Bleazard, W., J.M. McCaffery, E.J. King, S. Bale, A. Mozdy, Q. Tieu, J. Nunnari, and J.M. Shaw. 1999. The dynamin-related GTPase Dnm1p regulates mitochondrial fusion in yeast. Nat. Cell Biol. 1:296–304.
Boeke, J.D., F. LaCounte, and G.R. Fink. 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. Med. Genet. Genet. 197:345–346.
Boldogh, I., N. Vojtov, S. Karmon, and L.A. Pom. 1998. Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. J. Cell Biol. 141:1371–1381.
Brachmann, C.B., A. Davies, G.J. Cost, E. Caputo, J. Li, P. Hieter, and J.D. Boeke. 1998. Designer deletion strains from Saccharomyces cerevisiae 28C; a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast. 14:115–122.
Cerveny, K.L., J.M. McCaffery, and R.E. Jensen. 2001. Division of mitochondria requires a novel Dnm1p-interacting protein, Net2p. Mol. Biol. Cell. 12: 309–321.
Dadarla, G., P.C. Böhmi, and G. Schatz. 1982. Import of proteins into mitochondria. Cytochrome b2 and cytochrome c peroxidase are located in the intermembrane space of yeast mitochondria. J. Cell Biol. 95:1302–1303.
Drubin, D.G., H.D. Jones, and K.F. Wartman. 1993. Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phallolidin-binding site. Mol. Biol. Cell. 4:1277–1294.
Dujon, B. 1981. Mitochondrial genetics and functions. In Molecular Biology of the Yeast Saccharomyces. J.M. Strathern, E.W. Jones, and J.R. Broach, edi-

Sesaki and Jensen Mitochondrial Fusion in Yeast
tors. Cold Spring Harbor Laboratory Press, Plainview, New York. 751 pp.

Emmert, J.L.T., and R.E. Jensen. 1993. MAP6 encodes an essential inner mem-
brane component of the yeast mitochondrial import pathway. J. Cell Biol. 122:1003–1012.

Fekkes, P., K.A. Shepard, and M.P. Yaffe. 2000. Gag3p, an outer membrane
protein required for fusion of mitochondrial tubules. J. Cell Biol. 151:333–
340.

Field, J., N. Ikawa, D. Brook, B. MacDonald, L. Rodgers, I.A. Wilson, R.A.
Lerner, and M. Wiger. 1988. Purification of a Ras-responsive adenyl cyclase
complex from Saccharomyces cerevisiae by use of an epitope addition
method. Mol. Cell. Biol. 8:2159–2165.

Fox, T.D., L.S. Folley, J.F. Mulero, T.W. McMullen, P.E. Thorness, L.O. He-
din, and M.C. Costanzo. 1991. Analysis and manipulation of yeast mitochondrial
genes. Methods Enzymol. 194:149–165.

Gammie, A.E., L.J. Kurilrah, R.B. Valee, and M.D. Rose. 1995. DNM1, a dy-
namin-related gene, participates in endosomal trafficking in yeast. J. Cell
Biol. 130:553–566.

Guan, K., L. Farh, T.K. Marshall, and R.J. Deschenes. 1993. Normal mitochondrial
structure and genome maintenance in yeast requires the dynamin-like
product of the MGM1 gene. Curr. Genet. 24:141–148.

Haid, A., and M. Suisa. 1983. Immunoochemical identification of membrane
proteins after sodium dodecyl-polyacrylamide gel electrophoresis. Methods Enzymol. 96:192–205.

Hales, K.G., and M.T. Fuller. 1997. Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. Cell. 90:121–129.

Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring
Harbor Press, Cold Spring Harbor, NY. 726 pp.

Hermann, G.J., and J.M. Shaw. 1998. Mitochondrial dynamics in yeast. Annu.
Rev. Cell Dev. Biol. 14:265–303.

Hermann, G.J., J.W. Thatcher, J.P. Mills, K.G. Hales, T.J. Muller, J. Nunnari,
and J.M. Shaw. 1998. Mitochondrial fusion in yeast requires the transmem-
brane GTPase Fzo1p. J. Cell Biol. 143:359–373.

Hoffman, C.S., and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation into
Escherichia coli. Gene. 57:267–272.

Hoffman, H.P., and C.J. Avers. 1973. Mitochondron of yeast: ultrastructural
evidence for one giant, branched organelle per cell. Science. 181:749–751.

Jensen, R.E., A.E. Aiken Hobbs, K.L. Cerveny, and H. Sesaki. 2000. Yeast mi-
dochondrial fission is a multistep process requiring the novel integral
membrane GTPase Fzo1p.

Jones, B.A., and W.L. Fangman. 1992. Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding
domain of dynamin. Genet. Dev. 6:380–389.

Kerscher, O., N.B. Sepuri, and R.E. Jensen. 2000. Tim18p is a new component of the Tim34p-Tim22p translocon in the mitochondrial inner membrane.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the
head of bacteriophage T4. Nature. 227:680–685.

Lawrence, C.W. 1991. Classical mutagenesis techniques. Methods Enzymol. 194:273–280.

Miyakawa, I., H. Aoi, N. Sando, and T. Kuroiwa. 1984. Fluorescence micro-
scope studies of mitochondrial nucleoids during meiosis and sporulation in the yeast, Saccharomyces cerevisiae. J. Cell Sci. 62:21–38.

Moody, A.D., J.M. McCaffery, and J.F. Shaw. 2000. Dnm1p GTPase-mediated mitochondrial fission is a multistep process requiring the novel integral
membrane component Fis1p. J. Cell Biol. 151:367–380.

 Munro, S., and H.R. Pelham. 1986. An Hsp70-like protein in the ER: identity
with the dynamin-related GTPase, dnm1p, to trigger mitochondrial division. J. Cell Biol. 151:353–366.

Nunnari, J., W.F. Marshall, A. Straight, A. Murray, J.W. Sedat, and P. Walter. 1997. Mitochondrial transmission during mating in Saccharomyces cerevisiae is determined by mitochondrial fusion and fission and the intramitochond-
rial segregation of mitochondrial DNA. Mol. Biol. Cell. 8:1233–1242.

Okamoto, K., P.S. Perlman, and R.A. Butow. 1998. The sorting of mitochon-
drial DNA and mitochondrial proteins in zygotex: preferential transmission of mitochondrial DNA to the medial bud. J. Cell Biol. 142:613–623.

Oldenburg, K.R., K.T. Vo, S. Michaelis, and C. Paddison. 1997. Reconstitution-
mediated PCR-directed plasmid construction in vivo. Nucleic Acids Res. 25:451–452.

Otsuga, D., B.R. Keegan, E. Brisch, J.W. Thatcher, G.J. Hermann, W. Bleaz-
ard, and J.M. Shaw. 1998. The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. J. Cell Biol. 143:333–349.

Pelham, H.R. 1999. SNAREs and the secretary pathway-lessons from yeast. Exp. Cell Res. 247:1–8.

Prinz, W.A., L. Grzyb, M. Veenhuis, I.A. Kahana, P.A. Silver, and T.A. Rapo-
port. 2000. Mutants affecting the structure of the cortical endoplasmic retic-
ulum in Saccharomyces cerevisiae. J. Cell Biol. 150:461–474.

Rapaport, D., M. Brunner, W. Neupert, and B. Westermann. 1998. Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of func-
tional mitochondria in Saccharomyces cerevisiae. J. Biol. Chem. 273:20150–
20155.

Reauve, S.E., and E.L. Tatum. 1949. Spontaneous and nitrogen mustard-in-
duced nutritional deficiencies in Saccharomyces cerevisiae. Arch. Biochem.
22:331–338.

Rothman, J.E., and G. Warren. 1994. Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. Curr. Biol. 4:220–233.

Ryan, K.R., M.M. Menold, S. Garrett, and R.E. Jensen. 1994. SMS1, a high-
copy suppressor of the yeast mat1α mutant, encodes an essential inner mem-
brane protein required for mitochondrial protein import. J. Cell Biol. 5:529–538.

Sesaki, H., and R.E. Jensen. 1999. Division versus fusion: Dnm1p and Fzo1p antagomically regulate mitochondrial shape. J. Cell Biol. 147:699–706.

Shepard, K.A., and M.P. Yaffe. 1999. The yeast dynamin-like protein, mgm1p, functions on the mitochondrial outer membrane to mediate mitochondrial inheritance. J. Cell Biol. 144:711–720.

Sikorski, R., and P. Hieter. 1989. A system of shuttle vectors and host strains
designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics. 122:19–28.

Stevens, B.J. 1977. Variation in number and volume of the mitochondria in yeast according to growth conditions. A study based on serial sectioning and
computer graphics reconstitution. Biol. Cell. 28:37–56.

Thomas, B.J., and R. Rothstein. 1989. Elevated recombination rates in tran-
scriptionally active DNA. Cell. 56:619–630.

Thomas, D.Y., and D. Wilkie. 1968. Recombination of mitochondrial drug-
resistance factors in Saccharomyces cerevisiae. Biochem. Biophys. Res. Com-
mun. 30:368–372.

Tieu, Q., and J. Nunnari. 2000. Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, dnm1p, to trigger mitochondrial division. J. Cell Biol. 151:335–366.

Tyler, D. 1992. The Mitochondrion. VCH Publishers, New York. 557 pp.

Vida, T.A., and S.D. Emr. 1995. A new vital stain for visualizing vacuolar mem-
brane dynamics and endocytosis in yeast. J. Cell Biol. 128:779–792.

White, J.M., T. Danielli, Y.I. Henis, G. Melikyan, and F.S. Cohen. 1996. Mem-
brane fusion by the influenza hemagglutinin: the fusion pore. Soc. Gen.
Physiol. Ser. 51:223–229.

Winston, F., C. Dollard, and S.L. Ricupero-Hovasse. 1995. Construction of a set
of shuttle vectors and host strains that are isogenic to S288C.

Yaffe, M.P. 1999. The machinery of mitochondrial inheritance and behavior. Science. 283:1493–1497.

Yaffe, M.P., and G. Schatz. 1984. Two nuclear mutations that block mitochon-
drial protein import in yeast. Proc. Natl. Acad. Sci. USA. 81:4819–4823.

Yaffe, M.P., R.E. Jensen, and E.C. Guido. 1989. The major 45-kDa protein of the yeast mitochondrial outer membrane is not essential for cell growth or
mitochondrial function. J. Biol. Chem. 264:21091–21096.