Expression of the *Saccharomyces cerevisiae* Gene *YME1* in the Petite-Negative Yeast *Schizosaccharomyces pombe* Converts It to Petite-Positive

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**ABSTRACT**

Organisms that can grow without mitochondrial DNA are referred to as “petite-positive” and those that are inviable in the absence of mitochondrial DNA are termed “petite-negative.” The petite-positive yeast *Saccharomyces cerevisiae* can be converted to a petite-negative yeast by inactivation of Yme1p, an ATP- and metal-dependent protease associated with the inner mitochondrial membrane. Suppression of this yme1 phenotype can occur by virtue of dominant mutations in the α- and γ-subunits of mitochondrial ATP synthase. These mutations are similar or identical to those occurring in the same subunits of the same enzyme that converts the petite-negative yeast *Kluyveromyces lactis* to petite-positive. Expression of *YME1* in the petite-negative yeast *Schizosaccharomyces pombe* converts this yeast to petite-positive. No sequence closely related to *YME1* was found by DNA-blot hybridization to *S. pombe* or *K. lactis* genomic DNA, and no antigenically related proteins were found in mitochondrial extracts of *S. pombe* probed with antisera directed against Yme1p. Mutations that block the formation of the F, component of mitochondrial ATP synthase are also petite-negative. Thus, the F, complex has an essential activity in cells lacking mitochondrial DNA and Yme1p can mediate that activity, even in heterologous systems.

**MITOCHONDRIAL** biogenesis requires the coordinated expression of genes encoded by mitochondrial and nuclear genomes, as well as the regulated assembly of a number of multicomponent protein complexes. Recent work in the yeast *Saccharomyces cerevisiae* has revealed the importance of a related set of mitochondrial proteases in the assembly of energy transduction complexes. Located in the inner mitochondrial membrane is a hetero-oligomer, composed of the homologous proteins Yta10p and Yta12p, that is necessary for the assembly of cytochrome oxidase and ATP synthase (Paul and Tzagoloff 1995; Arlt et al. 1996; Guélin et al. 1996). The yeast homolog of the Escherichia coli lon protease has also been implicated in the assembly of inner mitochondrial protein complexes (Rep et al. 1996). These proteases have an ATP requirement, and mutational analysis has led to a proposal that these proteins function largely as assembly factors with editing capability. Misfolded and damaged proteins or proteins present in excess that cannot be assembled into a higher-order complex are degraded by these proteases.

Yme1p forms another inner mitochondrial membrane complex with protease activity (Leonhard et al. 1996). Yme1p determines the stability of cytochrome oxidase subunit II that is present in excess or is not assembled into the multicomponent cytochrome oxidase complex (Nakai et al. 1995; Pearce and Sherman 1995; Weber et al. 1996). While Yme1p is envisioned to have a role as an assembly/editing factor similar to Yta10p/Yta12p and mitochondrial Lon, this has not yet been demonstrated experimentally. *YME1* was originally identified in a screen for mutations that increase the rate at which mitochondrial DNA (mtDNA) is transferred to the nucleus (Thorsson and Fox 1993). yme1 strains are also heat sensitive for growth on nonfermentable carbon sources (pet-ts) and cold-sensitive for growth on rich-glucose media and have altered mitochondrial morphology (Thorsson et al. 1993; Campbell et al. 1994). *S. cerevisiae* strains in which YME1 has been inactivated are petite-negative; that is, they are incapable of growth in the absence of mtDNA (Thorsson et al. 1993). Wild-type *S. cerevisiae* is a petite-positive strain, capable of growing on fermentable carbon sources in the absence of mtDNA. As a consequence of dysfunctional mitochondrial structure and function, yme1 strains have an increased rate of mitochondrial compartment turnover by the vacuole (Campbell and Thorsson 1998). Clearly, Yme1p is involved in a number of important mitochondrial processes that must extend beyond the turnover of unassembled Cox2p.

In an effort to understand the physiological role of Yme1p in mitochondrial biogenesis and function, a number of suppressors of *yme1* phenotypes have been characterized. A bypass suppressor of the *yme1* null allele that suppresses all *yme1* phenotypes was identified as *YNT1/RPT3*, a gene that encodes a regulatory subunit of the 26S protease (Campbell et al. 1994). Inactivation of *YNT20*, which encodes a putative 3′-5′ exonuclease...
located in mitochondria, suppresses the high rate of mtDNA escape in yme1 strains and also suppresses the synthetic nonrespiring phenotype of yme1 yme2 strains (Hanekamp and Thorsness 1998). Specific dominant mutations in the gene encoding the γ-subunit of the mitochondrial ATP synthase, ATP3, suppress the petite-negative phenotype of yme1 yeast (Weber et al. 1995). For none of these suppressors has it been possible to demonstrate a substrate/product relationship between the identified gene products and the Yme1p protease. In the work presented here, a number of additional mutations that result in the suppression of various yme1 phenotypes are described, and another suppressor of the petite-negative phenotype of yme1 yeast is fully described. Additionally, the heterologous expression of Yme1p in the petite-negative yeast Schizosaccharomyces pombe was used to define the genetic basis of yeast strains that require mtDNA for viability.

MATERIALS AND METHODS

Strains: The E. coli strains used for preparation and manipulation of DNA were DH5α (F−, endA1, hsdR17(rK−, m−), supE44, thi-1, λ recA1, gyrA96, relA1, Δ(lac-proAB) U169, o80, lacZΔM15) and XL1 Blue [recA1, endA1, gyrA96, thi-1, rdsR17, supE44, rda1, lac(f− proA B, lacZD15 M), Tn10 (tet)]. The E. coli strains ES1301 [lacZ53, mitS201: ::Tn5, thyA36, rha-5, metB1, F−, traD36, lacI qZ80, Tn9, supE44, relA1, Δlaci-proAB], (F−, traD36, proA−B+, lacZD15M) were used for in vitro mutagenesis and were obtained from Promega (Madison, WI). The genotypes of S. cerevisiae strains used in this study are listed in Table 1. Standard genetic techniques were used to construct and analyze the various yeast strains (Sherman et al. 1986; see below). The S. pombe strain PNY10 was a gift of Dr. Paul Nurse. The Kluyveromyces lactis strain Y11401 was a gift of Dr. Claudia Abeijon. The genotypes of both strains are listed in Table 1.

Media: E. coli strains containing plasmids were grown in Luria-Bertani (LB) medium (10 g bactotryptone, 10 g NaCl, 5 g yeast extract per liter; Maniatis et al. 1982) supplemented with 125 μg/ml of ampicillin. Yeast strains were grown in complete glucose medium (YPD) containing 2% glucose, 2% Bacto-peptone, 1% yeast extract; complete ethanol-glycerol medium (YPEG) containing 3% glycerol, 3% ethanol, 2% Bacto-peptone, 1% yeast extract; or minimal glucose medium (SD) containing 2% glucose, 6.7 g/liter yeast nitrogen base without amino acids (Difco, Detroit) and supplemented with the appropriate nutrients. Nutrients were uracil at 40 mg/liter, adenine at 40 mg/liter, tryptophan at 40 mg/liter, lysine at 60 mg/liter, and leucine at 100 mg/liter. For agar plates, Bacto-agar was added at 20 g/liter. Where indicated, ethidium bromide was added at 25 μg/ml (Weber et al. 1995).

yme1Δ1::URA3 suppressor isolation and analysis: Bypass suppressors of yme1Δ1::URA3 were isolated by screening for spontaneous revertants of the pet-1::ts phenotype of PTY52. Overnight cultures grown in YPD at 30° were plated on YPEG plates at ~2000 cells/plate and incubated for several days at 37°. Isolated revertants able to respire at high temperatures were colony purified, rescreened on YPEG at 37°, analyzed for suppression of other yme1Δ1::URA3 phenotypes, and screened for recessive collateral phenotypes by scoring for growth on different carbon sources at different temperatures. Each suppressed strain was mated to every other suppressed strain. Diploids were analyzed to identify complementation groups. Tetrad analysis of matings between the suppressed strains and a wild-type strain provided confirmation that any collateral phenotypes of PTY52. 0 were isolated in the same manner as the recessive suppressors except that the screen was performed using the diploid strain PTY52 × PTY60. Isolation of suppressors of the yme1 petite-negative phenotype was performed as described (Weber et al. 1995).

TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotypea</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTY44</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 [rho+, TRP1]</td>
<td>Thorsness and Fox (1993)</td>
</tr>
<tr>
<td>PTY52</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 [rhol+, TRP1]</td>
<td>Thorsness et al. (1993)</td>
</tr>
<tr>
<td>PTY62</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 [rhol+, TRP1]</td>
<td>Thorsness and Fox (1993)</td>
</tr>
<tr>
<td>PTY78</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ATP1-75 [rhol]</td>
<td>Weber et al. (1995)</td>
</tr>
<tr>
<td>PTY93</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ATP1-75 [rhol+, TRP1]</td>
<td>Weber et al. (1995)</td>
</tr>
<tr>
<td>DKY1</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 YNT9-1 [rhol+, TRP1]</td>
<td>This study</td>
</tr>
<tr>
<td>DKY21</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ynt4-1 [rhol+, TRP1]</td>
<td>This study</td>
</tr>
<tr>
<td>DKY22</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ynt5-1 [rhol+, TRP1]</td>
<td>This study</td>
</tr>
<tr>
<td>DKY25</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ynt6-1 [rhol+, TRP1]</td>
<td>This study</td>
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<tr>
<td>DKY26</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 yme1Δ1::URA3 ynt7-1 [rhol+, TRP1]</td>
<td>This study</td>
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<td>DKY27</td>
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<td>This study</td>
</tr>
<tr>
<td>DKY30</td>
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<td>This study</td>
</tr>
<tr>
<td>NTY1</td>
<td>MATa ura3-52 lys2 leu2-3,112 trpl-1Δ1 rent1-1 [rhol+, TRP1]</td>
<td>Campbel l et al. (1994)</td>
</tr>
<tr>
<td>W303ΔATP12</td>
<td>MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 trpl-1 atpl-1Δ1::LEU2 [rhol+]</td>
<td>Bowman et al. (1991)</td>
</tr>
<tr>
<td>PNY10</td>
<td>h− leu1-32</td>
<td>Paul Nurse</td>
</tr>
<tr>
<td>Y11401</td>
<td>MATa ura3</td>
<td>Claudia Abeijon</td>
</tr>
</tbody>
</table>

a The mitochondrial genotype is bracketed.

b S. pombe strain.

c K. lactis strain.
Nucleic acid techniques and DNA sequencing: All manipulations of DNA were performed using standard techniques (Maniatis et al. 1982). Restriction enzymes and DNA modification enzymes were purchased from New England Biolabs (Beverly, MA). Double-stranded DNA templates were prepared for sequencing by boiling lysis (Maniatis et al. 1982) and were sequenced by the nucleotide chain termination method (Sanger et al. 1977) using the Sequenase Version 2.0 DNA sequencing kit (United States Biochemical, Cleveland). DNA-blot hybridization analysis was performed using standard techniques (Maniatis et al. 1982). Blots were washed in 2× SSC with 0.5% SDS for 2 hr at 50°C. Random primed probes corresponding to the entire open reading frames (ORFs) of YME1 and ATP1 from S. cerevisiae were prepared using the NEBlot kit from New England Biolabs.

Isolation of ATP1-75: Briefly, genomic DNA was prepared as described (Rose and Broach 1991) from the yeast strain PTY78 bearing the ATP1-75 mutation. Genomic DNA was partially digested with Sau3A; 6–10-kb DNA fragments were isolated and cloned into the yeast CEN-vector pRS316 (Sikorski and Hieter 1989). Library DNA was prepared by large-scale alkaline lysis (Maniatis et al. 1982) and was used to transform PTY62 using the alkali cation treatment method (Ito et al. 1983). Approximately 2 × 10^5 transformants were inoculated into 10 ml of selective SD and incubated with agitation at 30°C for 2 hr. Aliquots (1 ml) of the SD culture were inoculated into each of 10 tubes containing 10 ml selective SD media plus ethidium bromide (25 μg/ml) and grown to saturation at 30°C (8 days). Ten microliters of each culture was inoculated into 10 ml of fresh SD plus ethidium bromide media and grown to saturation at 30°C (12 days). Dilutions of each culture were plated on YPD media. Colonies were replica plated to media containing 5-fluoroorotic acid (5-FOA; Boeke et al. 1984) to identify cells unable to grow without plasmid DNA. Thirty-two colonies unable to grow on 5-FOA were recovered from the YPD plates and the phenotype was rechecked. Plasmid DNA was prepared from four isolates. Three clones containing identical inserts of 2.4 kb. One of these, pDK1, was sequenced and found to contain ATP1. This clone was sent Yme1p, suppressors of a ynt5. These mutations suppress the petite-negative phenotype, as well as for inhernt mutations display no collateral phenotypes. The 2.2-kb ynt1 fragment containing the ATP1 gene was cloned into vector pRS316 and then digesting completely with HindIII. The 4-kb KpnI-HindIII fragment containing the atp1::LEU2 construct was gel purified and used to transform PTY44. The resulting strain, DKY30, was then tested for the ability to repress and whether it was petite-positive or petite-negative. Creation of the null mutant was verified by performing PCR with the oligonucleotides 5'-TAAGGCTCTATTGAGCCGTCG3' and 5'-TATTGTAGAGGCGGCTTCA3'.

S. pombe expression clones: The S. pombe expression constructs were made using the plasmid pART1, a gift from Dr. Paul Nurse. To isolate the YME1 coding sequence, the plasmid pYME1-N did was digested with NdeI and the ends were made blunt by filling in the site with Klenow. After digestion with ScaI, the 2-kb YME1 fragment was isolated and ligated into pART1 that had been digested with ScaI and Smal, generating pART1-YME1. The plasmid pyme1Δm was constructed by digesting pART1-YME1 with MluI. This site was then filled in using Klenow and the plasmid was religated. Transformation of S. pombe was performed by treatment of cells with alkali cations (Ito et al. 1983).

Preparation of cellular extracts and detection of Yme1p and Atp1p: Protein extracts were prepared as described (Pilus and Solomon 1986). Mitochondrial fractions were isolated by differential centrifugation (Daum et al. 1982). Protein fractions were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with antisera directed against Yme1p or Atp1p as described previously (Hanekamp et al. 1989) using the Sequenase Version 2.0 DNA sequencing kit (United States Biochemical, Cleveland).

RESULTS

Isolation and characterization of bypass suppressors of yme1: To identify gene products that interact with Yme1p, suppressors of a yme1::URA3 null allele were isolated. The yme1 null allele was used for this analysis because suppressors of yme1-1, a missense mutation, were all intragenic. Mutants were isolated either as dominant or recessive suppressors of the yme1-1 allele type or as suppressors of the yme1 petite-negative phenotype. The revertants were then screened for their ability to suppress other yme1 phenotypes, as well as for inherent collateral phenotypes. Nine strains were identified with mutations that suppress various yme1 phenotypes (Figure 1). Complementation analysis indicates that these suppressors represent mutations at distinct genetic loci. These mutations can be placed in classes based on the yme1 phenotypes suppressed and collateral phenotypes linked to the suppressing mutation. Class 1 contains the recessive suppressor ynt1, which suppresses all of the yme1 phenotypes and has a recessive collateral phenotype of cold-sensitive growth on the nonfermentable carbon sources ethanol and glycerol (Campbell et al. 1994). Class 2 includes the recessive mutants ynt4 and ynt5. These mutations suppress the yme1-1 ts, the high rate of mtDNA escape, and cold-sensitive growth on rich-glucose phenotypes. These suppressor mutations display no collateral phenotypes. The third class of suppressors includes the recessive
is the mutation YNT9-1, which is presumably a dominant suppressor of the yme1 pet-ts phenotype since it was isolated in a yme1-Δ1::URA3 homozygous diploid. However, further characterization of this mutation has proven difficult because this strain grows poorly on glucose media and does not sporulate. The final class of suppressors includes the dominant mutations ATP1-1 and ATP1-75, previously referred to as YNT3-1 (Weber et al. 1995). These mutants suppress only the petite-negative phenotype of yme1. Cells grown in the presence of ethidium bromide rapidly lose all DNA from their mitochondria (Slonimski et al. 1968; Fox et al. 1991) and are termed rho0. Unlike wild-type yeast, which readily grows in the presence of ethidium bromide, yme1 rho0 yeast are very slow growing. To better understand the basis for this petite-negative phenotype of yme1 strains, we pursued analysis of the ATP1-75 mutation.

**Isolation of ATP1-75, a dominant suppressor of the yme1 rho0 slow-growth phenotype:** Previous work in our laboratory demonstrated that mutations in the ATP-synthase γ-subunit suppress the petite-negative phenotype of yme1 yeast (Weber et al. 1995). Additionally, work in the petite-negative yeast K. lactis has shown that mutations in the ATP-synthase α-, β-, and γ-subunits convert this yeast to petite-positive (Chen and Clark-Walker 1995, 1996). To assess the similarities of petite negative suppressors in yme1 S. cerevisiae and K. lactis, we cloned the ATP1-75 mutation. A genomic DNA library was constructed from a yme1-Δ1::URA3 ATP1-75 strain. This genomic library was transformed into a yme1-1 strain, and transformed cells were screened for the ability to grow upon mtDNA loss. Several clones were isolated from this screen. Figure 2 shows the growth of a rho0 yme1-1 mutant after introduction of one of these plasmids, pDK1. DNA sequence analysis demonstrated that the plasmid insert contained the complete open reading frame of ATP1, the structural gene for the α-subunit of mitochondrial ATP synthase. Sequence analysis of the entire ORF identified a single nucleotide change that resulted in the conversion of the strictly conserved asparagine residue at position 102 to isoleucine. To demonstrate that the amino acid change at position 102 in ATP1-75 was responsible for suppression of the petite-negative phenotype, site-directed mutagenesis was performed. First, the residue was changed to the wild-type asparagine, followed by reversion of the asparagine residue back to the mutant isoleucine. As shown in Figure 2, a plasmid bearing the reverted wild-type ATP1 allele is unable to rescue the petite-negative phenotype, while both the original ATP1-75 allele and the reverted ATP1-75 allele restore growth on ethidium bromide.

A **requirement of functional mitochondrial ATP-synthase F1 component in rho0 yeast:** It has been reported that null mutations in the ATP-synthase γ-subunit (Weber et al. 1995) and the β-subunit (Giraud and Velours 1997) cause a petite-negative phenotype in S. *K. lactis*. These results suggest that the ATP-synthase γ-subunit plays an important role in maintaining mitochondrial function in rho0 yeast.
Basis for Petite-Positive Yeast

Figure 3.—Growth of rho<sup>+</sup> yeast bearing yme<sup>1</sup>, atp<sup>1</sup>, or atp<sup>12</sup> null alleles. Yeast cells were streaked on a synthetic glucose plate (A) and incubated for 3 days at 30°C and on a synthetic glucose plate containing 25 μg/ml ethidium bromide (B) and incubated for 5 days at 30°C. The indicated yeast strains are wild type (PTY44), yme<sup>1</sup>Δ (PTY52), atp<sup>1</sup>Δ (DKY30), and atp<sup>12</sup>Δ (W303ΔATP12).

and <i>K. lactis</i>: The results presented above suggest that the activity of Yme1p in <i>S. cerevisiae</i> is essential to maintain the petite-positive phenotype of this yeast. Because of the striking similarities between a yme<sup>1</sup> mutant and the petite-negative yeasts <i>S. pombe</i> and <i>K. lactis</i>, we wondered if this growth characteristic was due to the absence of a Yme1p-like activity. To address this issue DNA-blot hybridization analysis was performed to determine whether the genomes <i>S. pombe</i> and <i>K. lactis</i> encode a YME1 homolog. Total DNA prepared from the strains PTY44, PTY52, PNY10, and Y11401 was digested with EcoRI or SspI, blotted, and probed with a random-primed probe prepared from a PCR product corresponding to the YME1 ORF under low stringency conditions. Figure 4 shows that a strong signal was detected for the <i>S. cerevisiae</i> DNA (Figure 4, lanes 5, 6, 9, and 10), corresponding to YME1. An additional weak signal was detected in the SspI-digested <i>S. cerevisiae</i> DNA at ~6.5 kb (Figure 4, lanes 9 and 10). These fragments correspond to the AFG3 gene (data not shown). Digestion of the AFG3 locus with EcoRI produces a fragment of ~15 kb; thus the AFG3 signal in lanes 5 and 6 was

An absence of closely related <i>YME1</i> genes in <i>S. pombe</i> and <i>K. lactis</i> does not appear to contribute to the petite-negative phenotype.
Figure 4.—Detection of DNA sequences homologous to YME1. DNA-blot hybridization was performed on genomic DNA prepared from *S. cerevisiae*, *S. pombe*, and *K. lactis*. Designations for the DNA sources are as follows: Sc (lanes 1, 5, 9, 13, 16), wild-type *S. cerevisiae* (PTY44); Sc9 (lanes 2, 6, 10), yme1-D1::URA3 *S. cerevisiae* (PTY52); Sp (lanes 3, 7, 11, 15, 18), *S. pombe* (PYN10); Kl (lanes 4, 8, 12, 14, 17), *K. lactis* (Y11401). Restriction enzyme digestions were as indicated. Lanes 1–12 were probed with *S. cerevisiae* YME1 sequences and lanes 13–18 with *S. cerevisiae* ATP1 sequences. Hybridization signals were detected with a Bio-Rad (Richmond, CA) phosphorimager.

Figure 5.—Heterologous expression of YME1 in *S. pombe*. *S. cerevisiae* and *S. pombe* were transformed with the indicated plasmids and streaked on a synthetic glucose plate (A) and incubated for 5 days at 30°C or streaked on a synthetic glucose plate containing 25 µg/ml ethidium bromide (B) and incubated for 7 days at 30°C. The indicated strains are as follows: *S. c. wild type*, *S. cerevisiae* wild-type strain PTY44; *S. c. yme1Δ*, *S. cerevisiae* yme1 null mutant strain PTY52; *S. p. wild type*, *S. pombe* wild-type strain PYN10. The indicated plasmids are as follows: pART1, *S. pombe* expression vector; pYME1, the vector plasmid pART1 bearing YME1; pyme1Δm, the vector plasmid pART1 bearing an inactivated yme1 allele.

**Heterologous expression of YME1 in *S. pombe***: To determine if Yme1p activity was able to convert a petite-negative yeast to petite-positive, YME1 was expressed in *S. pombe*. The YME1 gene was cloned into the *S. pombe* expression vector pART1. As an additional control, the pART1-YME1 construct was digested with Mu1, filled in, and religated. This plasmid, pyme1Δm, has a frameshift in the YME1 ORF that creates a nonfunctional gene product. These two plasmids, along with the vector, were transformed into a *S. cerevisiae* yme1 mutant and the *S. pombe* strain PYN10. As shown in Figure 5, expression of YME1 in both the *S. cerevisiae* yme1 and *S. pombe* strains allows growth in the absence of mtDNA, while the pART1 vector and the inactivated yme1 allele do not. Thus, Yme1p provides an activity that is essential for viability of two yeast when they lack mtDNA. Western blot analysis using whole cell extracts from these strains detected Yme1p in mitochondria of *S. pombe* transformed with pART1-YME1 (Figure 6).

**DISCUSSION**

To identify possible Yme1p substrates and to elucidate the role of Yme1p in yeast mitochondria, we undertook a suppressor analysis of a yme1 null mutant. Several
genes were identified that, when mutated, lead to suppression of various yme1 phenotypes (Figure 1). Because mutation of different genes suppress different spectrums of yme1 phenotypes, we anticipate multiple roles for Yme1p in mitochondrial biogenesis and function.

Previous work in our laboratory identified mutations in the ATP3 gene that suppress the petite-negative phenotype of yme1 strains (Weber et al. 1995). Here we identify a mutation in the ATP1 gene that suppresses the same phenotype. The F1 complex of the mitochondrial ATP synthase is a multi-subunit structure with a subunit stoichiometry of α3β3γε. Deletion of ATP1 leads to the inability to use nonfermentable carbon sources (Takeda et al. 1986) and causes a petite-negative phenotype (Figure 3). Mutational inactivation of the γ- and δ-F1 subunits (Weber et al. 1995; Giraud and velours 1997) and of ATP12p, a protein involved in F1 subunit assembly (Bowman et al. 1991), also cause this phenotype (Figure 3). These results indicate that Yme1p, as well as the F1 portion of the ATP synthase, are important for the growth of S. cerevisiae in the absence of mtDNA. One extensively characterized mutation that causes S. cerevisiae to become petite-negative is op1, a mutation in the major ADP/ATP translocator encoded by AAC2 (Kovacova et al. 1968; Lawson and Douglas 1988; Kolarov et al. 1990). Both op1 and a null mutation of ACC2 render cells unable to grow on nonfermentable carbon sources (Kovacova et al. 1968; Lawson et al. 1990). The petite-negative nature of op1 strains has been taken as evidence for the absolute requirement for ATP in the matrix of mitochondria.

The yeasts K. lactis and S. pombe are petite-negative. Work in K. lactis has shown that specific mutations in the ATP-synthase α-, β-, and γ-subunits convert this yeast to a petite-positive organism (Chen and Clark-Walker 1995, 1996). Based on the similarities between these petite-negative strains and an S. cerevisiae yme1 mutant, we surmised that these yeasts may be petite-negative because they lack a yme1p-like activity. DNA-blot analysis indicated that neither K. lactis nor S. pombe encode a close YM E1 homolog (Figure 4), nor is there an anti- genically related Yme1p homolog in S. pombe (Figure 6). Additionally, heterologous expression of YM E1 in S. pombe converted this petite-negative yeast to petite-positive. Thus, Yme1p has an activity that is missing in S. pombe, and presumably also in K. lactis, that results in these yeast being petite-negative.

We utilized the bovine F1-ATPase crystal structure (Abrahams et al. 1994) to identify the relative location of mutations in the α- and γ-subunits of mitochondrial ATP synthase that convert petite-negative yeast to petite-positive. The amino acid changed in ATP1-75, Asn3, is located near the interface of the α- and γ-subunits in the region referred to as the “dimple” of the F1 complex, distal to the stalk connecting F1 to F0 (Abrahams et al. 1994). The two amino acid positions mutated in the K. lactis α-subunit, Ala333 and Phe443, are also located along this interface, in direct contact with the γ-subunit (Chen and Clark-Walker 1996). Thus, it is likely that the interaction of the α- and γ-subunits is altered in these mutant F1 complexes.

The information presented here suggests that Yme1p must be involved in the regulation of mitochondrial ATP synthase, although the nature of this regulation is unknown. The consequences of the absence of Yme1p in yeast that contain a complete mitochondrial genome is not deleterious at 30°, as these cells can use nonfermentable carbon sources. Upon loss of mtDNA, however, the absence of Yme1p leads to the petite-negative phenotype. It has previously been shown that Yme1p is a metallo-protease that is involved in the turnover of cytochrome oxidase subunit II (Nakai et al. 1995; Pearce and Sherman 1995; Weber et al. 1996). One possibility is that Yme1p may also have a direct role in the turnover of F1 subunits. In mitochondria lacking mtDNA, which encodes the F0 subunits Atp6p, Atp8p, and Atp9p, excess F1 subunits might be degraded in a Yme1p-dependent manner. Without Yme1p, F1 subunits may accumulate, leading to aberrant complex formation. This scenario seems unlikely since the accumulation of ATP3p has been shown to be independent of Yme1p (Weber et al. 1995). Alternatively, Yme1p may affect the F1 complex indirectly by interacting with F0 subunits, at least six of which are encoded in the nucleus. In the absence of the mitochondrially encoded F0 subunits, the nuclearly encoded subunits may be subject to degradation by Yme1p, allowing the F1 portion of the ATP synthase to function properly. In the absence of Yme1p, these F0 subunits may accumulate and interfere with an F1 activity that is essential for cells lacking mtDNA. The mutations that have been identified in ATP1 and ATP3 would thus allow the F1 complex to assume a conformation that is not subject to interference by inhibitory factors that exist in the absence of Yme1p activity.
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LITERATURE CITED


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