Regulation of Mitochondrial Morphology and Inheritance by Mdm10p, A Protein of the Mitochondrial Outer Membrane

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Abstract. Yeast cells with the *mdm10* mutation possess giant spherical mitochondria and are defective for mitochondrial inheritance. The giant mitochondria display classical features of mitochondrial ultrastructure, yet they appear incapable of movement or division. Genetic analysis indicated that the mutant phenotypes resulted from a single nuclear mutation, and the isolated *MDM10* gene restored wild-type mitochondrial distribution and morphology when introduced into mutant cells. *MDM10* encodes a protein of 56.2 kD located in the mitochondrial outer membrane. Depletion of Mdm10p from cells led to a condensation of normally extended, tubular mitochondria into giant spheres, and reexpression of the protein resulted in a rapid restoration of normal mitochondrial morphology. These results demonstrate that Mdm10p can control mitochondrial morphology, and that it plays a role in the inheritance of mitochondria.

MITOCHONDRIAL inheritance is an essential component of cell proliferation (Yaffe, 1991*b*). This inheritance requires the growth and division of preexisting mitochondria and the distribution of mitochondria between daughter cells before cell division (Attardi and Schatz, 1988). Cytoskeletal elements have been implicated in the positioning and movement of mitochondria (Heggeness et al., 1978; Chen, 1988; McConnell and Yaffe, 1992; Drubin et al., 1993), but mechanisms underlying mitochondrial inheritance are poorly understood.

Mitochondria are usually found as snakelike tubules, widely distributed in the eukaryotic cytoplasm (Tzagoloff, 1982; Bereiter-Hahn, 1990). Often, these tubules are interconnected into extended mitochondrial reticula (Stevens, 1981; Chen, 1988). Microscopic studies of live cells have revealed that these mitochondrial networks are extremely dynamic, with tubular processes undergoing frequent fragmentation, branching, and fusion, as well as redistribution within the cytoplasm (Bereiter-Hahn, 1990; Koning et al., 1993). In addition to the dynamic properties of mitochondria found in most types of cells, certain pathological conditions lead to gross changes in mitochondrial morphology, including the development of giant mitochondria (Tandler and Hoppel, 1986; Inagaki et al., 1992). The molecular bases for these changes in mitochondrial morphology and distribution in both normal and diseased cells are unknown.

We recently described several *Saccharomyces cerevisiae* mutants defective for mitochondrial inheritance (McConnell et al., 1990). These mitochondrial distribution and morphology (*mdm*)[1] mutants were identified by screening collections of temperature-sensitive strains by fluorescence microscopy for cells that failed to transfer mitochondria into daughter buds during incubation at the nonpermissive temperature. Analysis of two of these mutant strains led to the identification of a novel cytoskeletal component that mediates mitochondrial inheritance (McConnell and Yaffe, 1992, 1993), and it indicated a role for unsaturated fatty acids in mitochondrial movement (Stewart and Yaffe, 1991). Here, we describe a new *mdm* mutant that displays aberrant mitochondrial inheritance and gross changes in mitochondrial morphology.

Materials and Methods

Strains and Mutant Isolation

The *mdm10-1* mutant (MYY501) was isolated from a collection of 872 temperature-sensitive strains prepared by mutagenesis of yeast strain MYY290 (*MATa, leu2, his3, ura3*) (Smith and Yaffe, 1991) with ethyl methanesulfonate as described previously (Yaffe and Schatz, 1984). Mutant cultures were screened for defects in mitochondrial distribution and morphology by fluorescence microscopy as previously described (McConnell et al., 1990). Strains MYY291 (*MATα, leu2, his3, ura3*) and MYY297 (*Mata/α, leu2/leu2, his3/his3, ura3/ura3*) were described previously (Atencio and Yaffe, 1992). A yeast strain containing a deletion mutation in the *MDM10* gene, MYY503 (*MATa, mdm10::URA3, leu2, his3*), was created as described below. Media were prepared and standard genetic manipulations were performed as described by Rose et al. (1990). Yeast cells were transformed by the lithium acetate method (Ito et al., 1983). Plasmid DNA was prepared in *Escherichia coli* strain DH5α.

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1. *Abbreviations used in this paper*: DASPMI, 2-(4-dimethylaminostryl)-1-methylpyridinium iodide; *mdm*, mitochondrial distribution and morphology (mutant).

Isolation and Sequence Analysis of the MDM10 gene

The *MDM10* gene was isolated by complementation of the temperature-sensitive growth defect at 39°C. Mutant strain MYY501 was transformed with a yeast genomic library in the yeast centromere vector p366 (obtained from M. Hoekstra). One temperature-resistant Leu$^+$ clone was isolated out of 1500 transformants. The complementing plasmid, p366-M10, contained a 9.0-kb insert of yeast DNA.

Complementation analysis of various subclones and deletion constructs derived from the 9.0-kb fragment indicated that the *MDM10* gene occupied portions of a 1.1-kb HindIII fragment and an adjacent 3.8-kb HindIII-BamHI fragment. These fragments were cloned into Bluescript KS (Stratagene, La Jolla, CA), and templates for sequencing were generated using ExonucleaseIII to create nested deletions. Additional templates included several small restriction fragments subcloned into the Bluescript KS vector. Nucleotide sequences of these fragments were determined by using a Sequenase 2.0 DNA sequencing kit (U.S. Biochemical Corp., Cleveland, OH). The predicted amino acid sequence of Mdm10p was compared to sequences in the GenBank database with the FASTA program (Lipman and Pearson, 1985).

Integrative Transformation

A 3.5-kb BglII fragment isolated from plasmid p366-M10 was ligated into the BamHI site of plasmid YIp5 (Struhl et al., 1979). The resulting plasmid, pIM2-2, was linearized with BstX1 and transformed into yeast strain MYY291. Ura$^+$ transformants were crossed to MYY501 (*mdm10-1*), and the resulting diploid was sporulated. Tetrad dissection revealed that 23/23 tetrads analyzed were of the parental ditype (2 Ura$^+$, 2ts$^+$:2 Ura$^-$, 2ts$^-$). These results indicated that the integration event occurred within 2.2 cM of the *mdm10* locus.

Gene Disruption of MDM10

A yeast mutant lacking most of the *MDM10* coding region was constructed as follows. A 1.9-kb BglI-KpnI fragment containing the entire *MDM10* gene was isolated from plasmid p366-M10 and ligated into plasmid Bluescript KS to yield plasmid BSMDM10. The 1.0-kb EcoRI fragment within the *MDM10* gene in the BSMDM10 was deleted and replaced with the yeast *URA3* gene on a HindIII fragment isolated from plasmid pFL1 (Chevallier et al., 1980). The disrupted *MDM10* gene was excised from the plasmid by digestion with XbaI and KpnI, isolated by gel electrophoresis, and transformed into MYY297. Ura$^+$ transformants were selected, and the diploids sporulated at 30°C. All four spore tetrads yielded two large spores and two small spores. The small spores were temperature sensitive and Ura$^+$.

Preparation and Characterization of Antisera

Antibodies against Mdm10p were raised against a β-galactosidase-Mdm10p fusion protein. The 1.1-kb HindIII fragment encoding the COOH-terminal 317 amino acids of Mdm10p was ligated into the HindIII site of plasmid pTRB1 (Burglin and DeRobertis, 1987) to create pTRB-MDM10. The fusion protein was expressed in *E. coli* strain 71-18 by induction with isopropyl thio-β-D-galactoside, purified by SDS-PAGE and electroelution, and used to immunize rabbits (Harlow and Lane, 1988). Antibodies were analyzed by immunoblotting (Towbin et al., 1979) of yeast cell homogenates or purified subcellular fractions.

Microscopy

Fluorescence and indirect immunofluorescence microscopy were performed as previously described (McConnell et al., 1990). Electron microscopy of wild-type (MYY290) and mutant (MYY501) cells was performed as described by Stevens (1977) with the following modifications. Cells were grown in YPD medium (1% yeast extract, 2% bactopeptone, 2% glucose) at 23°C to an OD_{600} of 1. Cells were isolated by centrifugation, resuspended in YPG medium (1% yeast extract, 2% bactopeptone, 2% glycerol) to the same OD_{600}, and incubated at 23°C for 6 h. Next, the cultures were shifted to 37°C for an additional 2 h. Subsequent to fixation, cells were stained with 2% uranyl acetate overnight at 4°C with constant shaking.

Construction and Analysis of Ubiquitin-MDM10 Fusions

An NdeI site was engineered in plasmid BSMDM10 at the start of the MDM10 open reading frame by oligonucleotide-directed mutagenesis (Kunkel et al., 1987). The resulting plasmid, BSMDM10-Nd, was cut with NdeI and the cut ends were filled with Klenow polymerase. The plasmid was digested with KpnI and the ends were blunted with T4 DNA polymerase. The 1.7-kb fragment containing *MDM10* was ligated into the blunted XbaI site of plasmid pGem-flu (Park et al., 1992) to create plasmid pFluM10. Plasmid pUBM10-R was constructed by a triple ligation of a 5.6-kb EcoRI-SphI fragment from plasmid pSE362 (Park et al., 1992), a 1.8-kb BamHI-SphI fragment from pFluM10, and a 1.7-kb EcoRI-BamHI fragment from pUB23-R (Bachmair et al., 1986).

Yeast strains MYY290 and MYY503 (*mdm10*-null) were transformed with pUBM10-R and cultured on synthetic medium without histidine. For analysis of ubiquitin-Mdm10p fusions, cells were grown overnight at 30°C in synthetic medium (without histidine) containing 2% raffinose and 2% galactose. Cells were collected on filters, washed, and resuspended in the same media without galactose. Cultures were back-diluted into fresh media (without galactose) after 8 and 24 h to maintain cells in a logarithmic phase of growth. At various times after removal of galactose, mitochondrial distribution and morphology were analyzed by fluorescence microscopy, and cellular homogenates were prepared by resuspending cells in a solution of 50 mM Tris-HCl, pH 7.5, 2 mM PMSF, 5 mM 2-mercaptoethanol, and vortexing with glass beads. Total protein amounts were quantified by BCA assays (Pierce Chemical Co., Rockford, IL). Individual protein species were analyzed by SDS-PAGE and Western blotting.

Subcellular Fractionation and Biochemical Analysis of Mdm10p

Subcellular fractions (Daum et al., 1982) and purified mitochondria (Daum et al., 1982; Yaffe, 1991*a*) were isolated from strain MYY290 by homogenization and differential centrifugation as described previously. Outer and inner membranes were purified from mitochondria isolated from yeast strain D273-10B (ATCC 24657) using a procedure involving osmotic shock, sonication, and fractionation of membranes by centrifugation in a sucrose-density gradient as described by Daum et al. (1982). For analysis of submitochondrial fractions, samples containing 0.2 mg of protein from each gradient fraction were diluted at least 10-fold with a solution containing 5 mM Hepes-KOH, pH 7.4, 10 mM KCL, 1 mM $MgCl_2$, and pelleted at 100 *g* for 1 h at 2°C. The pelleted membranes were resuspended in 0.5-ml ice-cold 0.1 M Na_2CO_3 and held on ice for 30 min. Membranes were recovered by centrifugation at 100 *g*, 1 h, 2°C, and proteins in the membrane pellets were analyzed by SDS-PAGE and Western blotting. Antiserum against cytochrome b_2 was the gift of A. Lewin (University of Florida, Gainesville, FL). Antiserum against cytochrome oxidase subunit IV was the gift of R. Jensen (Johns Hopkins University School of Medicine, Baltimore, MD). Antiserum against yeast glucose-6-phosphate dehydrogenase was purchased from Sigma Immunochemicals (St. Louis, MO). Antisera against $F_1\beta$, OM45, and glyceraldehyde-3-phosphate dehydrogenase were described previously (Jensen and Yaffe, 1988).

To analyze Mdm10p partitioning in detergent, 0.3 mg of purified mitochondria were resuspended in a solution containing 2% Triton X-114 in Breaking buffer (0.6 M mannitol, 20 mM Hepes-KOH, pH 7.4). The mixture was held on ice for 30 min and centrifuged for 2 min at 0°C. The supernatant was warmed to 4°C for 2 min, incubated for 10 min at 37°C, and then centrifuged for 1 min at room temperature. Proteins were precipitated from the separated aqueous and detergent layers by addition of TCA to 10%. Protein samples were processed and analyzed by SDS-PAGE and Western blotting.

Sodium carbonate extraction of mitochondria was performed by resuspending 0.3 mg of purified mitochondria in 350 μl of an ice-cold solution of 0.1 M Na_2CO_3. Samples were held on ice for 30 min, and membrane and supernatant fractions were recovered by centrifugation at 100,000 *g* for 1 h at 4°C.

For protease treatment, purified mitochondria (2 mg/ml) were treated with proteinase K at 0.2 mg/ml. After 10 min on ice, digestion was stopped by addition of PMSF to 2 mM, and proteins were precipitated by addition of TCA to 10%. Samples were processed and analyzed by SDS-PAGE and Western blotting.

Results

Giant Mitochondria in the mdm10 Mutant

The *mdm10* mutant was isolated by screening a collection of temperature-sensitive yeast strains for cells that possessed

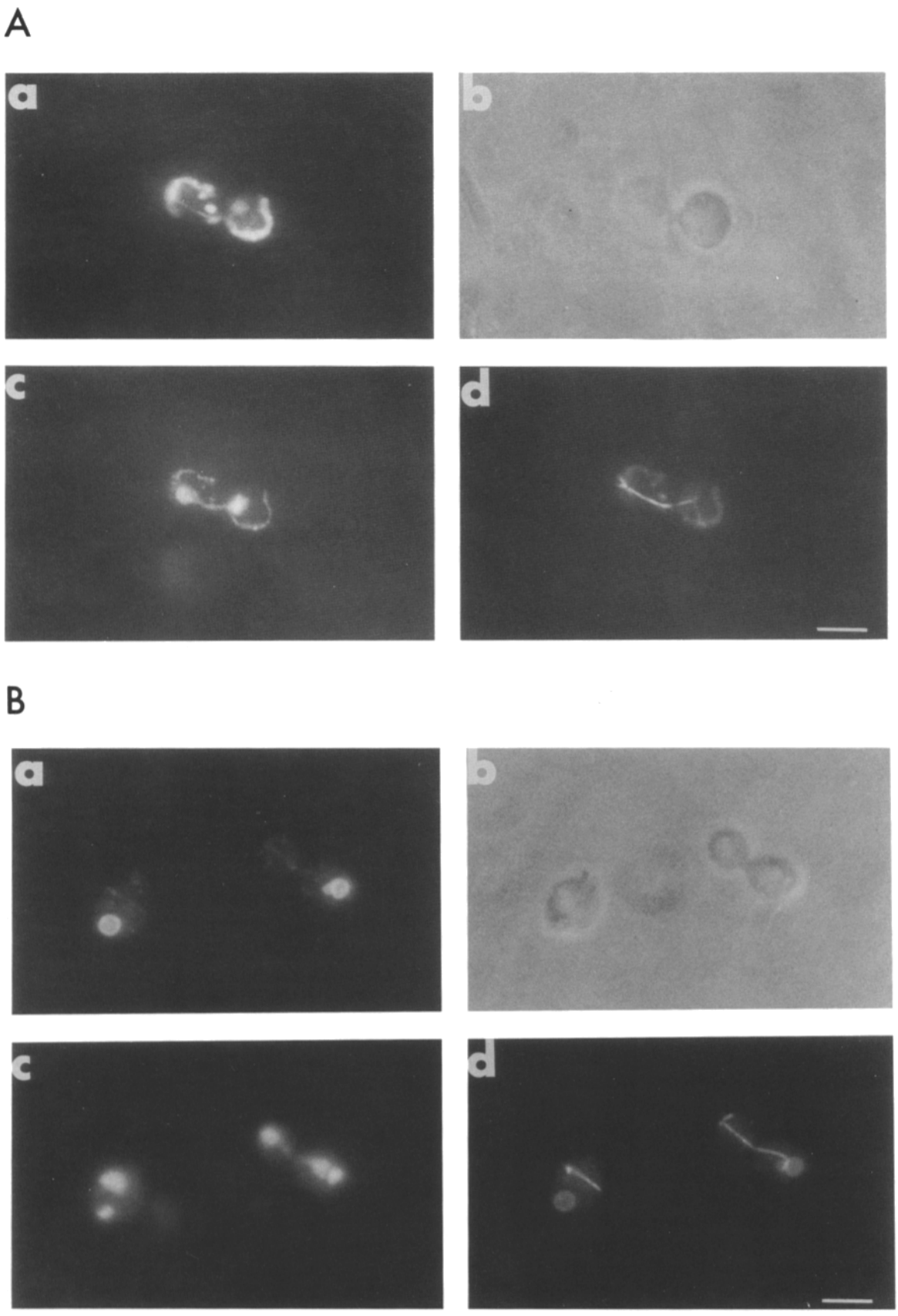

Figure 1. mdm10 cells display defects in mitochondrial distribution and morphology. Wild-type (*A*) or mutant (*mdm10-1*) (*B*) cells were grown overnight in YPD medium at 23°C, incubated at 37°C for 2 h, and fixed with formaldehyde. Mitochondria (*a*) were detected with a mouse monoclonal antibody against OM14 (a mitochondrial outer-membrane protein) followed by rhodamine-conjugated goat anti-mouse IgG. Nuclear and mitochondrial DNA (*c*) were visualized by staining with 4′, 6′ diamidino-2-phenylindole. Microtubules (*d*) were detected with a rat monoclonal antibody against tubulin followed by FITC-conjugated goat anti-rat IgG. Some fluorescence from the rhodamine channel (mitochondria) appears in the FITC channel (microtubules). Whole-cell, bright-field images of the corresponding cells are represented in the *b* panels. Bar, 2 μm.

aberrant mitochondrial distribution and morphology after incubation at 37°C. The screening procedure involved examination of yeast cultures by fluorescence microscopy after staining with the mitochondrial-specific dye 2-(4-dimethylaminostyryl)-1-methylpyridinium iodide (DASPMI). One strain displayed both deficient transfer of mitochondria into daughter buds and abnormally large mitochondrial structures. Indirect immunofluorescence microscopy of these cells confirmed the presence of large spherical mitochondrial structures and the absence of mitochondria from a large fraction of daughter buds (Fig. 1). The large mitochondria were present at both permissive and restrictive temperatures. Aberrant mitochondrial morphology was found in virtually all mutant cells. The enlarged mitochondria and defective mitochondrial inheritance were observed in cells grown on either fermentable (glucose or galactose) and nonfermentable (glycerol, ethanol, or lactate) carbon sources. In addition, growth of *mdm10* cells on nonfermentable carbon sources led to the production of multiple daughter buds, with 60–65% of these buds failing to receive mitochondria. Cells with the *mdm10* mutation grew about three fold slower than wild-type cells on rich glucose medium and about 14-fold slower than wild-type on semisynthetic lactate medium. Microscopic examination also indicated that the *mdm10* lesion had no effect on nuclear transfer to buds or on the apparent function of the microtubule cytoskeleton. Genetic analysis involving backcrosses of the *mdm10* mutant to the wild-type parental strain and tetrad analysis demonstrated that the defects in mitochondrial inheritance, mitochondrial morphology, and growth at 37°C were all caused by a single recessive nuclear mutation (data not shown).

Examination of *mdm10* cells by electron microscopy confirmed the presence of giant, spherical mitochondria (Fig. 2). These giant mitochondria displayed classical structural features, including double membranes and elaborate cristae. However, cross-sectional diameters of the giant mitochondria were 1.0–1.6 μm compared to a diameter of 0.3–0.5 μm in wild-type cells. Most mutant cells contained only a single giant mitochondrion, although a few cells contained two or three such structures. Frequently, a small mitochondrion of wild-type size also was present (Fig. 2).

The giant mitochondria in *mdm10* cells were at least partially competent for oxidative phosphorylation because cells were able to grow (albeit slowly) on nonfermentable carbon sources at 23°C or 30°C. In addition, the giant mitochondria rapidly accumulated potential sensitive dyes.

Isolation and Analysis of the MDM10 Gene

To determine the molecular basis for defects in the *mdm10* cells, the wild-type *MDM10* gene was cloned by complementation of the temperature-sensitive growth phenotype. Out of 1500 Leu$^+$ transformants, a single colony that contained a plasmid conferring growth at 37°C was isolated. This plasmid also complemented the mitochondrial morphology and distribution phenotypes. Restriction analysis demonstrated that the complementing plasmid contained a 9.0-kb insert of yeast genomic DNA. Complementation analysis with various fragments of this insert suggested that the *MDM10* gene was contained within a 1.9-kb BglI-KpnI fragment (Fig. 3). The cloned DNA was shown to correspond to sequences from the *mdm10* locus (rather than encoding an extragenic suppressor) by integrative transformation and mapping (see Materials and Methods).

Nucleotide sequence analysis revealed that the complementing fragment contained a single large open reading frame of 1482 bases encoding a potential protein product of 56.2 kD (Fig. 4). Comparison of the putative amino acid sequence with sequences in the GenBank database indicated that *MDM10* was previously identified as an open reading frame adjacent to the *SP07* gene on chromosome I and was designated *FUN37* (*FUN*=function unknown now) (Whyte et al., 1990) and as *YAL010* (Ouellette et al., 1993). The Mdm10p amino acid sequence does not display significant similarities to other known proteins, common motifs for functional sites, or characteristic targeting sequences for subcellular localization. The sequence does contain a large number of hydroxylated amino acids (26% of the total residues are serine, threonine, or tyrosine) and, consequently, a number of potential phosphorylation sites.

To analyze further the cellular requirements for *MDM10*, a null mutation was created by replacing a large portion of the coding region of one of two chromosomal copies of *MDM10* with the yeast *URA3* gene in a diploid cell (Fig. 3 and Materials and Methods). The gene disruption was confirmed by Southern analysis (data not shown). The transformed diploid was sporulated, and growth of the meiotic progeny was examined. Spores containing the gene disruption (Ura$^+$) grew slowly at 23°C (with rates similar to that of the *mdm10-1* cells) and were unable to grow at 37°C (data not shown). Microscopic analysis of the cells containing the *mdm10*-null allele revealed phenotypes very similar to those of the original *mdm10* isolate: large spherical mitochondria were present in virtually all cells and daughter buds deficient in mitochondria were present at both 23°C and 37°C (Fig. 5). Transformation of the null mutant with a centromere-based plasmid encoding the wild-type *MDM10* gene restored the wild-type phenotypes (data not shown). These results indicated that *MDM10* is essential for viability at elevated temperatures and for optimal growth at lower temperatures.

Mdm10p is a Component of the Mitochondrial Outer Membrane

To determine the intracellular location of Mdm10p, antibodies were raised in rabbits against a β-galactosidase-Mdm10p fusion protein. This antiserum recognized polypeptides of 56, 57, and ~170 kD on a Western blot of proteins from a yeast cell homogenate (Fig. 6 *A*). The 56- kD band was absent in extracts prepared from the *mdm10*-null strain (Fig. 6 *A*). Antibodies affinity-purified on the fusion protein also recognized the 56-kD band (data not shown). These results demonstrate that the 56-kD polypeptide is the product of the *MDM10* gene. Antibodies affinity purified on the 56-kD band (Mdm10p) recognized both the 56- and 57-kD polypeptide species (data not shown). This observation indicates that the two proteins share one or more epitopes, even though they are unrelated genetically.

The subcellular distribution of Mdm10p was analyzed by Western blotting of proteins extracted from subcellular fractions. Mdm10p was found in the mitochondrial fraction (Fig. 6 *B*), and it was deficient in fractions depleted of mitochondria. Mitochondrial subfractionation revealed that Mdm10p was enriched in the outer membrane (Fig. 6 *C*) and fraction-

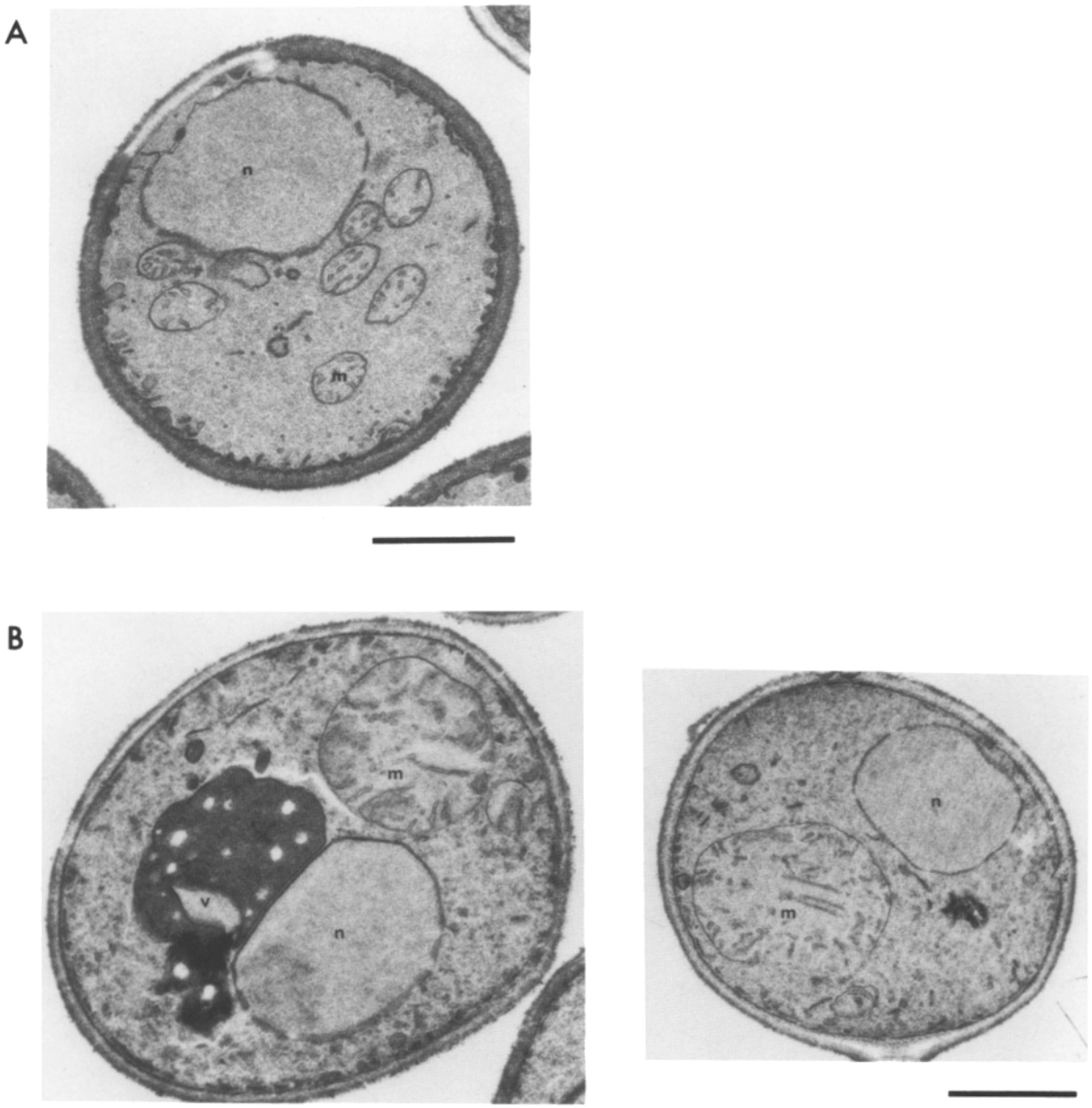

Figure 2. Transmission electron microscopy reveals giant mitochondria in *mdm10* cells. Wild-type (*A*) or mutant (*mdm10-1*) (*B*) cells were cultured as described in Materials and Methods, prefixed with glutaraldehyde, fixed in $KMnO_4$, and stained in uranyl acetate. Sections were stained with lead citrate. *m*, mitochondria; *n*, nuclei; *v*, vacuole. Bar, 1 μm.

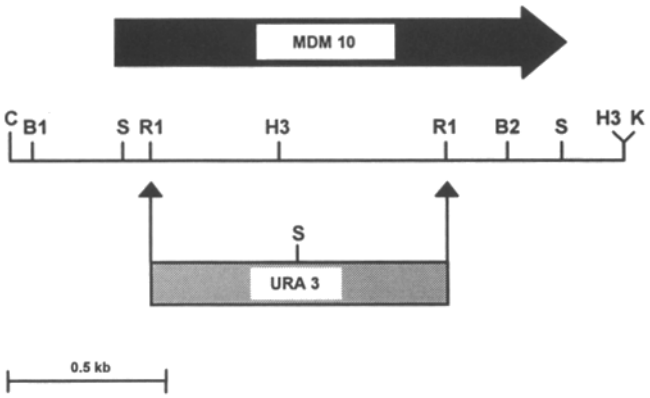

Figure 3. Restriction map and disruption of the *MDM10* gene. Map of some restriction endonuclease sites in the 2.0-kb complementing fragment. The solid arrow indicates the MDM10 open reading frame. The *mdm10*-null stain was created by replacement of most of the MDM10 open reading frame with the URA3 gene as indicated. Restrictions sites: *C*, ClaI; *B1*, BglI; *S*, ScaI; *H3*, HindIII; *R1*, EcoRI; *B2*, BglII; *K*, KpnI.

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-180 TTATTTAGCT TTCCACTACC TTTCTTCCTT TGTGTGTATA ATCAATAGAT TTTCGCTAAA TTTCGCATCT TCCCTTTTAA GAATAATTAG

-90  TATTTAATTT TACTTTGACT TTATTGTTGA GTGAAGAAAC AAAGCAAATA TACGTTAGGA AAAAGACACG AACAGAGAAG ACCGATCTTG

1    ATG CTA CCC TAT ATG GAC CAA GTA CTA AGG GCA TTT TAT CAG AGC ACC CAT TGG AGT ACG CAA AAT AGC TAC GAG
1    MET Leu Pro Tyr MET Asp Gln Val Leu Arg Ala Phe Tyr Gln Ser Thr His Trp Ser Thr Gln Asn Ser Tyr Glu

76   GAT ATA ACG GCC ACA TCG AGA ACA TTA TTA GAT TTC CGA ATT CCC TCA GCA ATA CAC CTG CAA ATT TCC AAC AAA
26   Asp Ile Thr Ala Thr Ser Arg Thr Leu Leu Asp Phe Arg Ile Pro Ser Ala Ile His Leu Gln Ile Ser Asn Lys

151  TCT ACT CCC AAT ACA TTC AAT TCT TTA GAT TTT TCT ACG AGG TCC AGG ATA AAT GGT TCT CTG AGT TAT TTA TAC
51   Ser Thr Pro Asn Thr Phe Asn Ser Leu Asp Phe Ser Thr Arg Ser Arg Ile Asn Gly Ser Leu Ser Tyr Leu Tyr

226  TCC GAT GCA CAG CAA TTG GAG AAA TTC ATG CGC AAC TCT ACT GAT ATC CCA TTA CAA GAT GCC ACC GAA ACA TAC
76   Ser Asp Ala Gln Gln Leu Glu Lys Phe MET Arg Asn Ser Thr Asp Ile Pro Leu Gln Asp Ala Thr Glu Thr Tyr

301  AGA CAA TTG CAA CCA AAC CTC AAT TTC AGT GTT AGT AGT GCG AAT ACG TTG AGT AGT GAC AAC ACC ACA GTC GAC
101  Arg Gln Leu Gln Pro Asn Leu Asn Phe Ser Val Ser Ser Ala Asn Thr Leu Ser Ser Asp Asn Thr Thr Val Asp

376  AAT GAC AAG AAA TTA CTA CAT GAC TCG AAA TTT GTT AAA AAA TCC CTT TAT TAT GGT AGA ATG TAC TAC CCC AGC
126  Asn Asp Lys Lys Leu Leu His Asp Ser Lys Phe Val Lys Lys Ser Leu Tyr Tyr Gly Arg MET Tyr Tyr Pro Ser

451  TCT GAT TTA GAA GCA ATG ATA ATA AAA CGA CTA AGT CCA CAA ACC CAA TTT ATG CTT AAG GGT GTC AGT AGT TTC
151  Ser Asp Leu Glu Ala MET Ile Ile Lys Arg Leu Ser Pro Gln Thr Gln Phe MET Leu Lys Gly Val Ser Ser Phe

526  AAA GAA AGC TTA AAC GTT TTA ACG TGC TAT TTT CAA AGA GAT TCT CAC CGC AAT TTA CAG GAG TGG ATA TTT TCC
176  Lys Glu Ser Leu Asn Val Leu Thr Cys Tyr Phe Gln Arg Asp Ser His Arg Asn Leu Gln Glu Trp Ile Phe Ser

601  ACC AGT GAT CTA TTA TGT GGT TAT AGA GTA TTA CAC AAT TTC CTT ACC ACG CCT TCC AAG TTT AAC ACC TCA CTG
201  Thr Ser Asp Leu Leu Cys Gly Tyr Arg Val Leu His Asn Phe Leu Thr Thr Pro Ser Lys Phe Asn Thr Ser Leu

676  TAC AAT AAT TCT TCG TTG TCG CTT GGT GCT GAA TTT TGG TTA GGG TTA GTA AGT TTA AGC CCC GGT TGT TCG ACA
226  Tyr Asn Asn Ser Ser Leu Ser Leu Gly Ala Glu Phe Trp Leu Gly Leu Val Ser Leu Ser Pro Gly Cys Ser Thr

751  ACT TTA AGA TAT TAC ACA CAT TCT ACA AAC ACA GGA CGA CCA CTA ACT TTG ACA TTA TCT TGG AAT CCA TTA TTC
251  Thr Leu Arg Tyr Tyr Thr His Ser Thr Asn Thr Gly Arg Pro Leu Thr Leu Thr Leu Ser Trp Asn Pro Leu Phe

826  GGC CAT ATA TCC TCC ACA TAT TCG GCC AAG ACA GGG ACA AAT TCT ACT TTT TGC GCG AAG TAT GAT TTT AAT CTT
276  Gly His Ile Ser Ser Thr Tyr Ser Ala Lys Thr Gly Thr Asn Ser Thr Phe Cys Ala Lys Tyr Asp Phe Asn Leu

901  TAT TCG ATT GAA TCA AAT CTT TCA TTT GGG TGC GAA TTT TGG CAA AAA AAG CAT CAT TTG CTT GAA ACC AAT AAA
301  Tyr Ser Ile Glu Ser Asn Leu Ser Phe Gly Cys Glu Phe Trp Gln Lys Lys His His Leu Leu Glu Thr Asn Lys

1051 AAC AAT AAT GAT AAA TTA GAA CCA ATC TCC GAC GAA TTG GTT GAT ATA AAT CCA AAC AGC AGA GCG ACT AAA CTA
351  Asn Asn Asn Asp Lys Leu Glu Pro Ile Ser Asp Glu Leu Val Asp Ile Asn Pro Asn Ser Arg Ala Thr Lys Leu

1126 CTG CAC GAA AAT GTA CCG GAT CTG AAT TCA GCT GTT AAC GAT ATT CCT TCT ACA CTA GAT ATA CCT GTT CAC AAA
376  Leu His Glu Asn Val Pro Asp Leu Asn Ser Ala Val Asn Asp Ile Pro Ser Thr Leu Asp Ile Pro Val His Lys

1201 CAA AAG CTA TTA AAT GAT TTA ACT TAT GCA TTC TCG TCG TCA TTA AGA AAA ATC GAT GAA GAA AGA TCT ACC ATC
401  Gln Lys Leu Leu Asn Asp Leu Thr Tyr Ala Phe Ser Ser Ser Leu Arg Lys Ile Asp Glu Glu Arg Ser Thr Ile

1276 GAA AAA TTT GAT AAC AAA ATA AAT AGT TCC ATT TTT ACC AGT GTT TGG AAA TTA AGC ACG TCA TTA CGT GAC AAG
426  Glu Lys Phe Asp Asn Lys Ile Asn Ser Ser Ile Phe Thr Ser Val Trp Lys Leu Ser Thr Ser Leu Arg Asp Lys

1351 ACT TTA AAA CTA TTA TGG GAA GGC AAA TGG AGG GGA TTT TTA ATA TCT GCC GGG ACA GAG CTG GTA TTC ACT AGA
451  Thr Leu Lys Leu Leu Trp Glu Gly Lys Trp Arg Gly Phe Leu Ile Ser Ala Gly Thr Glu Leu Val Phe Thr Arg

1426 GGC TTT CAA GAA AGT TTA TCC GAT GAT GAA AAG AAT GAT AAT GCA ATA TCT ATA TCA GCA ACT GAT ACA GAA AAC
476  Gly Phe Gln Glu Ser Leu Ser Asp Asp Glu Lys Asn Asp Asn Ala Ile Ser Ile Ser Ala Thr Asp Thr Glu Asn

1501 GGC AAT ATA CCA GTT TTC CCG GCA AAG TTT GGC ATA CAA TTC CAG TAC TCC ACA
501  Gly Asn Ile Pro Val Phe Pro Ala Lys Phe Gly Ile Gln Phe Gln Tyr Ser Thr

1555 TGATGAGTAA TGTGAAATAA AATAAAGGTT TTAATATAC AGGTTAAAAAA TAAGTAATAC AATGTGTATA ATCATAATGG CGTTAAAGCA
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Figure 4. Sequence of the *MDM10* gene and its predicted protein product. These sequence data are available from EMBL/GenBank/DDBJ under accession number X80874.

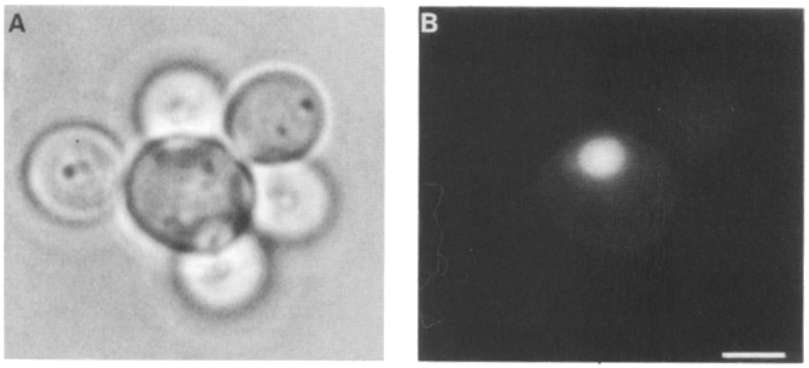


Figure 5. Giant mitochondria and empty buds in the null mutant. MYY503 (*mdm10-null*) cells were cultured in semisynthetic-lactate medium at 23°C, stained with the mitochondrial-specific dye, DASPMI, and embedded in 1% low melting agarose before photomicroscopy. Shown is a typical cell with a single giant mitochondrion and multiple empty buds. (*A*) Phase contrast microscopy. (*B*) Fluorescence microscopy. Bar, 3 μm.

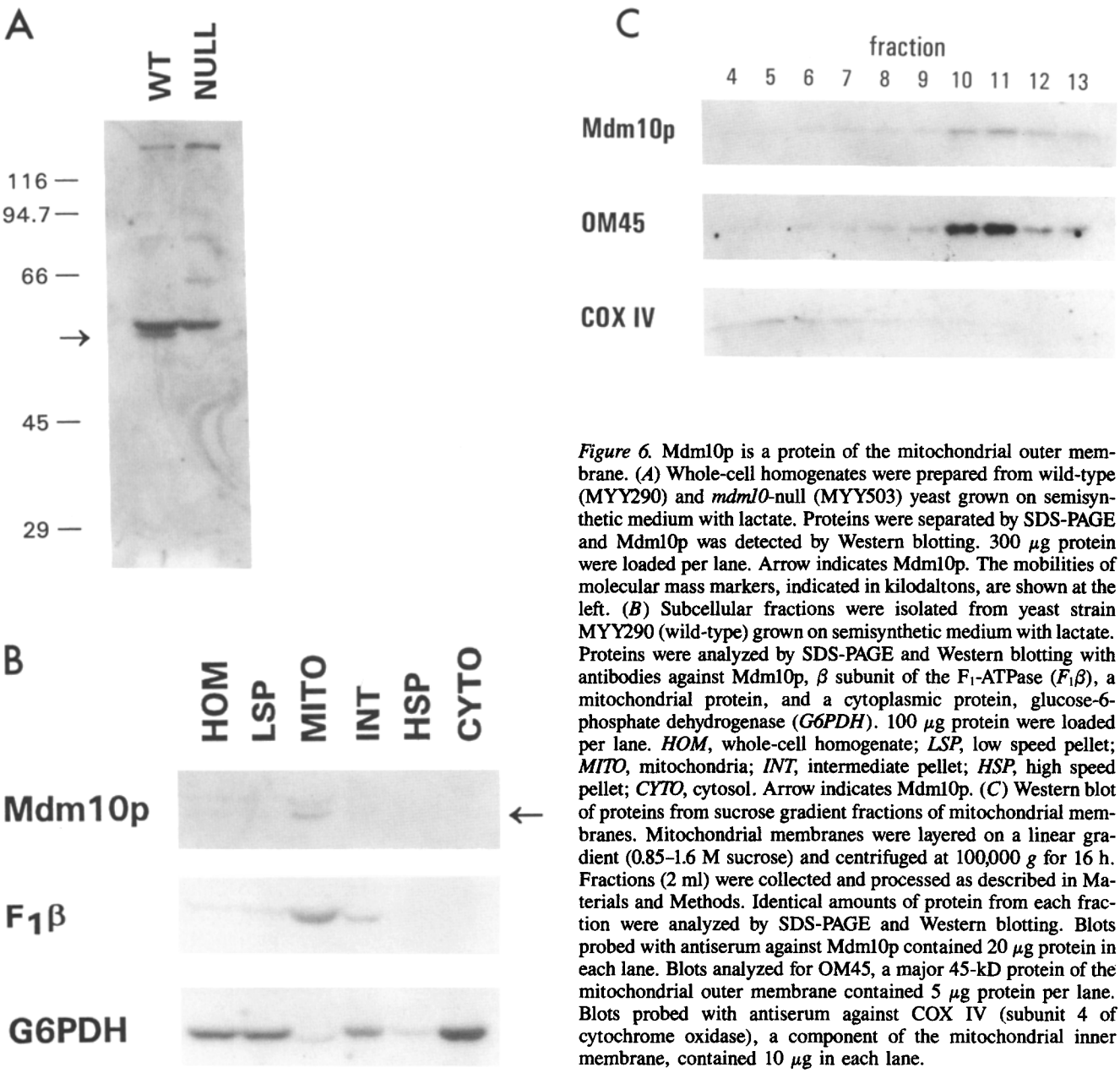


Figure 6. Mdm10p is a protein of the mitochondrial outer membrane. (*A*) Whole-cell homogenates were prepared from wild-type (MYY290) and *mdm10*-null (MYY503) yeast grown on semisynthetic medium with lactate. Proteins were separated by SDS-PAGE and Mdm10p was detected by Western blotting. 300 μg protein were loaded per lane. Arrow indicates Mdm10p. The mobilities of molecular mass markers, indicated in kilodaltons, are shown at the left. (*B*) Subcellular fractions were isolated from yeast strain MYY290 (wild-type) grown on semisynthetic medium with lactate. Proteins were analyzed by SDS-PAGE and Western blotting with antibodies against Mdm10p, β subunit of the F_1-ATPase ($F_1\beta$), a mitochondrial protein, and a cytoplasmic protein, glucose-6-phosphate dehydrogenase (*G6PDH*). 100 μg protein were loaded per lane. *HOM*, whole-cell homogenate; *LSP*, low speed pellet; *MITO*, mitochondria; *INT*, intermediate pellet; *HSP*, high speed pellet; *CYTO*, cytosol. Arrow indicates Mdm10p. (*C*) Western blot of proteins from sucrose gradient fractions of mitochondrial membranes. Mitochondrial membranes were layered on a linear gradient (0.85–1.6 M sucrose) and centrifuged at 100,000 *g* for 16 h. Fractions (2 ml) were collected and processed as described in Materials and Methods. Identical amounts of protein from each fraction were analyzed by SDS-PAGE and Western blotting. Blots probed with antiserum against Mdm10p contained 20 μg protein in each lane. Blots analyzed for OM45, a major 45-kD protein of the mitochondrial outer membrane contained 5 μg protein per lane. Blots probed with antiserum against COX IV (subunit 4 of cytochrome oxidase), a component of the mitochondrial inner membrane, contained 10 μg in each lane.

ated similar to OM45, a major protein of the mitochondrial outer membrane. These results demonstrated that Mdm10p is a protein of the mitochondrial outer membrane.

To confirm the outer membrane location of Mdm10p, the protein's accessibility to proteases added to isolated, intact mitochondria was examined. Mdm10p was degraded by proteinase K treatment (Fig. 7), under conditions where cytochrome b_2, a protein of the intermembrane space, was protected. Both Mdm10p and cytochrome b_2 were degraded by proteinase K added to mitochondria possessing broken outer membranes (data not shown). These results confirm the outer membrane location of Mdm10p and indicate that at least a portion of the protein is exposed to the cytoplasm.

The predicted amino acid sequence of Mdm10p does not include a characteristic hydrophobic membrane-spanning domain, so the nature of Mdm10p association with the outer membrane was investigated using various chemical treatments of purified mitochondria. Mdm10p was not extracted from the mitochondrial membranes by treatment with 0.2 M KCl or 0.3 M KI (data not shown), or by extraction with sodium carbonate at pH 10.8 (Fig. 7). The latter treatment generally is sufficient to release peripheral membrane proteins (Fujiki et al., 1982). When mitochondria were extracted with the detergent Triton X-114, Mdm10p partitioned into the detergent phase, similar to other integral membrane proteins (Bordier, 1981) (Fig. 7). Mdm10p was not released from the outer membrane by treatment with phospholipases (data not shown), and consensus sequences for lipid modification are not found in the predicted amino acid sequence. These results suggest that Mdm10p is an integral membrane protein.

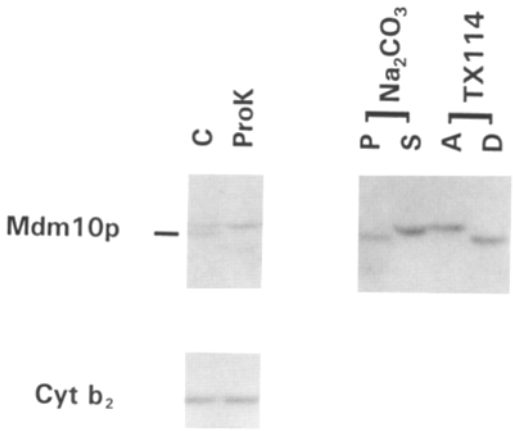


Figure 7. Mdm10p behaves as an integral membrane protein partially exposed to the cytosol. Mitochondria (100 μg) purified from wild-type cells were mock-treated (*C*) or incubated with 0.2 mg/ml proteinase K (*ProK*). Proteins were separated by SDS-PAGE and blotted with anti-Mdm10p (*top left panel*) and anti-cytochrome b_2 (*bottom panel*). The latter protein is a constituent of the mitochondrial intermembrane space (Daum et al., 1992) and indicates the intactness of the mitochondrial outer membrane. Mitochondria (125 μg) were resuspended in 0.1 M Na_2CO_3. Membranes were pelleted at 100,000 *g*, and pellet (*P*) and supernatant (*S*) fractions were recovered. For detergent extraction, 300 μg of mitochondria were separated into aqueous (*A*) and detergent (*D*) fractions by treatment with Triton X-114. Proteins from various fractions were separated by SDS-PAGE and Mdm10p was visualized by Western blotting (*top right panel*). Mdm10p is the lower band (*top panels*). The upper band is a 57-kD protein also recognized by antiserum against Mdm10p (see Fig. 6).

MDM10 Expression Can Control Mitochondrial Morphology

Since *mdm10* mutant cells contain giant mitochondria even at permissive temperatures, the origin of these enlarged organelles was unclear. To clarify the relationship between the giant mitochondria and Mdm10p, the *MDM10* gene was placed under control of the inducible *Gal10* promoter in an *mdm10*-null strain, and changes in mitochondrial morphology were analyzed after the termination of Mdm10p expression. To facilitate the turnover of preexisting Mdm10p, a gene fusion (pUBM10-R) was constructed that produced a chimeric protein containing ubiquitin-lacI sequences fused to the amino terminus of Mdm10p. Previous studies (Bachmair et al., 1986; Park et al., 1992) have demonstrated that the ubiquitin moiety of such a fusion protein is rapidly cleaved in yeast cells, and that the stability of the remaining protein depends on the identity of the new amino-terminal residue. This residue was designed to be an arginine that was predicted (Bachmair et al., 1986) to confer a short half-life on the protein. After cleavage of the ubiquitin, the resulting protein appeared ~6 kD larger than native Mdm10p, yet it localized to mitochondria (data not shown), and was fully functional for complementing the *mdm10*-null mutation (see Fig. 9 *B*, 0 h).

Western blot analysis of protein samples extracted from cells at various times after removal of galactose from the culture media (Fig. 8) suggested that Mdm10p levels were initially higher than that found in wild-type cells. No effect of this overexpression of Mdm10p on mitochondrial distribution or morphology was apparent. Mdm10p subsequently decreased to undetectable levels by 15 h after removal of galactose, while cellular levels of two other proteins, β subunit of the F_1-ATPase (a mitochondrial component) and the cytoplasmic protein, glyceraldehyde-3-phosphate dehydrogenase, showed only minimal changes after termination of *MDM10* expression (Fig. 8 *B*). Mitochondria in these cells initially appeared as snakelike structures and extended reticulated networks as observed by fluorescence microscopy. This wild-type morphology was still present after 8 h. After 15 h, the mitochondria in a large fraction of cells appeared to have condensed into one or two thick loglike structures (Fig. 9 *B*, 15 h). After 24 h, in addition to a substantial fraction of cells possessing condensed mitochondria (Fig. 9 *B*, 24 h, *top panel*), many cells were observed with large mitochondrial balls (Fig. 9 *B*, 24 h, *lower panel*). By this time, a number of cells also possessed empty daughter buds devoid of mitochondria (Fig. 9 *C*). The number of cells with giant mitochondria and with empty buds increased further during the next 24 h (Fig. 9 *B*, 48 h). Mitochondrial morphologies in wild-type cells harboring the pUBM10-R construct showed little change after removal of galactose (Fig. 9 *A*). Although it was not technically possible to follow the fate of individual cells, quantitative analysis of populations of cells (Fig. 10 *A*) suggested that depletion of Mdm10p led to the progressive condensation of mitochondrial structures into giant mitochondria. These results demonstrate that the depletion of Mdm10p leads to progressive changes in mitochondrial morphology that result in giant spherical mitochondria.

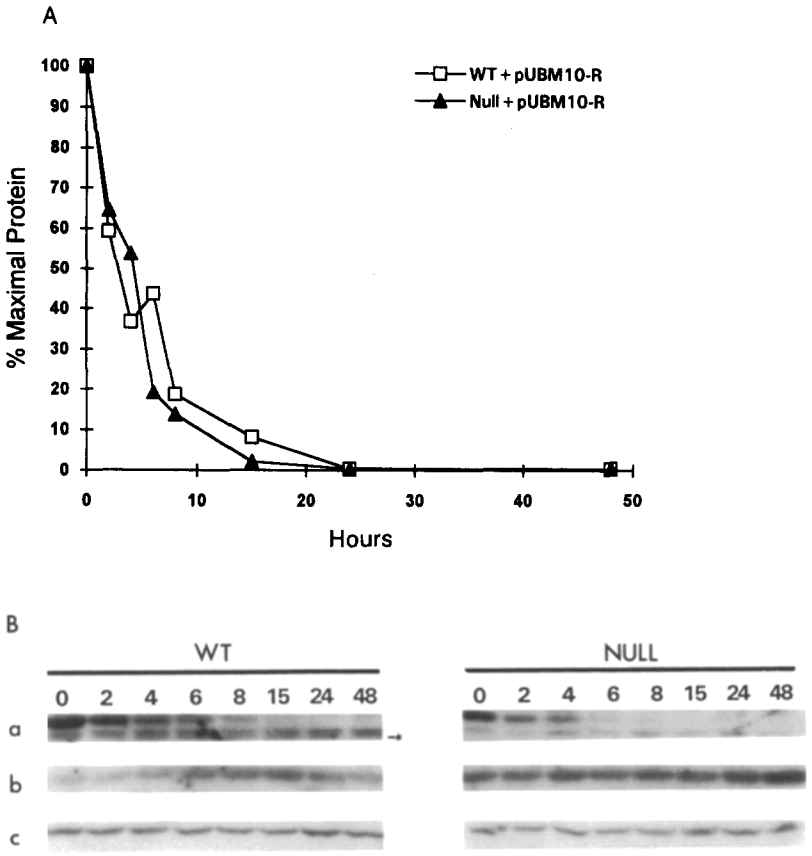


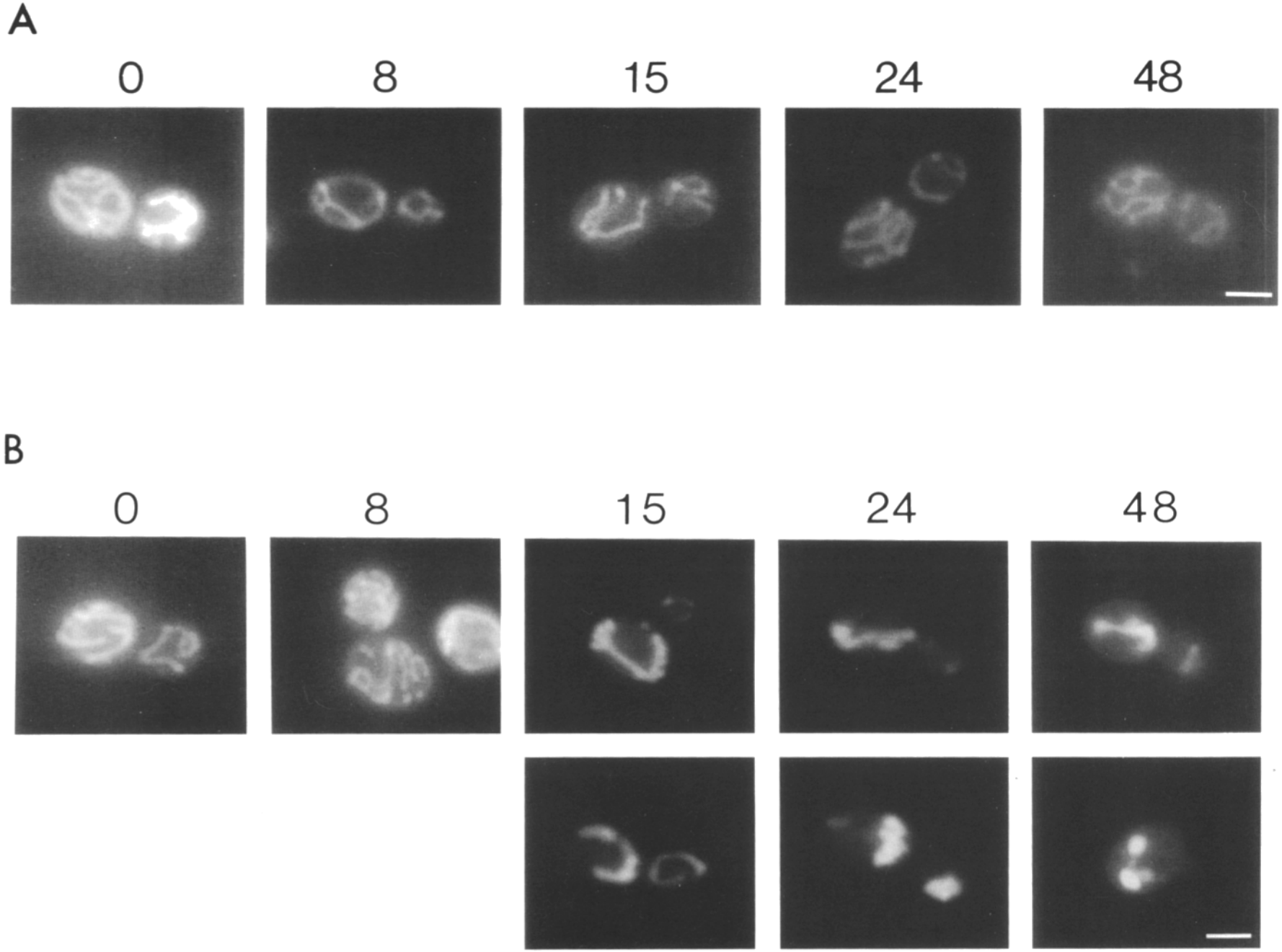
Figure 8. Depletion of the Mdm10p-chimera after galactose removal. (*A*) Wild-type (MYY290) and *mdm10*-null (MYY503) cells harboring plasmid pUBM10-R were cultured on selective medium containing 2% galactose and 2% raffinose. Cells were collected on filters and resuspended in selective medium without galactose (with 2% raffinose). At the times indicated, cellular aliquots were removed, and proteins were extracted. Protein samples were analyzed by SDS-PAGE and Western blotting. Individual bands on autoradiographs were quantified with a linear CCD camera and the NIH Image 1.49 program. (*B*) Autoradiograph of proteins blotted with antisera against Mdm10p (*a*), glyceraldehyde-3-phosphate dehydrogenase (*b*), and $F_1\beta$-subunit of the mitochondrial ATPase (*c*). The Mdm10p protein encoded by pUBM10-R is the top band (disappears at later time points). The endogenous Mdm10p in the wild-type strain is the lower band of the doublet (denoted by asterisk). The upper band of the doublet in wild-type lanes and the single lower band visible in samples from the *mdm10*-null strain is the 57-kD cross-reacting species recognized by the anti-Mdm10p antiserum.

The function of *MDM10* in establishing normal mitochondrial morphology was explored further by reexpressing Mdm10p in cells first depleted of the protein. The addition of galactose, which induced the expression of the chimeric Mdm10p (encoded by pUBM10-R), led to the rapid reversal of the morphological defects (Fig. 10 *B*). After 1 h, 70% of cells possessed mitochondria with extended stringy morphologies, and the number of cells with wild-type mitochondria increased further during the next hour. These results indicate that restoring expression of Mdm10p can lead to the reestablishment of normal mitochondrial morphology.

Discussion

Mdm10p can regulate mitochondrial morphology. Mitochondria normally comprise an extended reticulated network distributed throughout the periphery of the yeast cytoplasm (Stevens, 1981; Koning et al., 1993). Loss of Mdm10p led to the appearance of a condensed mitochondrial mass and the eventual development of giant, spherical, or oval mitochondria. This process appeared to occur by a collapse of elongated mitochondrial tubules and a rearrangement of preexisting mitochondrial components. The reexpression of Mdm10p resulted in a rapid return to an extended snakelike mitochondrial morphology. A similar reversal of mutant morphology was observed after the mating of haploid *mdm10* cells to wild-type cells of the opposite mating type (data not shown). The dumbbell-shaped zygotes resulting from such matings initially possessed giant spherical mitochondria on one side of the cell and wild-type mitochondria on the other side. Over time, the giant mitochondria in these zygotes appeared to fragment into elongated tubules and eventually only mitochondria of normal morphology were present. These observations suggest that Mdm10p can influence a dynamic equilibrium between different mitochondrial morphologies. Alterations in mitochondrial morphology have also been reported in another yeast mutant, *mgm1* (Guan et al., 1993). *MGM1* encodes a dynamin-like protein that is required for maintenance of the mitochondrial genome (Jones and Fangman, 1992), however, the specific function and subcellular location of this component has not been established.

Immunological and biochemical analyses indicated that Mdm10p is an integral protein of the mitochondrial outer membrane. The tight membrane association of Mdm10p was unexpected because the deduced amino acid sequence lacks a characteristic hydrophobic domain of sufficient length to span the membrane (Kyte and Doolittle, 1982). Additionally, we have been unable to obtain evidence for the presence of covalently attached lipids that could anchor the protein to the outer membrane. The predicted protein sequence does contain four stretches of 15–21 uncharged amino acids (residues 102-119, 221-235, 237-252, and 264-284), and one or several of these domains potentially could span the membrane. Interestingly, another important protein of the mitochondrial outer membrane in yeast, ISP42, also behaves as an integral membrane protein but lacks a typical membrane-spanning domain in its sequence (Baker et al., 1990). Similarly, the α subunit of the signal recognition particle receptor is firmly embedded in the endoplasmic reticulum membrane but does not contain a classical transmembrane domain (Lauffer et al., 1985).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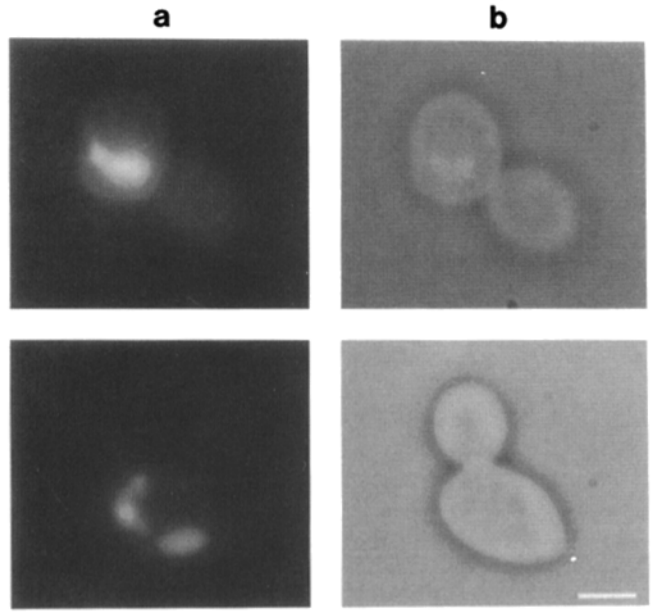

Figure 9. Mdm10p depletion produces altered mitochondrial morphology. Wild-type (*A*) and *mdm10*-null (*B*) strains harboring the pUBM10-R plasmid were grown as described for Fig. 8. Cells were stained with the mitochondrial-specific dye DASPMI and embedded in 1% low melting agarose before photomicroscopy. Photographs show cells displaying representative mitochondrial morphologies at various times (hours) of incubation in medium lacking galactose. Proportions of cellular populations represented by the various phenotypes are indicated in Fig. 10 *A*. Bar, 3 μm. (*C*) Mutant (*mdm10-null*) cells grown for 24 h without galactose and representative of a cellular population having empty buds and large, spherical mitochondria. Cells were stained and photographed as in *a* and *b*.

MDM10 is essential for cell proliferation at elevated temperatures and necessary for optimal growth at lower temperatures. This requirement of Mdm10p is likely to reflect defective mitochondrial inheritance in the *mdm10* mutant. Previous studies have suggested that mitochondria supply some essential cellular functions in addition to their role in energy metabolism (Yaffe and Schatz, 1984; Gbelska et al., 1983; Kovacova et al., 1968), so daughter cells that fail to receive mitochondria are unlikely to proliferate. Mutant *mdm10* cells do proliferate (albeit slowly) at permissive temperatures, and a fraction of daughter buds receive some mitochondria. The source of these inherited mitochondria is unclear because the mutant cells contain giant mitochondria, even at permissive temperatures, and the giant mitochondria do not appear to be transferred to daughter buds. A possible explanation is that the small mitochondria that are often present along with the giant organelles in *mdm10* cells (Fig. 2) are transferred to buds during mitotic growth. The presence of these small mitochondria in the mutant cells suggests that an alternative or bypass pathway for mitochondrial division and inheritance may operate in *mdm10* cells. This bypass pathway may be inadequate to support mitochondrial inheritance at higher temperatures.

Several observations suggest that the giant spherical mito-

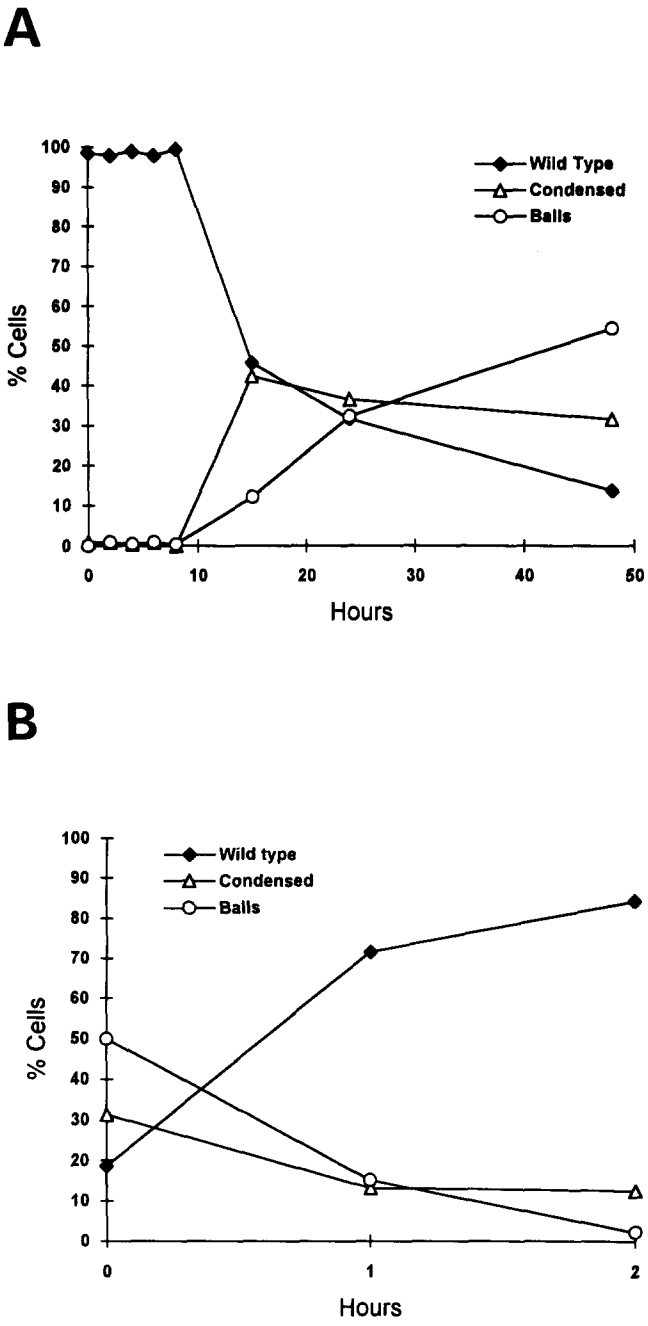

Figure 10. Quantification of mitochondrial morphologies during Mdm10p depletion and reexpression. (*A*) Mdm10p was depleted as described for Fig. 8. Periodically, cellular aliquots were treated with DASPMI to stain mitochondria, and cells were counted to determine the proportion of cell populations displaying various mitochondrial morphologies. An average of 330 cells were counted for each time point. Cells were classified as having wild-type mitochondria (extended tubular networks), condensed mitochondria (thickened masses with limited distribution), or ball-like mitochondria. The graph shows the effect of Mdm10p-chimera depletion in the *mdm10*-null strain. Depletion of Mdm10p-chimera in the wild-type strain showed no quantifiable change in mitochondrial morphology. (*B*) *mdm10*-null cells with the pUBM10-R plasmid were depleted of Mdm10p by culturing without galactose for 48 h as described for Fig. 8. Galactose was added to a final concentration of 2%, and samples were removed after 0, 1, and 2 h incubation. Mitochondria were stained and morphologies were quantified as described above.

chondria are generally defective for division and inheritance. First, structural features suggesting mitochondrial division, such as division furrows or constricted regions, were not apparent in electron micrographs of *mdml0* cells. Second, examination of multibudded *mdml0* cells (a common phenotype on nonfermentable carbon sources) generally revealed one or two giant mitochondria in the mother portion of the cell and many buds lacking mitochondria. Although these cells had gone through multiple rounds of bud formation, the number of mitochondria had not increased, consistent with a defect in mitochondrial division. Third, the giant mitochondria were rarely found by microscopy in daughter buds, further suggesting that these mitochondria were not competent for inheritance. Finally, in contrast to the rapid and frequent alteration of mitochondrial distribution in wild-type cells (Koning et al., 1993), little mitochondrial movement was detected in time-lapse photomicroscopic analysis of *mdml0* cells (Yaffe, M., unpublished data). An inefficient process may operate in *mdml0* cells to allow occasional division or budding of mitochondria, and this process could provide a source of the small mitochondria that frequently accompany the giant organelles.

How can loss of Mdm10p lead to defects in mitochondrial morphology, division, and inheritance? One model that connects these different phenotypes is that mitochondrial morphology, division, and inheritance all depend on interaction of mitochondria with the cytoskeleton and that this interaction requires Mdm10p. Since Mdm10p appears to be anchored in the mitochondrial outer membrane with a portion of its sequence accessible to the cytoplasm, this protein might provide a direct physical link between cytoskeletal components and the organelle. Such a "handle" (Mdm10p or associated protein) on the outer membrane could function in both the transport of mitochondria along extended cytoskeletal tracks and the pulling out of a mitochondrion to produce the characteristic tubular morphology. Similarly, pulling of a mitochondrion in opposite directions might facilitate the division of the organelle. In the absence of Mdm10p, the reticulated mitochondria would lose their connections to the cytoskeleton and collapse into a basic spherical structure. Alternatively, Mdm10p could regulate the binding of a second outer membrane protein to elements of the cytoskeleton. A second model for Mdm10p function is that the protein's primary role is for the determination of an extended, tubular, mitochondrial morphology, and that this morphology, rather than the direct function of Mdm10p per se, is required for mitochondrial division and movement. The identification of proteins that interact with Mdm10p will provide further insight into molecular mechanisms mediating the morphology, division, and inheritance of mitochondria.

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References

Atencio, D. P., and M. P. Yaffe. 1992. MAS5, a yeast homolog of DnaJ involved in mitochondrial protein import. *Mol. Cell. Biol.* 12:283–291.

Attardi, G., and G. Schatz. 1988. Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* 4:289–333.

Bachmair, A., D. Finley, and A. Varshavsky. 1986. In vivo half-life of a protein is a function of its amino-terminal residue. *Science (Wash. DC).* 234:179–186.

Baker, K. P., A. Schaniel, D. Vestweber, and G. Schatz. 1990. A yeast mitochondrial outer membrane protein essential for protein import and cell viability. *Nature (Lond.).* 348:605–609.

Bereiter-Hahn, J. 1990. Behavior of mitochondria in the living cell. *Int. Rev. Cytol.* 122:1–64.

Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* 256:1604–1607.

Burglin, T. R., and E. M. DeRobertis. 1987. The nuclear migration signal of *Xenopus laevis* nucleoplasmin. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2617–2625.

Chen, L. B. 1988. Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol.* 4:155–181.

Chevallier, M.-R., J. C. Bloch, and F. Lacroute. 1980. Transcriptional and translational expression of a chimeric bacterial-yeast plasmid in yeast. *Gene (Amst.).* 11:11–19.

Daum, G., P. C. Bohni, 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.* 257:13028–13033.

Drubin, D. G., H. D. Jones, and K. F. Wertman. 1993. Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Biol. Cell.* 4:1277–1294.

Fujiki, Y., A. L. Hubbard, S. Fowler, and P. B. Lazarow. 1982. Isolation of intracellular membranes by means of sodium carbonate treatment. Application to endoplasmic reticulum. *J. Cell Biol.* 93:97–102.

Gbelska, Y., J. Subik, A. Goffeau, and L. Kovac. 1983. Intramitochondrial ATP and cell functions: yeast cells depleted of intramitochondrial ATP lose the ability to grow and multiply. *Eur. J. Biochem.* 130:281–286.

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.

Harlow, E., and D. Lane. 1988. Antibodies. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. pp. 55–137.

Heggeness, M. H., M. Simon, and S. J. Singer. 1978. Association of mitochondria with microtubules in cultured cells. *Proc. Natl. Acad. Sci. USA.* 75:3863–3866.

Inagaki, T., S. Kobayashi, N. Ozeki, M. Suzuki, Y. Fukuzawa, K. Shimizu, and K. Kato. 1992. Ultrastructural identification of light microscopic giant mitochondria in alcoholic liver disease. *Hepatalogy.* 15:46–65.

Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163–168.

Jensen, R. E., and M. P. Yaffe. 1988. Import of proteins into yeast mitochondria: the nuclear MAS2 gene encodes a component of the processing protease that is homologous to the MAS1-encoded subunit. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3863–3871.

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. *Genes & Dev.* 6:380–389.

Koning, A. J., P. Y. Lum, J. M. Williams, and R. Wright. 1993. DiOC6 staining reveals organelle structure and dynamics in living yeast cells. *Cell Motil. Cytoskeleton.* 25:111–128.

Kovacova, V., J. Irmlerova, and L. Kovac. 1968. Oxidative phosphorylation in yeast. IV. Combination of a nuclear mutation affecting oxidative phosphorylation with a cytoplasmic mutation to respiratory deficiency. *Biochim. Biophys. Acta.* 162:157–163.

Kunkel, T. A., J. D. Roberts, and R. A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367–382.

Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105–132.

Lauffer, L. P. D. Garcia, R. N. Harkins. L. Coussens, A. Ullrich, and P. Walter. 1985. Topology of signal recognition particle receptor in endoplasmic reticulum membrane. *Nature (Lond.).* 318:334–338.

Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. *Science (Wash. DC).* 227:1435–1441.

McConnell, S. J., L. C. Stewart, A. Talin, and M. P. Yaffe. 1990. Temperature-sensitive yeast mutants defective in mitochondrial inheritance. *J. Cell Biol.* 111:967–976.

McConnell, S. J., and M. P. Yaffe. 1992. Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the MDM1 protein. *J. Cell Biol.* 118:385–395.

McConnell, S. J., and M. P. Yaffe. 1993. Intermediate filament formation by a yeast protein essential for organelle inheritance. *Science (Wash. DC).* 260:687–689.

Ouellette, B. F. F., M. W. Clark, T. Keng, R. K. Storms, W. Zhong, B. Zeng, N. Fortin, S. Delaney, A. Barton, D. B. Kaback, and H. Bussey. 1993. Sequencing of chromosome I from Saccharomyces cerevisiae: analysis of a 32 kb region between LTE1 and SPO7. *Genome.* 36:32–42.

Park, E.-C., D. Finlay, and J. Szostak. 1992. A strategy for the generation of conditional mutations by protein destabilization. *Proc. Natl. Acad. Sci. USA.* 89:1249–1252.

Rose, M. D., F. Winston, and P. Heiter. 1990. Methods in Yeast Genetics: A Laboratory Course Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 198 pp.

Smith, B. J., and M. P. Yaffe. 1991. A mutation in the yeast heat-shock factor gene causes temperature-sensitive defects in both mitochondrial protein import and the cell cycle. *Mol. Cell. Biol.* 11:2647–2655.

Stevens, B. 1981. Mitochondrial structure. *In* The Molecular Biology of the Yeast *Saccharomyces cerevisiae*. Vol. 1. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. pp. 471–504.

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 reconstruction. *Biol. Cell.* 28:37–56.

Stewart, L. C., and M. P. Yaffe. 1991. A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J. Cell. Biol.* 115:1249–1257.

Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis. 1979. High frequency transformation of yeast: Autonomous replication of hybrid DNA molecules. *Proc. Natl. Acad. Sci. USA.* 76:1035–1039.

Tandler, B., and C. L. Hoppel. 1986. Studies on giant mitochondria. *Ann. NY Acad. Sci.* 488:65–81.

Towbin, H., T. H. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

Tzagoloff, A. 1982. Mitochondria. Plenum Press Publishing Corp., New York. 342 pp.

Whyte, W., L. H. Keopp, J. Lamb, J. C. Crowley, and D. B. Kaback. 1990. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae:* isolation, characterization and regulation of the SPO7 sporulation gene. *Gene.* 95:65–72.

Yaffe, M. P. 1991*a*. Analysis of mitochondrial function and assembly. *Methods Enzymol.* 194:627–643.

Yaffe, M. P. 1991*b*. Organelle inheritance in the yeast cell cycle. *Trends Cell Biol.* 1:160–164.

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