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Inactivation of *YME1*, a Member of the *ftsH-SEC18-PAS1-CDC48* Family of Putative ATPase-Encoding Genes, Causes Increased Escape of DNA from Mitochondria in *Saccharomyces cerevisiae*

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The yeast nuclear gene *YME1* was one of six genes recently identified in a screen for mutations that elevate the rate at which DNA escapes from mitochondria and migrates to the nucleus. *yme1* mutations, including a deletion, cause four known recessive phenotypes: an elevation in the rate at which copies of *TRP1* and *ARS1*, integrated into the mitochondrial genome, escape to the nucleus; a heat-sensitive respiratory-growth defect; a cold-sensitive growth defect on rich glucose medium; and synthetic lethality in *rho*$^-$ (cytoplasmic petite) cells. The cloned *YME1* gene complements all of these phenotypes. The gene product, Yme1p, is immunologically detectable as an 82-kDa protein present in mitochondria. Yme1p is a member of a family of homologous putative ATPases, including Sec18p, Pas1p, Cdc48p, TBP-1, and the FtsH protein. Yme1p is most similar to the *Escherichia coli* FtsH protein, an essential protein involved in septum formation during cell division. This observation suggests the hypothesis that Yme1p may play a role in mitochondrial fusion and/or division.

The intracellular movement of DNA from mitochondria to the nucleus can be experimentally detected in strains of *Saccharomyces cerevisiae* that contain nuclear genetic markers in their mitochondrial genomes (43, 44). This phenomenon is of interest since it has probably played a role in the transfer of genetic information from mitochondrial to nuclear genomes that has occurred during eucaryotic evolution (10, 13, 19, 25, 31, 47). Equally if not more importantly, an examination of the mechanism by which DNA escapes from mitochondria should provide a novel avenue for identifying and studying factors involved in maintaining the integrity of mitochondria during the dynamic processes of cell division and fusion.

To study genetically the mechanism by which DNA escapes mitochondria, we devised a screen for mutations that elevate the rate at which escape occurs (44). A fragment of yeast nuclear DNA bearing the *TRP1* gene and its associated origin of nuclear DNA replication, *ARS1*, was integrated into a fully functional mitochondrial chromosome. Strains containing the mitochondrially located *TRP1* gene and a nuclear *trp1* deletion were phenotypically tryptophan auxotrophs (Trp$^-$). However, such strains produced Trp$^+$ derivatives which contained the *TRP1-ARS1* fragment and portions of mitochondrial DNA (mtDNA) replicating as plasmids in their nuclei. To identify mutations affecting the rate of escape, we screened for elevated production of Trp$^+$ derivatives. Twenty-one recessive nuclear mutations that fell into six complementation groups, designated *YME1* through *YME6* (for yeast mitochondrial escape), were obtained.

The *YME1* gene is of particular interest since *yme1* mutations cause a heat-sensitive defect in respiratory growth and a cold-sensitive defect in growth on fermentable carbon sources (44). In addition, as reported here, *yme1* mutations cause lethality in *rho*$^-$ (cytoplasmic petite) cells, thereby generating respiration-competent (at temperatures of 30° or below) petite-negative strains of *S. cerevisiae*. We also describe here the isolation and nucleotide sequence of *YME1* and the detection of its product, Yme1p, in mitochondria. Yme1p has significant homology to a family of putative ATPases whose members are involved in processes ranging from organelle biogenesis to gene expression to cell division. Yme1p appears to be most related to the *Escherichia coli* FtsH protein, an essential protein involved in septum formation during cell division (46).

MATERIALS AND METHODS

Strains, strain constructions, and genetic methods. The *E. coli* strain used for preparation and manipulation of DNA was DH5α [F$^-$ *endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1* λ *recA gyrA96 relA1* Δ(*argF-lacZYA*) *U169* φ80 *lacZ*ΔM15].

The genotypes of the *S. cerevisiae* strains used in this work are listed in Table 1. Standard genetic techniques were used to construct and analyze the various yeast strains (38).

Media. *E. coli* containing plasmids was grown in LB (10 g of Bacto Tryptone, 10 g of NaCl, and 5 g of yeast extract per liter) plus 125 μg of ampicillin per ml. Yeast strains were grown in complete glucose medium (YPD medium), complete ethanol-and-glycerol medium (YPEG), or minimal glucose medium plus the indicated nutrients (SD medium) (44). Ampicillin and nutrients were obtained from Sigma.

Isolation of *yme1*-complementing plasmids. The temperature-sensitive respiratory-growth phenotype caused by the *yme1-1* mutation was used as the basis for cloning *YME1*. PTY62 was transformed (18) with 70 μg of DNA prepared from a YCp50-based *S. cerevisiae* genomic DNA bank (34). Twelve thousand Ura$^+$ colonies grew on minimal SD medium after incubation for 4 days at 30°C. The transformants were prewarmed at 37°C for 1 h and then replica plated onto YPEG plates and incubated at 37°C. Three transformants were able to grow at the restrictive condition in a plasmid-dependent fashion. Total yeast DNA was prepared from

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
PTY33	*MATa ade2 ura3-52 leu2-3,112 trp1-Δ1 [rho⁺ TRP1]*	44
PTY33×PTY44	*MATa ade2 LYS2 ura3-52 leu2-3,112 trp1-Δ1 [rho⁺ TRP1]* *MATα ADE2 lys2 ura3-52 leu2-3,112 trp1-Δ1*	This study
PTY44	*MATα lys2 ura3-52 leu2-3,112 trp1-Δ1 [rho⁺ TRP1]*	44
PTY51	*MATa ade2 ura3-52 leu2-3,112 trp1-Δ1 YME1::URA3 [rho⁺ TRP1]*	This study
PTY52	*MATα lys2 ura3-52 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 [rho⁺ TRP1]*	This study
PTY62	*MATα, lys2 ura3-52 leu2-3,112 trp1-Δ1 yme1-1 [rho⁺ TRP1]*	44
PTH78	*MATa ade2-101 ura3-52 pet123::URA3*	16
LSF236	*MATa ade2 ura3-52 trp1-Δ1 pet494-41*	L. S. Folley
TF197	*MATα, leu2-3,112 ura3-52 yme1-1 [rho⁺ TRP1]*	This study
A236-57B	*MATa, leu2-3 trp1 met4 aro7 his3 lys11 SUC2 MAL3 can1*	12
X12-6B	*MATa, rad1-1 ade2-1 gal2*	YGSC[a]

[a] YGSC, Yeast Genetic Stock Center, Berkeley, Calif.

these three transformants and used to transform *E. coli*. Two of the rescued plasmids were identical (pPT31 in Fig. 2), and the third contained a smaller but overlapping insert (pPT32 in Fig. 2).

Nucleic acid techniques and plasmid constructions. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories and New England Biolabs. Standard techniques for generating recombinant DNAs and performing DNA blot hybridizations were used (26).

Plasmids pPT31ΔX and pPT31ΔB (see Fig. 2) were constructed by digestion of pPT31 with *Xba*I and *Bam*HI, respectively, followed by gel purification of the large restriction fragment and subsequent recircularization with T4 ligase. Both pPT31ΔX and pPT31ΔB are YCp50-based CEN plasmids. Plasmids pPT34 and pPT35 are, respectively, *Sal*I and *Bam*HI fragments of pPT32 that were gel purified and ligated into their corresponding sites in the polylinker of the *URA3 CEN* vector pRS316 (40). Plasmid pPT49 was constructed by digesting pPT32 with *Mlu*I and *Eco*RI and then purifying the 3-kb insert fragment and ligating it to the vector fragment created by digesting pPT34 with *Mlu*I and *Eco*RI. Five kilobase pairs of yeast DNA plus 378 bp of vector sequence contained in this plasmid was excised by digestion with *Xho*I and *Eco*RI and ligated into the same sites of pRS315, a *LEU2 CEN* vector (40), to create pPT49.

The *yme1-Δ1::URA3* mutation was constructed as follows. pPT42 DNA was digested with *Hpa*I, removing 1,370 nucleotides of internal *YME1* sequence. pPT42 has the same insert DNA as pPT49 in the corresponding sites in the polylinker of pBluescript KS⁺. In place of these nucleotides was ligated a 1.5-kb *Sma*I fragment containing the *URA3* gene, generating pPT45 (see Fig. 2). Sixteen micrograms of pPT45 DNA was digested with *Bgl*II and *Hin*dIII, and this was used to transform the yeast diploid PTY33×PTY44 to uracil prototrophy. A Ura⁺ transformant was sporulated, and tetrads were dissected. A spore from one of those tetrads is PTY52.

The DNA sequence was determined on double-stranded templates by the nucleotide chain termination method (37). Templates were generated from pPT34 and pPT35 by subcloning with restriction enzymes and exonuclease III-generated deletions. Several gaps in the sequence were closed by having oligonucleotides synthesized and using them as primers.

Detection of Yme1p and mitochondrial fractionation. A 1.5-kb *Hpa*I-*Eco*RI fragment of DNA, encoding the carboxy-terminal 105 residues of Yme1p, was ligated into the glutathione *S*-transferase vector pGEX-3X (Pharmacia) to generate a chimeric gene encoding a glutathione *S*-transferase–Yme1p fusion protein. The resulting plasmid, pPT44, was transformed into *E. coli* DH5α, and the fusion protein was induced and isolated as described previously (41). The fusion protein was used to immunize rabbits as previously described (5).

Total protein extracts were prepared from YPEG-grown yeast cells. Cells were collected and washed once in 1 M sorbitol–100 mM EDTA, pH 7.5. Cells were resuspended in 0.05 times the original volume of the same buffer with 160 μg of Zymolyase 20T (ICN Immunobiologicals, Inc.) per ml and incubated for 3 h at 37°C. The spheroplasted cells were pelleted in 10 pellet volumes of 50 mM Tris–20 mM EDTA–1% sodium dodecyl sulfate (SDS), pH 7.4, and heated at 65°C for 30 min. The protein extracts were then frozen at −80°C until needed. A crude cell extract, purified mitochondria, and postmitochondrial supernatant were prepared from YPEG-grown PTY44 yeast cells essentially as described by Yaffe (50). Briefly, cells were spheroplasted by treatment with Zymolyase and broken in a Dounce homogenizer. Unbroken cells and debris were removed by centrifugation at 3,000 × *g*. A crude mitochondrial pellet was generated by centrifuging the extract at 9,500 × *g* and collecting the pellet. The supernatant from this spin constituted the postmitochondrial supernatant. Mitochondria were washed by twice resuspending the mitochondrial pellet and pelleting the mitochondria again at 9,500 × *g*. Mitochondria were purified further by banding them in a Percoll density gradient.

Protein fractions were subjected to SDS-polyacrylamide gel electrophoresis as described elsewhere (23). Protein-containing fractions were separated on a 10% gel and electroblotted at 15 V for 45 min to 0.2-μm nitrocellulose (Bio-Rad) by using a Bio-Rad Trans-Blot SD cell. The transfer buffer was 48 mM Tris–39 mM glycine, pH 9.2. After transfer, the filter was stained with Ponceau S in 5% acetic acid and subsequently destained with distilled water. The filter was blocked with 20 mM Tris–500 mM NaCl–3% gelatin, pH 7.5. The filter was then washed in 20 mM Tris–500 mM NaCl–0.05% Tween 20, pH 7.5. The rabbit antisera were diluted 1:200 in the wash solution containing 1% gelatin and incubated with the filter for 1 h at 37°C with shaking. Immune complexes were identified by reaction with a secondary antibody, purified goat anti-rabbit immunoglobulin G heavy-plus-light-chain gold conjugate (Bio-Rad), for 16 h at 37°C with shaking.

Nucleotide sequence accession number. The sequence for *YME1* has been assigned GenBank accession number L14616.

RESULTS

Phenotypes associated with *YME1* mutations. Among the six genes identified in our previous screen for mutations affecting the rate at which mtDNA escapes to the nucleus, *YME1* was of particular interest since *yme1* mutations caused several phenotypes. In addition to having an increased rate of mtDNA escape (Fig. 1A), *yme1* strains were also unable to grow on nonfermentable carbon sources at 37°C and showed significantly decreased growth rates on rich medium containing glucose or galactose at 14°C (Fig. 1B).

During the preliminary phenotypic characterization of *yme1* mutants (44), we found that there was no elevation in the frequency of cytoplasmic petite, or *rho*$^-$, mutants. Indeed, a more careful examination suggested that the *yme1-1* mutation might actually prevent accumulation of *rho*$^-$ mutants: among 14,000 clones of a *yme1-1* mutant strain, no *rho*$^-$ colonies were found, in contrast to an isogenic wild-type strain, in which approximately 1% of the cells were *rho*$^-$.

The incompatibility of the *yme1-1* mutation with *rho*$^-$ mutations was confirmed in two ways. First, *yme1-1* strains could not grow on medium containing a 25-μg/ml concentration of ethidium bromide (Fig. 1C), a drug that very efficiently induces *rho*$^-$ mutations (14). Second, the *yme1-1* allele caused inviability in strains that also carried a null *pet123* mutation, which is known to destabilize *rho*$^+$ mtDNA (16). The *yme1* mutant strain PTY62 was crossed with the *pet123*::*URA3* strain PTH78, and the resulting diploids were sporulated. Twenty tetrads were dissected on YPD medium and scored for growth, respiratory ability, and uracil prototrophy (to follow segregation of *pet123*::*URA3*). One-quarter of the spores did not yield viable haploids, although they germinated and formed microcolonies. In all cases the viable spores either were wild type or contained the *yme1-1* or *pet123*::*URA3* mutation, but not both.

The *yme1-1* mutation was not simply incompatible with unconditional respiratory defects. This was demonstrated by crossing a *yme1-1* strain (PTY62) and a strain (LSF236) carrying a *pet494* mutation, which prevents respiration but does not destabilize *rho*$^+$ mtDNA (8, 29). The viability of spores from this cross was normal, and one-quarter of the progeny were double mutants. Thus, *yme1-1* appears to be specifically incompatible with *rho*$^-$ (cytoplasmic petite) mutations and effectively produces respiration-competent (at temperatures of 30°C or below) petite-negative strains of *S. cerevisiae*.

Isolation of yeast genomic DNA that complements *yme1* mutations. The yeast strain PTY62, bearing the *yme1-1* and *ura3-52* mutations, was transformed with the YCp50-based genomic library of Rose et al. (34). About 12,000 transformants were obtained, replica plated to YPEG, and incubated at 37°C. Three transformants were capable of growth on YPEG at 37°C. Total DNA was prepared from these transformants, and their plasmids were recovered by transformation of *E. coli*. Reintroduction of these plasmids into PTY62 demonstrated that they complemented the heat-sensitive respiration phenotype of *yme1-1*. Restriction analysis of the plasmids revealed that two of the recovered plasmids were identical (pPT31 in Fig. 2). The third plasmid, pPT32, contained a somewhat smaller insert from the same region of

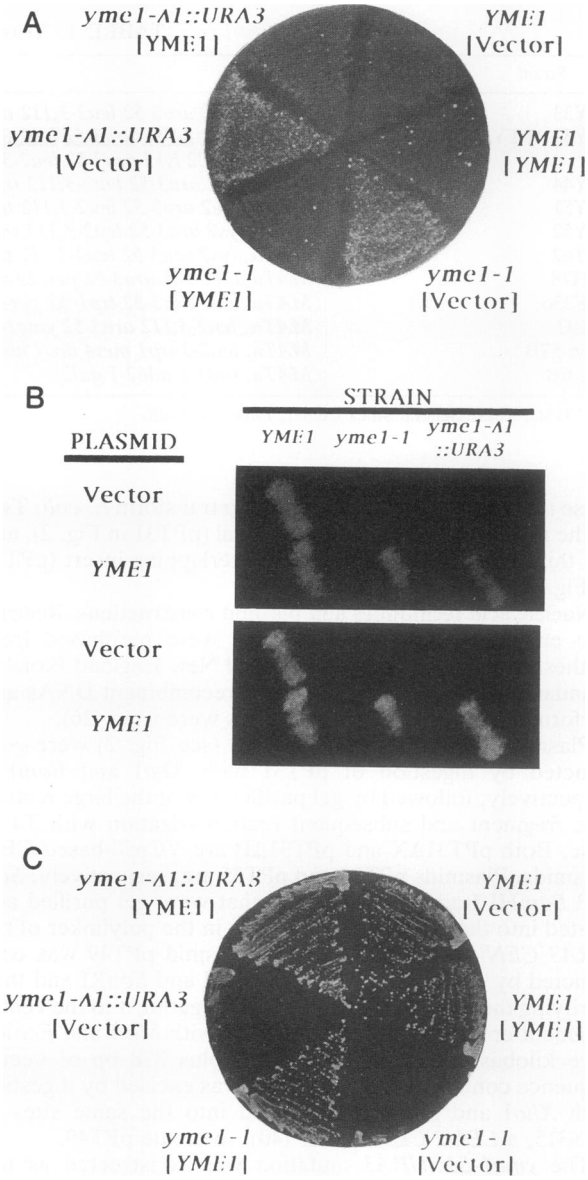

FIG. 1. Complementation of *yme1* phenotypes with cloned yeast genomic DNA. A *YME1* strain (PTY44), a *yme1-1* mutant (PTY62), and a *yme1-Δ1*::*URA3* mutant (PTY52) were transformed with either the *YME1*-carrying plasmid pPT49 or the vector pRS315. The transformants, indicated with the plasmid in brackets when appropriate, 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 replica plated to SD medium supplemented with adenine, uracil, and lysine (the plate shown) to detect Trp$^+$ colonies resulting from the escape of *TRP1* from mitochondria. The plate was photographed after 5 days of incubation at 30°C. (B) Respiratory growth at 37°C and growth on rich glucose medium at 14°C. Cells patched onto YPD medium were printed to YPEG and incubated for 2 days at 37°C (upper panel) and printed to YPD medium and incubated for 7 days at 14°C (lower panel). (C) Growth on glucose in the presence of ethidium bromide. The cells were streaked to SD medium containing 25 μg of ethidium bromide per ml, adenine, uracil, lysine, leucine, and tryptophan and incubated at 30°C for 3 days. Cells were picked from isolated colonies (for strains containing *YME1*) or from the area of heaviest growth (for *yme1* strains), streaked to the same medium, and incubated at 30°C for 4 days to give the growth shown. We have found it necessary to reculture strains several times in the presence of ethidium bromide to ensure the generation of *rho*$^-$ or *rho*^{0} mitochondrial genomes.

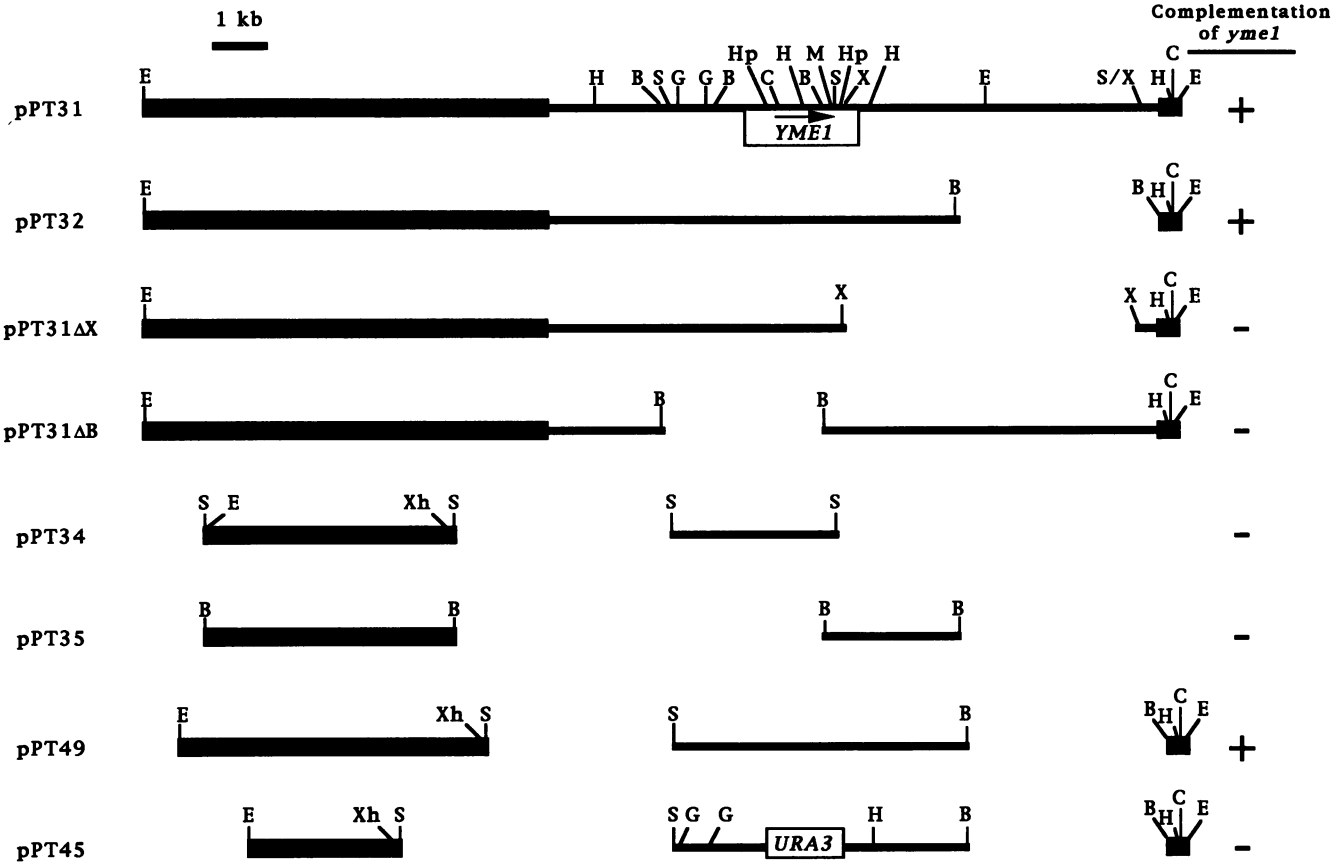

FIG. 2. Restriction map, complementation analysis, and gene disruption of the *YME1* region. The thick lines represent vector sequences, and the thinner lines represent yeast genomic sequences. The genomic sequences in the various subclones are aligned. The position and orientation of the *YME1* open reading frame are indicated on plasmid pPT31 by the box and arrow, respectively. Plasmids pPT31, pPT32, pPT31ΔX, and pPT31ΔB are YCp50-based plasmids. Plasmids pPT34 and pPT35 are pRS316 based, and pPT49 is pRS315 based. The insert in plasmid pPT45 is in pBluescript KS+. pPT45 was used in a one-step gene replacement at the *YME1* locus. When the resulting locus was placed in *trans* to *yme1-1* in a diploid, it did not complement, so this plasmid has been indicated as noncomplementing. Restriction endonuclease cleavage sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; Hp, *Hpa*I; M, *Mlu*I; S, *Sal*I; X, *Xba*I; Xh, *Xho*I.

the genome. Subsequent subcloning and transformation of PTY62 defined the complementing region of DNA more accurately (Fig. 2). The subcloned segment of DNA in plasmid pPT49 complemented all four phenotypes associated with the *yme1-1* mutation (Fig. 1).

Genetic mapping of the *YME1* locus. A 3.5-kb *Cla*I fragment from pPT32, containing much of the *YME1* gene and some 3′ sequence, was introduced into an integrating *URA3* vector. That plasmid, pPT37, was linearized with the restriction endonuclease *Mlu*I and used to transform the wild-type strain PTY33. The Ura$^+$ transformant, PTY51, was mated to the *yme1-1* strain TF197, and the resulting diploid was sporulated. There were no recombinants among 18 tetrads scored for uracil prototrophy and heat-sensitive respiratory growth, indicating that the cloned chromosomal DNA corresponded to the *YME1* locus.

The plasmid pPT35 was used to probe a chromosome blot of *S. cerevisiae* DNA. The probe was found to hybridize to the filter only at the position of chromosome 16 (data not shown). Meiotic-mapping data for *YME1*, from tetrad analysis, is summarized in Table 2. *YME1* is located on the right arm of chromosome 16 about 16 centimorgans from the centromere and about 35 centimorgans from *aro7*.

Primary sequence analysis of *YME1*. Dideoxynucleotide chain termination reactions were used to determine the sequence of 2,839 nucleotides on both strands (Fig. 3). This sequence has a single open reading frame that encodes a protein of 747 amino acids with a predicted relative molecular weight of 81,679. The sequence of the predicted protein, Yme1p, was used to search sequence data bases. We found significant sequence homology to a family of proteins that

TABLE 2. Tetrad analysis of linkage between markers in the *YME1* region of chromosome 16[a]

Interval	Ascus type (no. of asci)			Segregation (no. of asci)[b]		Distance (cM)
	PD	NPD	T	FD	SD	
yme1-aro7[c]	33	2	45			35
yme1-CEN16[c]				53	24	16
yme1-rad1[d]	21	5	37			53

[a] PD, parental ditype; NPD, nonparental ditype; T, tetratype; FD, first division; SD, second division; cM, centimorgans.
[b] Scored relative to the centromere-linked marker *trp1*.
[c] From the cross TF197 × A236-57B.
[d] From the cross TF197 × X12-6B.

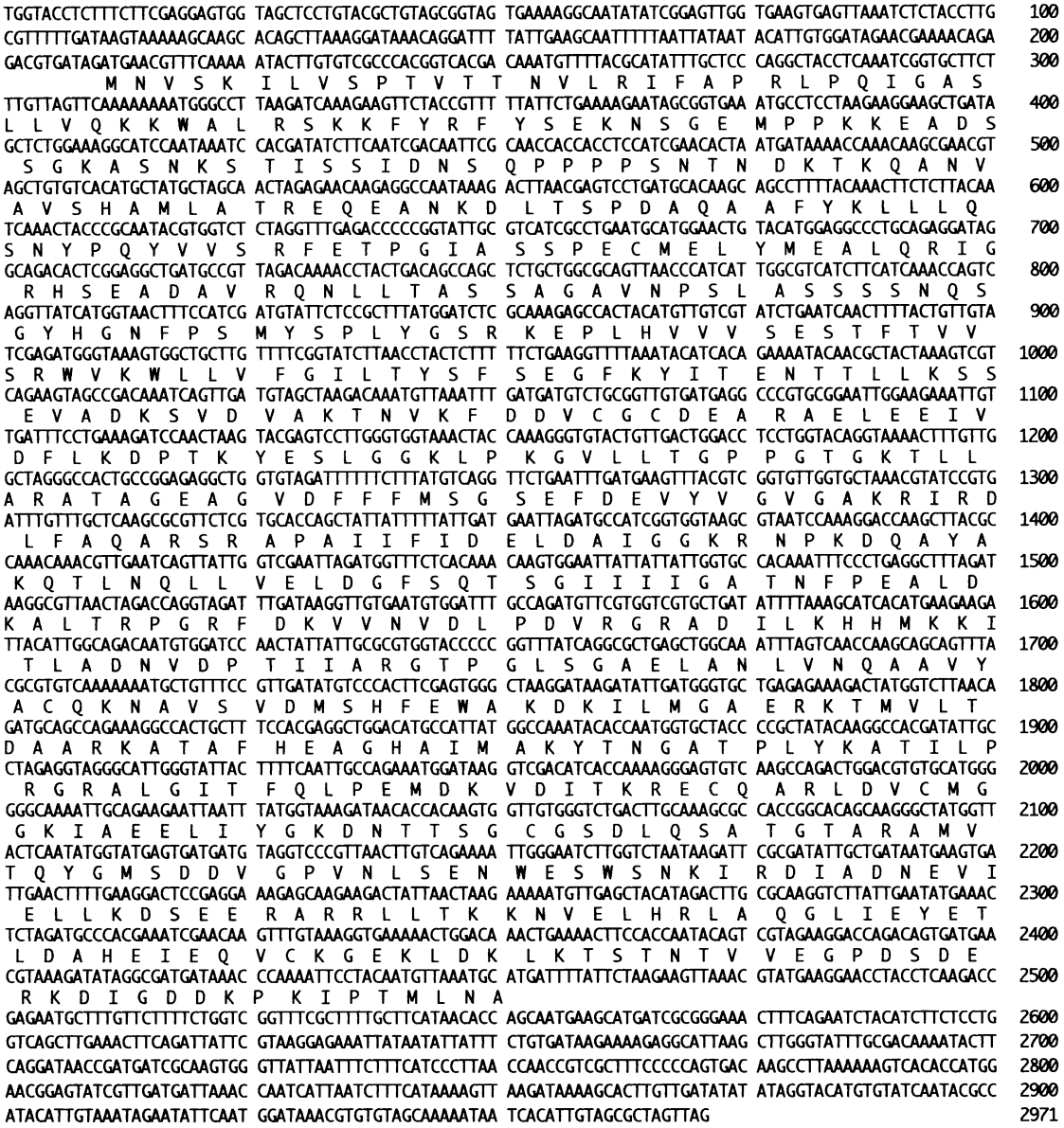

FIG. 3. Nucleotide sequence of *YME1* and predicted amino acid sequence of Yme1p. The sequence of the 2,971 bp was determined by sequencing both the coding and anticoding strands.

are putative ATPases, one of which (32) has been biochemically demonstrated to have ATPase activity. Yme1p is most closely related to the FtsH protein from *E. coli* (46). The FtsH protein is apparently involved in the posttranslational processing of PBP 3, a protein necessary for septation during cell division in *E. coli* (46). Yme1p is also related to the 84-kDa yeast protein Sec18p, which is required for several membrane fusion events involving the endoplasmic reticulum, vesicles, and the Golgi apparatus in the secretory pathway (7). The mammalian homolog of Sec18p is NSF, the *N*-ethylmaleimide-sensitive fusion protein (49). Two other large yeast proteins in this family are Pas1p, which is involved in peroxisome assembly (9), and Cdc48p, which appears to be involved in nuclear division (11). Two Cdc48p homologs, VCP (21) and p97-ATPase (32), have been found in higher eucaryotes. Yme1p is also homologous to several shorter proteins: Sug1p (42) and Afg1p (24) from yeast cells and the HIV-Tat-associated proteins TBP-1 (30) and Mss1 (39) from mammalian cells.

A comparison of the sequences of Yme1p and the FtsH protein is presented in Fig. 4. Over their full lengths, Yme1p and the FtsH protein share 30% amino acid identity, and an additional 3% of the residues are conserved substitutions. The amino-terminal portions of these proteins have no significant homology. The amino terminus of the FtsH protein contains two hydrophobic stretches that span the cytoplasmic membrane twice, leaving the bulk of the protein in the cytoplasm (45). There are no obvious sequence elements in Yme1p that would suggest association of the protein with membranes. From residue 272 of Yme1p and residue 145 of the FtsH protein to their respective C termini, these proteins are 46% identical. These sequences include

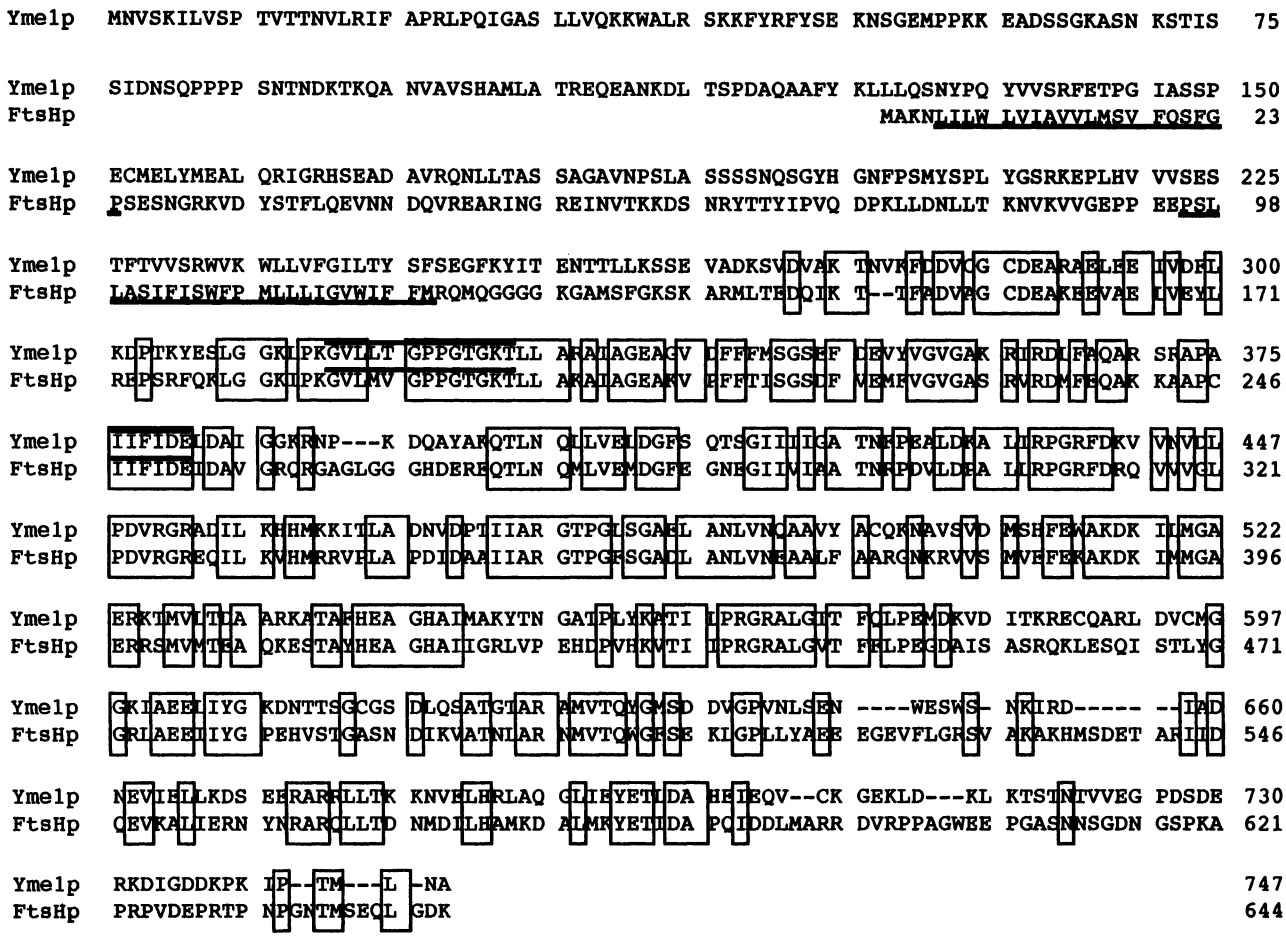

FIG. 4. Amino acid sequence comparison of Yme1p and the FtsH protein (FtsHp). The sequences were aligned with the aid of the alignment program of GeneWorks 2.2 from IntelliGenetics. Identical amino acids are boxed, the nucleotide-binding motifs (3) are indicated by lines above the sequence elements, and the membrane-spanning sequences of the FtsH protein (45, 46) are underlined. No significant level of homology between these two proteins is evident in the amino-terminal portions. Consequently, the boxed identities and gaps introduced by the alignment program prior to residue 272 for Yme1p and residue 145 for the FtsH protein have been eliminated in this alignment.

the region of homology shared among all members of this family of putative ATPases. As has been previously noted, this region contains two elements believed to be important for ATP binding (underlined sequences in Fig. 4) (3), but it also contains homologous sequence elements in addition to those assigned to ATP-binding or ATPase activity. The duplication of the conserved nucleotide-binding region noted for Pas1p, Sec18p, and Cdc48p (9, 11) is not evident in either Yme1p or the FtsH protein.

Generation and phenotypic consequences of a *yme1* null mutation. To destroy the function of *YME1*, 1,370 nucleotides internal to the reading frame were deleted and replaced by the *URA3* gene. The resulting DNA fragment (pPT45; Fig. 2) carried *URA3* flanked by approximately 2 kb of 5′ *YME1* sequence, including the presumptive *YME1* promoter and the first 185 codons, and approximately 1.5 kb of 3′ *YME1* sequence which included the C-terminal 105 codons (Fig. 2). This DNA, in linear form, was used to transform the diploid strain PTY33×PTY44 to Ura$^+$, deleting one copy of *YME1* (36). A Ura$^+$ transformant was purified and sporulated. The resulting tetrads each contained four viable spores, two of which were Ura$^+$ and two of which were Ura$^-$. Total DNA was prepared from each spore of one tetrad and subjected to DNA blot hybridization analysis (not shown) to verify that the Ura$^+$ spores contained the expected mutation, termed *yme1-Δ1::URA3*. In each case, the Ura$^+$ spores exhibited the same phenotypes caused by the *yme1-1* mutation (Fig. 1): a high rate of DNA escape from mitochondria to the nucleus, temperature-sensitive respiratory growth at 37°C, slow growth on rich glucose medium at 14°C, and lethality when combined with *rho*$^-$ mtDNA.

The *yme1-Δ1::URA3* mutation deleted over 60% of the structural gene, including all of the highly conserved sequence associated with ATP binding and hydrolysis, suggesting that it is a null mutation. If this is the case, then Yme1p is dispensable for growth and respiration at 30°C, although its absence results in high rates of DNA escape from mitochondria.

Six independent alleles of *yme1* were isolated in the original mutant screen (44), and the phenotypes caused by all are essentially the same as those caused by the *yme1-Δ1::URA3* mutation. The *yme1-1* mutation appears to be a missense mutation since full-length Yme1p can be detected in a *yme1-1* strain by antibodies (see below) directed against Yme1p. It is interesting that *yme1-1* exhibits weak intragenic complementation with the *yme1-3* allele, suggesting that Yme1p may function in a multimeric complex.

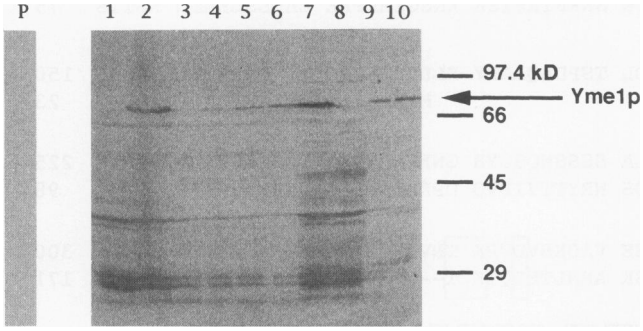

FIG. 5. Immunological detection of Yme1p in yeast extracts with anti-Yme1p antisera. Lanes: P, preimmune serum probing of wild-type whole-cell extract; 1, PTY52 (*yme1-Δ1::URA3*) whole-cell extract; 2, PTY44 (wild type) whole-cell extract; 3, PTY52/pRS315 (vector) whole-cell extract; 4, PTY52/pPT49 (*YME1* plasmid) whole-cell extract; 5, PTY44/pRS202 (multicopy vector) whole-cell extract; 6, PTY44/pPT48 (*YME1* in multicopy vector) whole-cell extract; 7, crude cell extract; 8, postmitochondrial supernatant; 9, purified mitochondrial pellet; 10, Percoll gradient-purified mitochondria. The crude cell extract in lane 7, the postmitochondrial supernatant in lane 8, and the mitochondrial pellet in lane 9 were prepared such that the concentrations of both soluble protein and protein associated with mitochondria remained constant, and equal volumes were loaded on the polyacrylamide gel.

Yme1p is associated with mitochondria. To generate an antibody against Yme1p, DNA encoding the C-terminal 105 amino acids of Yme1p was fused in frame to the 3′ end of the glutathione *S*-transferase-encoding gene (41), and the resulting fusion protein was isolated and injected into rabbits. The antisera were first used to probe immunoblots of total protein extracts from the wild-type yeast strain PTY44 and from the *yme1-Δ1::URA3* strain PTY52 that had been electrophoresed on a 10% polyacrylamide–SDS gel. A protein of the expected size of 82 kDa was detected by immunogold labeling in the wild-type extract but was absent in the *yme1-Δ1::URA3* strain (Fig. 5). The 82-kDa protein was restored in the *yme1-Δ1::URA3* strain by introducing a plasmid expressing the *YME1* gene. Thus, the antisera raised against the fusion protein detect Yme1p in crude extracts of *S. cerevisiae*. It is interesting that when the *YME1* gene is carried by a multicopy plasmid, there is apparently no increase in the amount of Yme1p present in the cell.

Because *yme1* mutations cause phenotypes associated with mitochondria, it seemed likely that Yme1p might be a mitochondrial protein. To test this idea, crude extracts of wild-type yeast cells were fractionated into mitochondrial pellets and postmitochondrial supernatants. These fractions were electrophoresed and blotted as before and then probed with the antisera. Yme1p was found to be depleted from the postmitochondrial fraction and enriched in the mitochondrial fraction (Fig. 5). To confirm the mitochondrial location of Yme1p, the mitochondrial pellet was resuspended and subjected to buoyant density centrifugation in a Percoll gradient. These gradient-purified mitochondria still contained Yme1p (Fig. 5).

DISCUSSION

Mutations in the gene *YME1* were isolated by screening for respiration-competent cells at 30°C that exhibited an increased rate (25 times higher than the wild-type rate for *yme1-1*) of DNA escape from mitochondria to the nucleus (44). Here, we have shown that this phenotype, as well as others associated with *yme1* mutations, is caused by the loss of the protein product, Yme1p, which is normally located in mitochondria.

In addition to increasing the rate of DNA escape from mitochondria, Yme1p deficiency causes two other phenotypes that are clearly related to mitochondrial function. First, *yme1* mutants are unable to grow on nonfermentable carbon sources at 37°C. The second phenotype is lethality of cells carrying both a *yme1* nuclear mutation and *rho*$^-$ (cytoplasmic petite) deletion mutations affecting the mitochondrial genome. All three of these phenotypes, caused by the loss of Yme1p, could be the result of decreased integrity (increased leakiness) of the mitochondrial compartment.

rho$^-$ cells cannot carry out any mitochondrial protein synthesis. Therefore, their mitochondrial inner membranes lack several major protein components (reviewed in references 6 and 33), a condition likely to result in decreased mitochondrial integrity. Indeed, we have observed that DNA escapes from *rho*$^-$ mitochondria at a 30-fold-higher rate than from *rho*$^+$ organelles (44). Intact mitochondrial compartments are required for viability in *S. cerevisiae* (1). Thus, one interpretation of the synthetic lethality of *rho*$^-$ *yme1* double mutants is that the combined absence of all mitochondrial translation products as well as Yme1p reduces mitochondrial integrity below a threshold necessary for viability.

Yme1p exhibits significant homology to a family of proteins that contain sequence elements implicated in nucleotide binding. One member of this protein family, mammalian p97-ATPase, has been shown biochemically to have ATPase activity (32). Yme1p is most homologous to a protein from *E. coli*, encoded by the *ftsH* gene, which is involved in formation of the septum during cell division (46). The next-most-homologous protein to Yme1p is the yeast protein Sec18p (19% identical to Yme1p). Sec18p and the corresponding mammalian protein, NSF, are required for membrane fusion events involving the endoplasmic reticulum, vesicles, and the Golgi apparatus in the secretory pathway (7, 49). NSF is assembled into a membrane-associated "fusion machine" containing several other components (4; reviewed in references 35 and 48).

The two other large yeast proteins in this family, Pas1p and Cdc48p, are less closely related to Yme1p. While their functions are less well defined than those of Sec18p, they also appear to be involved in organellar assembly and/or rearrangement. Pas1p is required for the assembly of peroxisomes (9). Cold-sensitive mutations affecting Cdc48p block spindle pole body duplication and nuclear division (11).

The homology between Yme1p and the FtsH protein, and to a lesser extent Sec18p, suggests the hypothesis that Yme1p could be involved in promoting efficient mitochondrial fusion and/or division. This hypothesis could easily account for the three mitochondrion-related phenotypes of *yme1* mutants described above if, in the absence of Yme1p, organellar fusion and/or division structures were not tightly sealed. For example, breaches in mitochondrial membranes during aberrant fusion or division events could allow DNA to leak into the cytoplasm. The fourth known phenotype of *yme1* mutants is cold-sensitive growth on rich glucose-containing medium (Fig. 1) but not on medium containing nonfermentable carbon sources (44). This too could be due to a defect in mitochondrial division if one assumed that at low temperatures division of mitochondria lacking Yme1p was too slow to keep pace with rapid cell growth on glucose and that no checkpoint function held up cell division for

completion of mitochondrial division (17). A total block of mitochondrial division would be expected to produce a lethal phenotype (27, 28, 51).

Whatever role Yme1p may play in mitochondrial function, the polypeptide appears to be part of a multimeric complex. We observed intragenic complementation between the apparent missense allele *yme1-1* and the uncharacterized *yme1-3* mutation, suggesting that the functional unit contains more than one copy of Yme1p. In addition, an elevated gene dosage of *YME1* did not cause overaccumulation of the protein, suggesting that the level of a complex containing Yme1p is limited by the level of some other component and that unassembled Yme1p may be degraded. Our previous observation that a *yme1 yme2* double mutant had a synthetic nonrespiratory phenotype (44) suggests the possibility that the *YME2* product could be part of such a complex.

A novel consequence of *yme1* mutations is that they convert the petite-positive yeast *S. cerevisiae* into respiring (at permissive temperatures) petite-negative strains by preventing the growth of *rho*$^-$ cells. This property will be useful for many genetic screens and selections in which the very frequent *rho*$^-$ mutations represent background that can now be easily eliminated. The op_1 (*pet9*) mutation also kills *rho*$^-$ cells. However, this mutation, which inactivates a major mitochondrial ADP-ATP translocator, also prevents respiratory growth (20, 22). Interestingly, other recent evidence also indicates that the differences between petite-positive and petite-negative yeasts may not be very great. Nuclear mutations have been selected in the petite-negative yeasts *Schizosaccharomyces pombe* (15) and *Kluyveromyces lactis* (2) that allow them to tolerate the loss of mtDNA.

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