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Inactivation of *YME2*/*RNA12*, Which Encodes an Integral Inner Mitochondrial Membrane Protein, Causes Increased Escape of DNA from Mitochondria to the Nucleus in *Saccharomyces cerevisiae*

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Inactivation of the yeast nuclear gene *YME2* causes an increased rate of DNA escape from mitochondria to the nucleus. Mutations in *yme2* also show genetic interactions with *yme1*, a second gene that affects DNA escape from mitochondria to the nucleus. The *yme1* cold-sensitive growth phenotype is suppressed by *yme2* mutations. In addition, *yme1 yme2* double mutants exhibit a synthetic growth defect on ethanol-glycerol medium at 30°C. *YME2* was isolated by complementation of the synthetic growth defect of *yme1 yme2* strains and was found to be identical with the previously cloned *RNA12* gene. The dominant temperature-sensitive mutation *RNA12-1* prevents growth of yeast cells at 37°C. *YME2* encodes a protein with a predicted molecular weight of 96,681 and is an integral inner mitochondrial membrane protein. The larger carboxyl-terminal domain of the *YME2* gene product faces the intermembrane space. Null alleles of *yme2* display the same genetic interactions with *yme1* and high rate of DNA escape from mitochondria as do the originally isolated *yme2* mutant strains. Disruption of *yme2* causes a strain-dependent growth defect on nonfermentable carbon sources.

Proper maintenance of the mitochondrial compartment is essential for the viability of yeast cells. To identify mutations that affect the integrity of the mitochondrial compartment in yeast cells, a screen that allowed the detection of DNA that escaped from mitochondria and migrated to the nucleus was developed (31). The yeast nuclear *TRP1* gene and its associated origin of replication, *ARS1*, were introduced into the mitochondrial chromosome of a strain carrying a nuclear *trp1* deletion (32). The *TRP1* gene is not expressed within mitochondria, but because of the escape of mitochondrial DNA (mtDNA) to the nucleus, this Trp^- strain readily gives rise to respiration-competent Trp^+ clones that contain the *TRP1*/*ARS1* fragment, as well as portions of associated mtDNA, replicating in their nuclei. We have identified 10 nuclear genetic loci that when mutated increase the rate at which DNA escapes mitochondria and migrates to the nucleus. Nine of these loci are characterized by recessive mutations, and one is characterized by a dominant mutation (32). The genes identified by these mutations have been designated *YME*, for yeast mitochondrial escape.

YME1 encodes a putative ATP- and zinc-dependent protease that is located in the inner mitochondrial membrane (35). The lack of Yme1p causes structural and functional defects in mitochondria. In addition to an increase in the escape of DNA from mitochondria and its subsequent migration to the nucleus, *yme1* strains are unable to utilize nonfermentable carbon sources for growth at 37°C, grow slowly on rich glucose media at 14°C, and grow very slowly when mtDNA is completely absent from the cell (33, 36). The morphology of mitochondrial compartments in *yme1* strains differs from that of the reticulated network found in wild-type strains, showing punctate compartments and poorly defined inner membranes (7).

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In addition to the inability of *yme1* strains to utilize nonfermentable carbon sources at 37°C, *yme1 yme2* double-mutant strains exhibit a slow-growth phenotype on nonfermentable carbon sources at 30°C. Furthermore, mutation of *yme2* suppresses the cold-sensitive growth phenotype seen in *yme1* strains grown on rich glucose media. This genetic interaction between *yme1* and *yme2* allowed us to isolate the *YME2* structural gene and further investigate the nature of genes involved in maintaining the integrity of the mitochondrial compartment.

In this study, we show that *YME2* encodes an integral inner mitochondrial membrane protein with the carboxyl-terminal portion facing the intermembrane space. The cellular location of the *YME2* protein along with the three phenotypes associated with *yme2* mutations, (i) the high frequency of mtDNA escape to the nucleus, (ii) the inability to utilize nonfermentable carbon sources when paired with *yme1* mutations, and (iii) the suppression of the cold-sensitive phenotype of *yme1*, support the notion that *YME2* may play an important role in determining the integrity and function of mitochondria.

MATERIALS AND METHODS

Strains, strain constructions, and genetic manipulations. The *Escherichia coli* strains used for preparation and manipulation of DNA were DH5α [F^- *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1* λ *recA gyrA96 relA1* Δ(*argF-lacZYA*) *U169* ϕ80 *lacZ*Δ*M15*] and GM2163 [F^- *ara-14 leuB6 ton A31 tsx-78 supE44 galK2 galT22 hisG4 rpsL136*(Str^r) *xyl-5 mtl-1 thi-1 dam13*::Tn*9* (Cam^r) *dcm-6 hsdR2* (r_K^- m_K^+) *mcrA mcrB1*]. The strain used for transformation after second-strand synthesis during site-specific mutagenesis was BMH71-18 *mutS* [*thi supE* Δ(*lac-proAB*)] [*mutS*::Tn*10*][F′ *proA*$^+$*B*$^+$ *lacI*^{q}*Z*Δ*M15*].

The genotypes of *Saccharomyces cerevisiae* strains used in this work are listed in Table 1. Standard genetic techniques, involving integrative transformations, were applied to construct and analyze the various yeast strains (25). A markerless *yme2* null allele was obtained by introducing the insert of pY2HUH (see below) into PTY44. Purified Ura^+ transformants (strain THY15) were then streaked out on 5-fluoro-orotic acid to select for Ura^- recombinants. Subsequently, the markerless *yme2* null allele strain, designated THY17, was tested for DNA escape.

Media. *E. coli* strains containing plasmids were grown in LB (10 g of Bacto Tryptone, 10 g of NaCl, and 5 g yeast extract per liter) plus 125 μg of ampicillin per ml. Yeast strains were grown in complete glucose medium (YPD), complete ethanol-glycerol medium (YPEG), or minimal glucose medium supplemented

TABLE 1. *S. cerevisiae* strains used

Strain[a]	Genotype[b]	Reference or source
PTY29rho^0	*MAT*α *lys2 leu2-3,112 trp1-Δ1* [*rho*^{0}]	32
PTY33	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1* [*rho*$^+$ *TRP1*]	32
PTY44	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1* [*rho*$^+$ *TRP1*]	32
PTY33 × PTY44	*MAT***a** *ura3-52 ade2 LYS2 leu2-3,112 trp1-Δ1* [*rho*$^+$ *TRP1*] *MAT*α *ura3-52 ADE2 lys2 leu2-3,112 trp1-Δ1*	33
PTY52	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3* [*rho*$^+$ *TRP1*]	33
PTY55	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-1 yme2-1* [*rho*$^+$ *TRP1*]	32
PTY60	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3* [*rho*$^+$ *TRP1*]	7
PTY62	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-1* [*rho*$^+$ *TRP1*]	32
PTY63	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1 yme2-1* [*rho*$^+$ *TRP1*]	This study
PTY64	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme2-1* [*rho*$^+$ *TRP1*]	32
THY1	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 yme2-1*[*rho*$^+$ *TRP1*]	This study
THY3	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 YME2::URA3* [*rho*$^+$ *TRP1*]	This study
THY4	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme2-Δ1::LEU2* [*rho*$^+$ *TRP1*]	This study
THY5	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme2-Δ1::URA3* [*rho*$^+$ *TRP1*]	This study
THY7	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1 yme2-Δ1::LEU2* [*rho*$^+$ *TRP1*]	This study
THY8	*MAT***a** *ura3-52 ade2 leu2-3,112 trp1-Δ1 yme2-Δ1::URA3* [*rho*$^+$ *TRP1*]	This study
THY9	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 yme2-Δ1::LEU2* [*rho*$^+$ *TRP1*]	This study
THY15	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme2-Δ1::hisG::URA3::hisG* [*rho*$^+$ *TRP1*]	This study
THY17	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme2-Δ1* [*rho*$^+$ *TRP1*]	This study
THY4 × THY8	*MAT*α *ura3-52 ADE2 lys2 leu2-3,112 trp1-Δ1 yme2-Δ1::LEU2* [*rho*$^+$ *TRP1*] *MAT***a** *ura3-52 ade2 LYS2 leu2-3,112 trp1-Δ1 yme2-Δ1::URA3*	This study
THY33	*MAT***a** *ura3-52 his3-Δ200 leu2Δ1 trp1Δ63 yme2-Δ1::URA3* [*rho*$^+$ *TRP1*]	This study
CCY13	*MAT***a** *ura3-52 his3-Δ200 leu2Δ1 trp1Δ63* [*rho*$^+$ *TRP1*]	This study
THY35	*MAT*α *ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 yme2-Δ1* [*rho*$^+$ *TRP1*]	This study
THY55	*MAT*α *ade2-1 his3-11 his3-15 trp1-1 ura3-1 leu2-3 leu2-112 yme2-Δ1::URA3* [*rho*$^+$ *TRP1*]	This study
W303	*MAT*α *ade2-1 his3-11 his3-15 trp1-1 ura3-1 leu2-3 leu2-112*	Sharon Ackerman
FL100	*MAT***a**	ATCC 28383
FLC1	*MAT***a** *ura3*	This study
FLC2	*MAT***a** *ura3-52 ade2 lys trp1 RNA12-1*	François Lacroute
FLC3	*MAT***a** *ura3 yme2-Δ1::URA3*	This study
FLC4	*MAT***a** *ura3-52 ade2 lys trp1 yme2-Δ1::URA3*	This study

[a] CCY13 contains the nuclear genome of S288C and the mitochondrial genome of PTY44. FLC1 and FLC3 are isogenic to FL100. FLC2 and FLC4 are isogenic with each other but not with FLC1 or FLC3.

[b] The mitochondrial genotype is bracketed.

with the indicated nutrients (SD medium) (32). Medium components were purchased from Difco. Ampicillin and nutrients were purchased from Sigma Chemical Co., St. Louis, Mo.

Isolation of *yme2*-complementing plasmids. The slow-growth phenotype on nonfermentable carbon sources caused by the *yme1-1 yme2-1* double mutant (PTY55) was used as a basis for cloning of *YME2*. PTY55 was transformed (12) with 6 μg of an *S. cerevisiae* genomic DNA library in the *CEN* vector YCp50 (23). In parallel, PTY55 was transformed with 8 μg of DNA prepared from a pRS202-based genomic DNA library of *S. cerevisiae*. Ura$^+$ transformants were grown on SD medium lacking uracil for 5 days at 30°C, replica plated onto YPEG plates, and incubated at 30°C. Total yeast DNA was isolated from Ura$^+$ transformants that were able to grow on YPEG at 30°C and introduced into *E. coli* DH5α. Plasmid DNA from ampicillin-resistant *E. coli* transformants was isolated and transformed into yeast strains containing a *yme2* mutation (PTY63) or *yme1 yme2* strain (PTY55). Colony-purified Ura$^+$ transformants of PTY63 and PTY55 were tested for complementation of the DNA escape phenotype (see below) and complementation of the slow-growth phenotype on YPEG at 30°C, respectively. Subclones were generated from plasmids that were able to complement both phenotypes, since these were potential candidates that contained the *YME2* gene.

Nucleic acid techniques and plasmid constructions. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs and Bethesda Research Laboratories. Standard techniques for generating recombinant DNAs and performing DNA blot hybridizations were used (18).

Overlapping DNA sequences from plasmids containing genomic sequences that complemented *yme2* were subcloned into the shuttle vector pRS316 (26) and tested for complementation of the slow-growth phenotype on nonfermentable carbon sources of a *yme1-1 yme2-1* mutant and DNA escape of *yme2* mutants. One complementing subclone from the Rose bank, pRS-R2H2, which contains a 6.1-kb *Hin*dIII fragment, was used to create a total of seven smaller subclones. The minimal complementing construct, designated pYME2, was obtained by subcloning a 3.6-kb *Nhe*I-*Bgl*II fragment containing the entire *YME2*/*RNA12* gene into the *Sma*I sites of the *URA3*/*CEN* vector pRS316. The same *Nhe*I-*Bgl*II fragment was subcloned into the *LEU2*/*CEN* vector pRS315 to generate pLCYME2. Strains containing *yme2* null alleles were constructed by excising the 3.6-kb insert of pYME2 with *Pst*I and *Bam*HI and inserting this fragment into the same sites of plasmid pBluescript KS+ to generate pBS-YME2. Demethylated plasmid DNA of pBS-YME2 was digested with *Bcl*I, which removed most of the *YME2* insert. A 1.5-kb *Bam*HI fragment from pPHB4 (provided by T. Fox), which contains the *URA3* gene, was inserted into the remaining pBS-YME2 plasmid. Alternatively, a 2.2-kb *Bam*HI fragment from pPHB6 (also provided by T. Fox), which contains the *LEU2* gene, was cloned into the *Bcl*I sites of digested pBS-YME2. The inserts of these two plasmids (pBS-*yme2*::*URA3* and pBS-*yme2*::*LEU2*) were excised, gel purified, and used to generate *yme2* null alleles by integrative transformation. In addition, a strain bearing a *yme2* null allele that did not include a genetic marker at the *YME2* locus was made. A 3.85-kb *Bam*HI-*Xba*I fragment from pBS31 (1) was blunt ended with the large fragment of DNA polymerase I and subcloned into the blunt-ended *Bcl*I sites of plasmid pBS-YME2. The resulting plasmid, pY2HUH, contained a *hisG*::*URA3*::*hisG* disruption of *yme2*. Genomic DNA from *yme2* deletion strains (except markerless null alleles) and *URA3*-linked *YME2* strains (see below) was isolated, and DNA blot hybridization was carried out to confirm that the correct genetic locus had been targeted.

DNA escape assay and analysis of yeast transformants. Purified Ura$^+$ or Leu$^+$ transformants containing *yme2* null alleles or the *YME2* allele linked to the *URA3* gene were tested for DNA escape as described previously (32). Briefly, transformants were spread out as sectors on minimal SD medium supplemented with adenine, lysine, tryptophan, and uracil for Leu$^+$ transformants or on SD medium containing adenine, lysine, tryptophan, and leucine for Ura$^+$ transformants and incubated at 30°C to a confluent lawn. Cells were replica plated onto SD medium containing the same supplements but lacking tryptophan to identify Trp$^+$ colonies. Replica plates were incubated for 5 days at 30°C before the number of Trp$^+$ papillae in each sector were scored.

DNA sequence analysis. Double-stranded DNA sequencing (24) of the 3.6-kb insert of plasmid pYME2 from both the 5′ and 3′ ends was carried out by using the T3 and T7 primers (Promega) and the Sequenase DNA sequencing system (United States Biochemical). Sequence data were also obtained from subclones of pYME2 and from subclones containing DNA sequences flanking the *YME2* gene. Sequence analysis was carried out by using BLAST server programs (2) and GeneWorks version 4.0.1 (IntelliGenetics, Mountain View, Calif.).

Linkage of *yme2* to cloned DNAs and mutant loci. Linkage of the cloned *YME2* DNA to the mutant locus defined by the *yme2* mutation was established by subcloning a 5.4-kb *Bam*HI-*Hin*dIII fragment of pRS-R2H2 into the same sites of the integrating pRS306. The resulting construct, pRS306HB, was linearized with *Eco*RI and introduced into PTY44 to generate THY3. Purified Ura^+ transformants of THY3 were backcrossed to PTY63, sporulated, and analyzed for segregation of DNA escape and the *URA3* marker.

Linkage of *YME2* to *RNA12-1* was established via a cross between THY5, which carries the *yme2*::*URA3* null allele, and FLC2 (provided by F. Lacroute), which contains the dominant temperature-sensitive (*ts*)-lethal *RNA12-1* allele (Table 1). Diploids were selected on SD minimal medium lacking uracil and leucine and then sporulated. In this cross, spore lethality was elevated, and only 11 full tetrads were recovered and tested for segregation of the *URA3* marker and the inability to grow on rich glucose medium (YPD) at 37°C. In parallel, FLC2 was mated to a strain bearing a *yme2*::*LEU2* null allele (THY4), zygotes were selected and sporulated, and tetrads were picked. A *MAT***a** *ts*-lethal $Ura3^-$ spore was identified and backcrossed to a strain bearing a *yme2*::*URA3* null allele, and diploids were selected on SD medium and sporulated. Eleven complete tetrads were tested for segregation of *ts* growth on YPD and growth on SD minimal medium lacking uracil.

Generation and analysis of site-directed mutations in *YME2*. The Altered Sites Mutagenesis System (Promega Corp., Madison, Wis.) was used to carry out site-directed mutagenesis of *YME2*. A 6.1-kb *Hin*dIII fragment from plasmid pRS-R2H2 containing the entire *YME2* gene was subcloned into the same sites of pALTER-1. Plasmid DNA of the resulting construct, designated pALTH2, was recovered from *E. coli* DH5α and transformed into *E. coli* JM109, and single-stranded DNA was prepared by using the helper phage R408. To allow modification of the AAT (=Asn) codon at bp 1503 to 1505 of the published *RNA12/YME2* DNA sequence to a TAT (=Tyr) codon, the synthetic oligonucleotide 5′-CCGGTGATGGGACAGTTTATGTTAAGGAAG-3′ (Integrated DNA Technologies Inc., Coralville, Iowa) was used as a primer for mutant-strand synthesis. Double-stranded DNA was transformed into *E. coli* BMH71-18 *mutS*. Total plasmid DNA was isolated and transformed into *E. coli* DH5α, and ampicillin-resistant transformants were identified. Plasmid DNA from several ampicillin-resistant transformants was prepared, and the DNA region surrounding the mutagenized area was sequenced (24) by using a second oligonucleotide, 5′-CGGTTTAGAATGTTA-3′, to confirm the A-to-T transition at nucleotide 1503. The mutagenized *YME2* allele, designated *yme2-12*, was subcloned into the shuttle vectors pRS315 and pRS316 and the integration vector pRS306 to generate pDLY2-12, pDUY2-12, and pLY2-12, respectively. Plasmids pDLY2-12 and pDUY2-12 as well as the *Eco*RI-linearized form of pLY2-12 were introduced into yeast strains THY4, FL100, W303, and CCY13, and the transformants were purified on appropriate minimal media and tested for growth on YPEG and YPD at room temperature and 37°C. Strain THY4 carrying plasmid pDUY2-12 was also tested for complementation of DNA escape. Furthermore, a whole cell protein extract from this strain was isolated, and Western blotting (immunoblotting) was carried out to show that the altered *YME2* gene is expressed from plasmid pDUY2-12.

Preparation of a polyclonal antibody against Yme2p. A 1.3-kb *Eco*RI fragment of plasmid pYME2 encoding the C-terminal 264 amino acid residues of Yme2p was ligated in frame into the same sites of the glutathione *S*-transferase vector pGEX-3X (Pharmacia) to generate a chimeric gene encoding a glutathione *S*-transferase–Yme2p fusion protein. The resulting construct, pECYME2, was transformed into *E. coli* DH5α, and the fusion protein was induced and isolated as described elsewhere (29). The partially purified fusion protein was used to immunize rabbits as described previously (8).

Preparation of cellular extracts, mitochondrial fractionation, and detection of Yme2p. Whole cell extracts were prepared by using standard protocols (3, 21). Briefly, cells were grown in 5 ml of YPEG to mid-log phase, harvested, and washed in 200 μl of phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, 1.7 mM KH_2PO_4 [pH 7.4]). Cells were lysed by agitation (five cycles of 1 min with 1-min intervals in an ice bath) on a vortex mixer in the presence of 200 μl of acid-washed glass beads (0.45-mm diameter) and 300 μl of PBS plus 0.1 mg of phenylmethylsulfonyl fluoride (PMSF) per ml; 300 μl of PBS plus 0.1 mg of PMSF and 200 μl of electrophoresis sample buffer (33.3% glycerol, 6.6% sodium dodecyl sulfate [SDS], 0.1 M dithiothreitol, 0.2% bromophenol blue) were then added, and samples were boiled for 5 min. Samples were frozen at −80°C or centrifuged for 1 min and loaded immediately onto SDS-polyacrylamide gels.

Isolation of intact mitochondria and mitochondrial subfractions was carried out as previously described (9). Briefly, yeast cells were grown in YPEG to mid-log phase at 30°C, harvested, converted to spheroplasts, homogenized, and separated into a 9,600 × *g* mitochondrial pellet and a postmitochondrial supernatant. Mitochondria were resuspended in TE buffer (10 mM Tris-HCl [pH 7.5], 0.1 mM EDTA) at a protein concentration of 5 mg/ml in the presence of 1 mM PMSF, 1 μg of aprotinin per ml, and 1 μg of leupeptin per ml, sonicated on ice with a Branson Sonifier 250 for five 3-s pulses at an output setting of 80%, and sedimented for 1 h at 3,500 × *g* at 4°C in a Beckman type 65 rotor. Inner and outer mitochondrial particles were then separated on a sucrose gradient (30 to 50%), and membrane fractions were collected as described previously (9).

Protein concentrations of whole cell extracts, mitochondrial vesicles, or mitochondrial subfractions were determined by using the bicinchoninic acid protein assay (Pierce). Protein fractions were resolved on an SDS–8% polyacrylamide gel, which was then equilibrated in transfer buffer (48 mM Tris-HCl, 39 mM glycine, 20% methanol [pH 9.2]) for 20 min. Proteins were electroblotted onto a 0.2-μm-pore-size nitrocellulose membrane (Bio-Rad) at 12 V for 30 min with a Bio-Rad Trans-Blot SD cell. Following transfer, the membrane was stained with 0.5% Ponceau S in 5% acetic acid, destained in 1× Tris-buffered saline (20 mM Tris-HCl, 137 mM NaCl, 3.8 mM hydrochloric acid [pH 7.6]), and then blocked with 5% dried milk powder in 1× TBS-T (Tris-buffered saline plus 0.1% Tween 20) for at least 2 h. Following three 30-min washes in 1× TBS-T supplemented with 1% milk powder, the membrane was decorated with rabbit polyclonal antibodies directed against epitopes in the carboxyl-terminal portion of Yme2p for 1 h at a dilution of 1:2,000 in 1× TBS-T containing 1% milk powder. Membranes were washed three times for 30 min each in 1× TBS-T plus 1% milk powder and then exposed to a secondary antibody, purified goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Amersham Corp., Arlington Heights, Ill.), at a dilution of 1:2,000 for 2 h at room temperature with agitation. Immune complexes were identified with an enhanced chemiluminescence detection system (Amersham Corp.). Immediately after detection of immune complexes, membranes were exposed to autoradiographic film (Kodak).

Where necessary, immune complexes were removed from membranes in stripping buffer (100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl [pH 6.7]) at 50°C for 30 min. Membranes were washed two times for 10 min each in 1× TBS-T and reprobed with a second antibody as described above. The purity of mitochondrial subfractions was confirmed by identifying marker proteins with antibodies to the γ subunit of the F_1-ATPase (Atp3p; provided by M. Douglas) and to the outer mitochondrial membrane protein Omp45p (provided by R. Jensen).

Solubility and membrane topology of Yme2p. To assess the membrane association of Yme2p, 100 μg of inner mitochondrial membrane proteins isolated from PTY44 was centrifuged at 12,000 × *g* for 10 min at 4°C. Mitochondrial membrane pellets were resuspended in 10 μl of either 1.5 M sodium chloride, 100 mM alkaline carbonate (pH 12), 7 M urea, or 0.5% Triton X-100 containing 1 μg of the proteinase inhibitor PMSF (Sigma) per ml. Membranes were solubilized for 30 min on ice and kept in solution by occasional pipetting up and down. Solubilized fractions were centrifuged for 10 min at 12,000 × *g* at 4°C. Equal volumes of supernatant and resuspended pellets were subjected to SDS-polyacrylamide gel electrophoresis (PAGE), and immunoblotting was carried out as follows. Western blots (immunoblots) were probed with an antibody against Yme2p, stripped as outlined above, and reprobed with antibodies (provided by M. Douglas) directed against the integral membrane protein Aac2p (the ADP/ATP carrier protein) and the α subunit of F_1-ATPase, Atp1p, which is loosely associated with the inner mitochondrial membrane.

To determine the orientation of Yme2p in the inner mitochondrial membrane, mitochondria were isolated from wild-type yeast strain PTY44 as described previously (9). Mitochondria were converted to mitoplasts which lack the outer mitochondrial membrane, and both intact mitochondria and mitoplasts were digested with trypsin at a final concentration of 50 μg/ml as described previously (5). One hundred-microgram aliquots of digested mitochondria and digested mitoplasts as well as undigested controls were subjected to SDS-PAGE and analyzed by immunoblotting as described above with antibodies against Yme2p, Atp1p (a gift from M. Douglas), the subunit II of cytochrome B ($Cytb_2p$) (also a gift from M. Douglas), and Omp45p (a gift from R. Jensen).

RESULTS

Phenotypes associated with *YME2* mutations. *YME2* was originally identified as one of six nuclear genes that, when mutated, caused an elevated rate of mtDNA escape and migration to the nucleus (32). Despite extensive efforts, no collateral phenotype was identified for *yme2* strains. Introduction of *yme2* mutations into *yme3*, *yme4*, *yme5*, and *yme6* mutant strains did not result in new synthetic phenotypes. However, in strain backgrounds containing a mutant *yme1* allele, two additional phenotypes were observed. First, *yme2* is able to suppress the cold-sensitive growth phenotype associated with *yme1* (Fig. 1C). Second, *yme1 yme2* double mutants are unable to grow on nonfermentable carbon sources at 30°C (32), while strains containing a single *yme1* or *yme2* mutation show normal growth on YPEG at 30°C (Fig. 1B and C). The growth deficiency of *yme1 yme2* mutant strains on medium at 30°C is leaky, and double-mutant strains grow slowly at this temperature upon prolonged incubation.

Isolation of the *YME2* gene. Cloning of *YME2* on the basis of reversion of mtDNA escape to wild-type levels is extremely difficult because even in wild-type strains, mtDNA escapes to the nucleus at a detectable rate (Fig. 1A). Hence, we used the

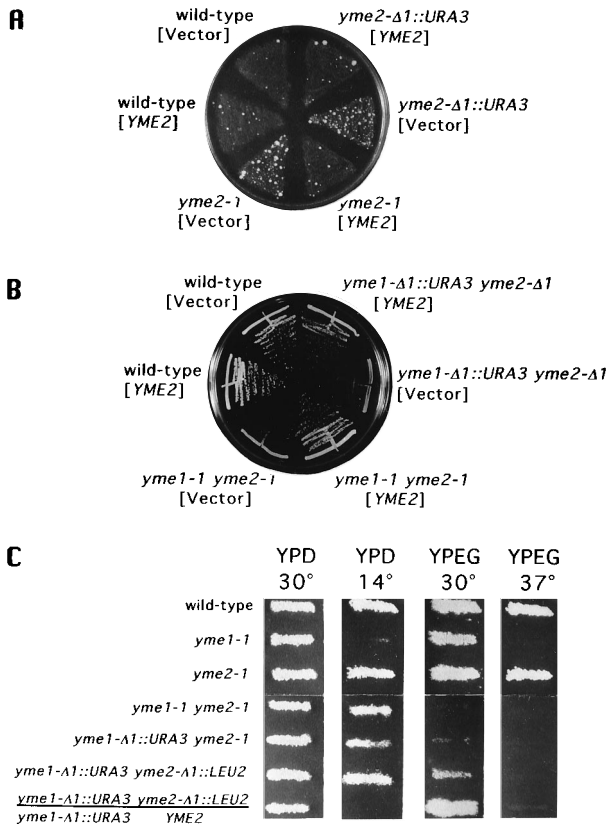

FIG. 1. Complementation of *yme2* and *yme1 yme2* phenotypes with cloned yeast genomic DNA. A wild-type strain (PTY44), a *yme2-1* mutant (PTY64), a *yme2-Δ1::URA3* mutant (THY5), a *yme1-1 yme2-1* double mutant (PTY55), and a *yme1-Δ1::URA3 yme2-Δ1* double-deletion mutant (THY35) were transformed with either the *YME2*-carrying plasmid pLCYME2 or the *LEU2/CEN* vector pRS315. Transformants carrying the plasmids indicated in brackets were analyzed as follows. (A) Detection of DNA escape from mitochondria to the nucleus. Confluent sectors were grown on SD medium supplemented with adenine, uracil, lysine, and tryptophan and printed onto SD medium supplemented with adenine, uracil, and lysine (the plate shown) to detect Trp^+ colonies resulting from the escape of *TRP1* from mitochondria to the nucleus. The plate was photographed after 5 days of incubation at 30°C. (B) Complementation of the growth deficiency of *yme1 yme2* mutants on YPEG at 30°C. Transformants were streaked on YPEG and incubated for 3 days at 30°C. (C) Suppression of the cold-sensitive phenotype of *yme1* by *yme2* mutations. A wild-type yeast strain (PTY44), a *yme1-1* mutant (PTY62), a *yme2-1* mutant (PTY64), a *yme1-1 yme2-1* double mutant (PTY55), a *yme1-Δ1::URA3 yme2-1* double mutant (THY1), a *yme1-Δ1::URA3 yme2-Δ1::LEU2* double-deletion mutant (THY9), and a diploid homozygous for *yme1-Δ1::URA3* and heterozygous for *YME2* (THY9 × PTY60) were streaked onto YPD and incubated at 30°C for 1 day. Cells were printed onto YPEG, and incubation was continued for 3 days at 30 or 37°C. In parallel, cells were printed onto YPD and incubated for 7 days at 14°C.

slow-growth phenotype on YPEG at 30°C of the *yme1-1 yme2-1* double mutant strain PTY55 to clone the wild-type *YME2* gene.

Plasmid DNA from a *CEN* library and, in parallel, from a 2μm library was transformed into PTY55. Approximately 10,000 Ura^+ transformants from each library were grown on minimal SD medium for 5 days at 30°C. Transformants were then replica plated onto YPEG plates and incubated at 30°C. A total of eight transformants (four each from the two libraries) that were able to grow on nonfermentable carbon sources at 30°C were identified. Four of the eight plasmids allowed growth of the *yme1 yme2* double-mutant strains on YPEG at 37°C, suggesting that those plasmids encoded *YME1* or an unspecified suppressing gene. Plasmid DNA was rescued from the four transformants that were able to grow on nonfermentable carbon sources at 30°C, but not at 37°C, and digested with restriction enzymes that recognize sites within the open reading frame of *YME1*. These plasmids had a restriction enzyme pattern that was clearly different from the expected *YME1* restriction enzyme pattern and also had several common restriction fragments. The largest DNA band that was present in these complementing clones was a 6.1-kb *Hin*dIII fragment (Fig. 2A). Overlapping subclones containing subsets of the 6.1-kb *Hin*dIII fragment were generated and, along with the original complementing clones, reintroduced into the *yme2* mutant and the *yme1 yme2* double mutant. A 3.6-kb *Nhe*I-*Bgl*II fragment was the smallest DNA sequence able to complement both the inability of *yme1 yme2* mutants to grow on nonfermentable carbon sources and the high rate of mtDNA escape phenotype of *yme2* strains (Fig. 1A and B).

Linkage of the cloned DNA to *yme2* was established by transforming the *Eco*RI-linearized construct pRS306HB, which contains a 5.0-kb *Hin*dIII-*Bam*HI fragment that spans the complementing region (Fig. 2A), into wild-type yeast cells. The Ura^+ transformant, THY3, was mated to the *yme2-1* strain PTY63, and the resulting diploid was sporulated. There were no recombinants among 12 tetrads scored for uracil prototrophy and DNA escape, indicating that the cloned chromosomal DNA corresponded to the *YME2* locus. The integration of the *URA3* gene at the cloned *YME2* locus was verified by DNA blot hybridization analysis (data not shown).

DNA sequence analysis of *YME2*. DNA sequencing of pRS-R2H2, as well as subclones of this plasmid, demonstrated that *YME2* is identical with *RNA12*. Restriction enzyme maps of the *YME2/RNA12* gene locus obtained from digests with numerous restriction endonucleases are in agreement with published DNA sequence data for this region. This published DNA sequence (GenBank accession number S92205) was used to construct site-directed mutations and additional subclones of *YME2/RNA12*. The *YME2/RNA12* gene is located on chromosome XIII downstream of the *ADH2* gene (17) and upstream of the recently identified *ATM1* gene (16), as shown in Fig. 2A. *RNA12/YME2* has an open reading frame of 2,550 bp that encodes a protein of 850 amino acid residues with an estimated pI of 8.96 and a predicted molecular weight of 96,681.

Sequence analysis of Yme2p did not reveal a typical mitochondrial signal sequence (22) at the amino terminus. However, the first 31 amino acid residues of Yme2p contain a large number of basic residues (pI 12.48) and present an overall hydrophobic stretch that distinguishes it from amino acid residues immediately following the putative leader sequence. Hydrophobicity plots (13) also indicated that a putative transmembrane domain is present between amino acid residues 287 and 305 which is followed by a long stretch of very hydrophilic amino acid residues (Fig. 2B). In addition, there is a rather acidic domain (pI 4.05) at residues 594 to 656 which is prominent in an otherwise alkaline protein (pI 8.96).

Phenotypic effects of *yme2* null alleles. To construct *yme2* null alleles, 2,445 bp of the *YME2* open reading frame and 151 bp of upstream DNA sequences were removed and the selectable marker *LEU2*, *URA3*, or *hisG:URA3:hisG* was inserted. These constructs were introduced into both haploid and diploid yeast strains. Diploids were sporulated, and the DNA escape phenotype of the resulting spores was tested. In each tetrad, the two spores that carried the disrupted *yme2* allele showed elevated levels of DNA escape comparable to the level for the original *yme2* isolate (Fig. 1A).

Strains containing *yme2* null alleles were also mated with strains containing the *yme1-1* point mutant allele or the *yme1* null allele (*yme1-Δ1::URA3*). The resulting diploids as well as

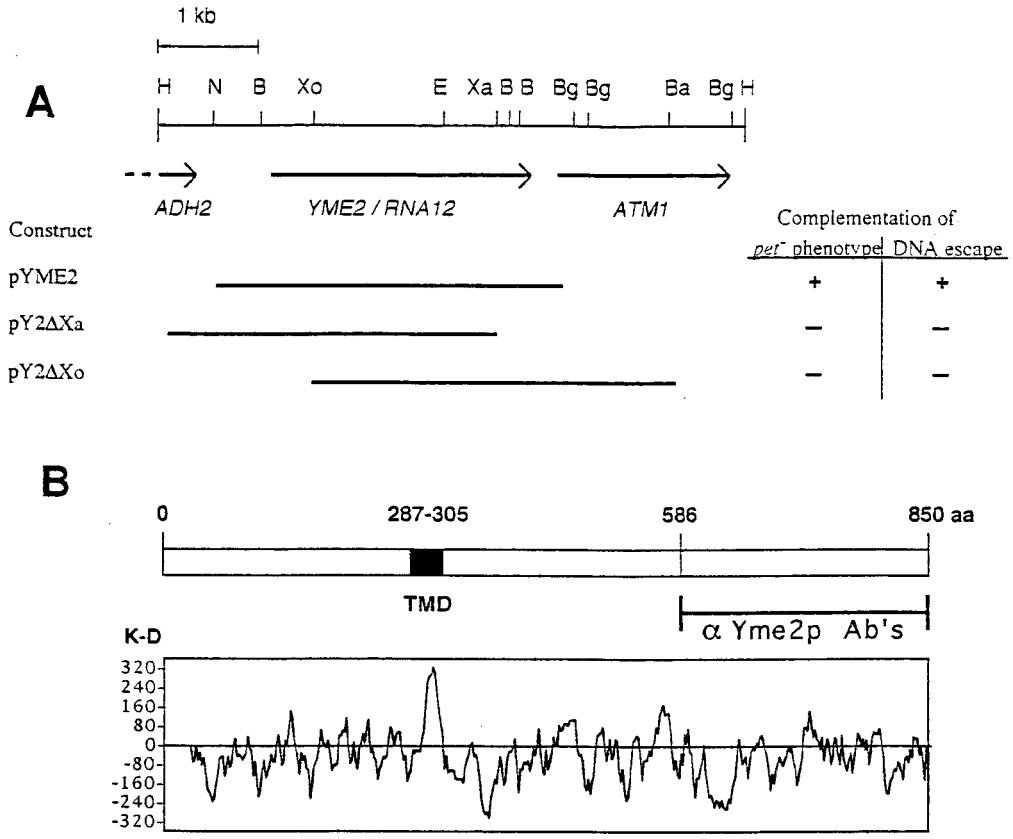

FIG. 2. (A) Physical and restriction map of *YME2/RNA12*. Abbreviations: B, *Bcl*I; Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; N, *Nhe*I; Xa, *Xba*I; Xo, *Xho*I. Restriction enzymes may have more recognition sites within the 6.1-kb *Hin*dIII region than indicated. The orientations and positions of open reading frames are indicated by arrows. DNA sequences that were subcloned into pRS316 are shown as bars, with the respective construct designation shown on the left. These constructs were tested for the ability to complement (+) or not complement (−) the growth deficiency of *yme1 yme2* mutants on nonfermentable carbon sources (*pet*⁻ phenotype) and the high rate of DNA escape of *yme2* mutants. (B) Yme2p has a putative transmembrane domain. Yme2p/Rna12p is shown as a long open block. Numbers above this block indicate the corresponding amino acid residues (aa). A solid block indicates the predicted transmembrane domain (TMD) of Yme2p. The carboxyl-terminal region that was used to produce polyclonal antibodies (Ab's) directed against Yme2p is indicated as a solid bar. A Kyte-Doolittle hydrophobicity plot of Yme2p is shown at the bottom. The hydrophobicity plot was generated by using the computer program GeneWorks 2.2 (IntelliGenetics).

haploid *yme1 yme2* double mutants derived from these crosses were tested for growth on YPEG and YPD at 14, 30, and 37°C (Fig. 1C). Like the original *yme2* mutation, *yme2* null alleles prevent growth on nonfermentable carbon sources at 30°C in a *yme1* background. Furthermore, *yme2* null alleles were able to suppress the cold-sensitive growth phenotype associated with *yme1* (Fig. 1C). Thus, suppression of the synthetic phenotypes of *yme1 yme2* strains is not due to direct interaction between mutant gene products but is caused by the absence of functional Yme1p and Yme2p. Plasmids containing the *YME2* gene are able to complement the *yme2*-induced phenotypes of both the *yme2-1* point mutation and the *yme2-Δ1* null allele (Fig. 1A and B). *YME2* carried on a multicopy plasmid does not generate any detectable phenotype, nor does it suppress any of the phenotypes found in *yme1* strains (data not shown).

Disruption of *yme2/rna12* generates strain-specific phenotypes. Null alleles of *yme2* were generated in several yeast strain backgrounds, including D273-10B, W303, CCY13 (nuclear genome of S288C and mitochondrial genome of PTY44), and FL100. Gene disruptions in the first three strain backgrounds resulted in no growth phenotypes. However, 30 independent transformants of FLC1 (isogenic to FL100), 18 that carried a *yme2*::*URA3* null allele (FLC3) and 12 that carried a *yme2*::*hisG*::*URA3*::*hisG* null allele (FLC5), were unable to utilize ethanol-glycerol (Fig. 3) or galactose (data not shown) as a carbon source. FLC1 transformants containing the *URA3* plasmid pRS316 as a control showed no growth defect. The inability of FLC3 and FLC5 to grow on nonfermentable carbon sources appears to be due to the loss of mitochondrial DNA, since diploid strains derived from crosses between FLC3 and PTY29rho0 or FLC5 and PTY29rho0 were unable to restore the growth defect on nonfermentable carbon sources.

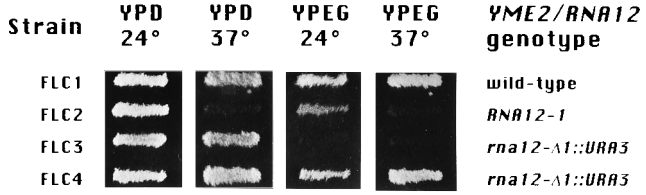

FIG. 3. A *yme2/rna12* disruption generates strain-dependent phenotypes. Deletion of *yme2/rna12* abolishes the *ts*-lethal phenotype caused by the dominant *RNA12-1* allele in FLC2 and creates a growth defect on nonfermentable carbon sources in FLC1. The *yme2/rna12-Δ1*::*URA3* mutation was introduced into two strains, FLC1 to generate FLC3 and FLC2 to generate FLC4. FLC1 is isogenic to FL100. FLC2 was derived from the *RNA12-1* strain but is not isogenic to *RNA12-1* (17). These strains were patched on YPD and incubated at room temperature (24°C) for 1 day. Cells were then printed onto either YPD or YPEG plates, and incubation was continued at 24 or 37°C for 2 to 3 days.

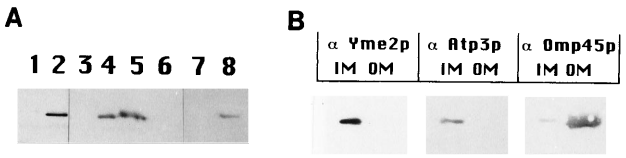

FIG. 4. Cellular localization of Yme2p. (A) Yme2p is a mitochondrial membrane protein, as determined by Western blot analysis. Lanes contained 100 μg of protein from whole cell extracts of the *yme2* deletion strain THY4 (lane 1) and the wild-type strain PTY44 (lane 2), intact mitochondria of THY4 (lane 3) and of PTY44 (lane 4), submitochondrial particles of PTY44 (lane 5), and sonicated supernatant of PTY44 (lane 6). A second immunoblot shows cross-reacting bands with protein fractions of the postmitochondrial supernatant (lane 7) and intact mitochondria (lane 8) from yeast strain FLC2, which carries the mutant *RNA12-1* allele. Both membranes were probed with an anti-Yme2p antibody. (B) Yme2p cofractionates with the inner mitochondrial membrane, as determined by immunoblot analysis of inner mitochondrial membrane fractions (IM) and outer mitochondrial membrane fractions (OM) of the wild-type strain PTY44 with antibodies against Yme2p (α Yme2p), Atp3p (α Atp3p), and Omp45p (α Omp45p).

Cellular localization of Yme2p. Whole cell protein extracts from a wild-type yeast strain and from a *yme2* null allele strain, as well as subcellular protein fractions from the wild-type strain, were separated on an SDS–8% polyacrylamide gel. Subsequent immunoblotting with antisera directed against Yme2p identified a cross-reacting band of about 96 kDa in whole cell extracts from the wild-type strain (Fig. 4A, lane 2). The observed molecular weight of Yme2p was consistent with the predicted molecular weight of 96,687 deduced from the DNA sequence of *YME2/RNA12* (17). A cross-reacting band of the expected molecular weight was also observed in intact mitochondria (lane 4) and submitochondrial particles (lane 5) of the wild-type strain. No cross-reacting band was detected in either the sonicated supernatant (lane 6) or the postmitochondrial supernatant (lane 7) from yeast strain FLC2, which carries the temperature-sensitive *RNA12-1* allele.

Submitochondrial particles from PTY44 were separated into inner and outer membrane fractions over a sucrose gradient, and 5 μg of protein from each fraction was subjected to immunoblot analysis with an anti-Yme2p antiserum. Cross-reacting protein was detected only in the inner membrane fraction (Fig. 4B). The Western blot was stripped and reprobed first with an anti-Atp3p antibody (20, 36) and then with an anti-Omp45p antiserum (37). The correct partitioning of Atp3p and Omp45p (Fig. 4B) demonstrates the purity of the inner and outer mitochondrial membrane fractions. Although long exposures of protein blots probed with the anti-Omp45p antiserum resulted in a faint cross-reacting band of 45,000 Da in the inner membrane fraction, suggesting that the inner membrane fraction was slightly contaminated with outer membrane proteins (Fig. 4B), this minute contamination has no effect on the observation that Yme2p is solely located in the inner membrane.

To test whether Yme2p is an integral membrane protein or loosely associated with the membrane, protein fractions from the inner mitochondrial membrane of wild-type yeast strain were extracted with either 1.5 M sodium chloride, 100 mM sodium carbonate (pH 12) (10), or 1% deoxycholate and subjected to immunoblotting with an anti-Yme2p antiserum. Yme2p was not extracted from the membrane when treated with 1.5 M NaCl or 100 mM sodium carbonate; however, the detergent deoxycholate solubilized the protein (Fig. 5). The same blot was stripped and reprobed with an antibody which recognizes the integral inner membrane protein Aac2p (15). The Western blot was stripped once again and then reprobed with an antiserum directed against a peripheral inner membrane protein, Atp1p. Atp1p, in contrast to Aac2p and Yme2p,

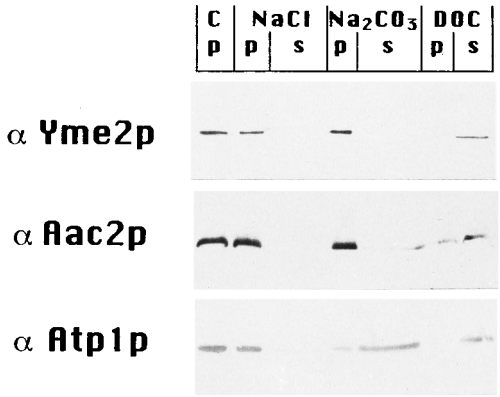

FIG. 5. Yme2p is an integral membrane protein, as determined by immunoblot analysis of inner mitochondrial membrane fractions of the wild-type yeast strain PTY44 after extraction with 10 mM Tris-HCl (pH 7.4) (C), 1.5 M sodium chloride (NaCl), 0.1 M sodium carbonate (pH 12) (Na_2CO_3), and 1% deoxycholate (DOC). Membrane extractions were pelleted, and the supernatant was removed. Pellets were resuspended in 10 mM Tris-HCl (pH 7.4) to obtain the same volume as was present prior to centrifugation. Equal volumes of supernatant (s) and resuspended pellet (p) of each extraction were loaded onto SDS-polyacrylamide gels. The same Western blot was probed with antibodies against Yme2p (α Yme2p), Aac2p (α Aacp2), and Atp1p (α Atp1p).

was washed off the membrane with 100 mM sodium carbonate. Yme2p behaves as an integral inner mitochondrial membrane protein.

Topology of Yme2p in the inner mitochondrial membrane. The putative transmembrane domain (Fig. 2B) divides Yme2p into a smaller amino terminus (about one-third of the protein) and a larger carboxyl terminus. The antiserum to Yme2p recognizes only epitopes at the carboxyl terminus of Yme2p (Fig. 2B), which allowed us to determine the orientation of Yme2p within the inner mitochondrial membrane. Intact mitochondria and mitoplasts, digested with trypsin, and undigested controls were subjected to immunoblot analysis with an anti-Yme2p antibody (Fig. 6). When intact mitochondria were treated with trypsin, Yme2p was not digested. This result is consistent with the predicted location of Yme2p in the inner mitochondrial membrane. Trypsin digestion of mitoplasts (mitochondria that lack the outer mitochondrial membrane) resulted in degradation of Yme2p (Fig. 6). Thus, we conclude that the larger carboxyl terminus of Yme2p faces the intermembrane space.

To demonstrate that mitoplasts with an intact inner mitochondrial membrane were generated, Western blots were stripped and reprobed with antisera directed against Atp1p

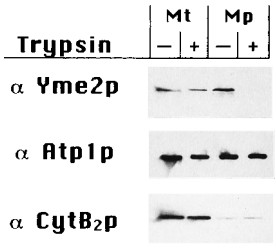

FIG. 6. The carboxyl terminus of Yme2p faces the intermembrane space in mitochondria, as determined by Western blot analysis of intact mitochondria (Mt) and mitoplasts (Mp) from wild-type yeast strain PTY44. Mitochondria and mitoplasts were digested with trypsin at a final concentration of 50 μg/ml (+) or not digested (–). The membrane was probed with antibodies directed against Yme2p (α Yme2p), Atp1p (α Atp1p), and $CytB_2$p (α $CytB_2$p).

and $CytB_2$p. Digestion of mitoplasts with trypsin had no effect on Atp1p, which is located within the mitochondrial matrix and is associated with the inner mitochondrial membrane (30). $CytB_2$p, which is located in the intermembrane space (11), was largely absent in the undigested mitoplast control (Fig. 6). These results demonstrate that in the mitoplast preparation, the outer mitochondrial membrane had been removed and the inner mitochondrial membrane remained intact.

Site-directed mutagenesis of *YME2*. It has been reported (17) that an A-to-T transition causes substitution of an asparagine by a tyrosine at amino acid 502 within Rna12p and is responsible for the dominant *ts* growth defect on rich glucose medium of strains carrying the *RNA12-1* allele. We reconstructed this mutation in *YME2* by using site-directed mutagenesis to generate a new allele, designated *yme2-12* (see Materials and Methods). The point mutation at amino acid residue 502 in *yme2-12* was confirmed via DNA sequencing across the mutagenized area. Plasmid pDUY2-12, which contains the *yme2-12* allele, was transformed into a diploid wild-type yeast strain (PTY33 × PTY44). Eight independent transformants were tested for growth on YPD at 37°C. None of these transformants possessed a *ts*-lethal phenotype. The genomic background of PTY33 × PTY44, which was derived from D273-10B, is very different from that of FL100, in which the original *RNA12-1* mutation was identified (14). Thus, the *yme2-12*-bearing plasmid pDUY2-12 was introduced into FLC1, a *ura3* derivative of FL100. The engineered *yme2-12* gene did not result in a *ts* phenotype. Immunoblot analysis of cellular extracts bearing a deletion of the chromosomal *yme2* locus and containing pDUY2-12 verified that Yme2p was produced (data not shown).

Unidentified differences in the DNA sequences of *YME2* and *RNA12*, which were isolated from genomic libraries constructed from yeast strains GRF88, an S288C derivative (23), and FL100 (17), respectively, may be responsible for the absence of a dominant *ts* growth defect of strains containing the artificially mutagenized *yme2-12* allele. However, plasmid FL38 (a gift from F. Lacroute), which contains the wild-type *RNA12* gene, is able to complement the growth defect of a *yme1 yme2* mutant on nonfermentable carbon sources at 30°C, suggesting that any speculative DNA sequence differences do not impair the biological function of *YME2*/*RNA12*. Additionally, we have obtained DNA sequence data for a segment of approximately 1,000 nucleotides of the *YME2* gene which is 99% identical with the published *RNA12* DNA sequence. Thus, a second, unidentified mutation in *RNA12-1* may also contribute to its *ts*-lethal phenotype.

Despite these unsuccessful efforts to reconstruct the dominant *ts*-lethal phenotype of *RNA12-1* in both our strain background and FL100, the *RNA12-1* mutation is apparently responsible for the *ts* growth defect. First, analysis of 22 full tetrads from two independent crosses between the *RNA12-1* mutant strain (FLC2) and a strain bearing a *yme2* null allele marked with either *URA3* or *LEU2* showed that the selectable markers always segregated away from the *ts* growth defect associated with *RNA12-1*. This finding suggests that *RNA12* and *YME2* are identical or at least very tightly linked. Second, to confirm that the *ts*-lethal phenotype was indeed caused by *RNA12-1*, we deleted the dominant *RNA12-1* allele in yeast strain FLC2. All 16 independent transformants of the resulting *yme2-Δ1*::*URA3* deletion strain (FLC4) that were tested lost the *ts* growth defect (Fig. 3), while FLC2 transformants carrying the control vector pRS316 were unable to grow at the restrictive temperature.

DISCUSSION

Inactivation of *YME2* in *S. cerevisiae* is characterized by three phenotypes: a high rate of DNA escape from mitochondria to the nucleus, an inability to grow on nonfermentable carbon sources at 30°C in combination with a *yme1* mutation, and the ability to suppress the cold-sensitive growth phenotype of *yme1* strains (32). There appears to be no phenotypic difference between the original *yme2* point mutation and artificially constructed null alleles of *yme2*. A number of observations, including DNA sequence data and linkage analysis, demonstrate that *YME2* is identical with *RNA12*. *RNA12-1* is a dominant *ts* mutant which exhibits a defect in RNA accumulation at 36°C (14). At 32°C the *RNA12-1* mutant is recessive, which made it possible to clone the wild-type *RNA12* gene by complementation of the *ts* phenotype (17). In contrast to the severe phenotype of the *RNA12-1* mutation, null alleles of *RNA12* constructed by Liang and coworkers had no discernible growth defect (17). Although the exact function of *YME2* is unknown, our results favor a direct role for *YME2*/*RNA12* in mitochondrial function and the maintenance of mitochondrial compartment integrity. Any effect that the *RNA12-1* mutation has upon the accumulation and maturation of pre-rRNA molecules (14, 17) is most likely the result of an indirect effect due to altered mitochondrial metabolism at the restrictive temperature.

Several pieces of evidence support a role in mitochondrial function for *YME2*. First, data obtained from Western blots show that Yme2p is exclusively located in the inner mitochondrial membrane, with the large COOH terminus facing the intermembrane space. Yme2p/Rna12p produced in strains containing the *ts RNA12-1* allele is also localized to mitochondria (Fig. 4). Independent of our results, a large-scale screen that allows rapid cellular localization of *lacZ*-tagged yeast genes also revealed that Yme2p/Rna12p is exclusively located in the mitochondrial compartment (6). The localization of Yme2p/RNA12p is inconsistent with a direct role in cytoplasmic pre-rRNA maturation. Second, *yme2* mutations have genetic interactions with mutations that inactivate the nuclear gene *YME1*, which also encodes a mitochondrial protein. Mutations in *yme2* are able to suppress the cold-sensitive growth phenotype of *yme1*. Furthermore, growth of *yme1* strains on nonfermentable carbon sources medium at 30°C is largely prohibited in a *yme2* background. Despite the synthetic phenotypes of *yme1 yme2* double mutants, the corresponding gene products do not appear to interact with each other, as assessed by coimmunoprecipitations using affinity-purified antibodies directed against Yme2p and by two-hybrid analysis (data not shown). Third, disruption of *yme2*/*rna12* in an FL100 background results in an apparent loss of mitochondrial DNA and thus an inability to utilize nonfermentable carbon sources. Fourth, the dominant *ts*-lethal *RNA12-1* mutant shows reduced growth on ethanol-glycerol medium at any temperature. This growth defect appears to be due to a dominant effect of the *RNA12-1* allele rather than the loss of part of the mitochondrial genome, since revertants to wild-type growth that are capable of growth on nonfermentable carbon sources appear frequently (data not shown). It seems most likely that the effects of *RNA12-1* on RNA accumulation at the restrictive temperature are in response to the interference with important metabolic activities occurring within mitochondria. The addition of a number of different growth inhibitors to yeast cultures that block cells at Start (34) or at random points in the cell cycle (28) can inhibit synthesis, cleavage, and methylation of pre-rRNA, suggesting that alteration of rRNA metabolism may be a general response to the arrest of cell growth.

There are no significant, full-length sequence similarities of Yme2p to any known proteins that would allow the prediction of a biological function for Yme2p. However, some regions of homology exist between Yme2p and small heat shock proteins (27). Whatever the biochemical function of Yme2p, it is likely to be interesting as there is a relative scarcity of dominant *ts* mutations that exert their effects through changes in mitochondrial function. One class of mutations that might cause such severe phenotypes includes genes that prohibit segregation of mitochondria to daughter cells, since the presence of mitochondrial compartments is an absolute requirement for cell viability (19). However, our unpublished observations in a study using fluorescence microscopy of 2-(4-dimethylaminostyryl)-*N*-methylpyridinium iodide (DAPSMI)-stained mitochondria (7) from the *RNA12-1* mutant strain FLC2 grown at the permissive and restrictive temperatures showed no obvious defects with respect to mitochondrial morphology or segregation of mitochondria to daughter cells, nor did any of the *yme2* mutations reveal any morphological abnormalities of the mitochondrial compartment. It thus appears to be unlikely that the *RNA12-1* gene product interferes with the segregation of mitochondria during cell division when cells are incubated at the restrictive temperature. A second class of mutations that can prevent cell growth include genes whose products are necessary for the import of proteins into mitochondria (4). Interference of protein import by the product of the *ts RNA12-1* allele would prevent cell growth. However, if Yme2p/Rna12p does associate with the protein import apparatus in some fashion, it must be largely dispensable because *yme2* null alleles are viable. The actual role of Yme2p in protein import, if any, remains to be determined.

ACKNOWLEDGMENTS

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