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Mutations in the Mitochondrial ATP Synthase Gamma Subunit Suppress a Slow-Growth Phenotype of *yme1* Yeast Lacking Mitochondrial DNA

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ABSTRACT

In *Saccharomyces cerevisiae*, inactivation of the nuclear gene *YME1* causes several phenotypes associated with impairment of mitochondrial function. In addition to deficiencies in mitochondrial compartment integrity and respiratory growth, *yme1* mutants grow extremely slowly in the absence of mitochondrial DNA. We have identified two genetic loci that, when mutated, act as dominant suppressors of the slow-growth phenotype of *yme1* strains lacking mitochondrial DNA. These mutations only suppressed the slow-growth phenotype of *yme1* strains lacking mitochondrial DNA and had no effect on other phenotypes associated with *yme1* mutations. One allele of one linkage group had a collateral respiratory deficient phenotype that allowed the isolation of the wild-type gene. This suppressing mutation was in *ATP3*, a gene that encodes the gamma subunit of the mitochondrial ATP synthase. Recovery of two of the suppressing *ATP3* alleles and subsequent sequence analysis placed the suppressing mutations at strictly conserved residues near the C terminus of Atp3p. Deletion of the *ATP3* genomic locus resulted in an inability to utilize nonfermentable carbon sources. *atp3* deletion strains lacking mitochondrial DNA grew slowly on glucose media but were not as compromised for growth as *yme1* yeast lacking mitochondrial DNA.

EUCARYOTIC cells require mitochondria for viability. Several lines of evidence suggest that this is not simply a reflection of a cellular requirement for oxidative phosphorylation and electron transport, but rather reflects a requirement for the mitochondrial compartment. The budding yeast *Saccharomyces cerevisiae* is able to grow on fermentable carbon sources without utilization of the electron transport chain or oxidative phosphorylation. In fact, this species can tolerate a partial deletion or complete absence of mitochondrial DNA (mtDNA) when grown on a fermentable carbon source (SLONIMSKI *et al.* 1968). Even so, strains grown under such conditions cannot tolerate the loss of the mitochondrial compartment. Two classes of mutations that impair mitochondrial biogenesis illustrate this fact. First, mutations that interfere with import of proteins into the mitochondria arrest cell growth (BAKER and SCHATZ 1991). Second, conditional mutations that prevent segregation of mitochondria to the emerging bud result in cessation of growth at the restrictive temperature (MCCONNELL *et al.* 1990). Thus, even when electron transport and oxidative phosphorylation are not required, the mitochondrial compartment is apparently essential for yeast cell growth.

Other organisms are also able to tolerate the loss of mtDNA, although it is more difficult to separate the need for respiratory capacity from the need for a mitochondrial compartment. It is possible to generate animal cells that lack mtDNA by treatment with ethidium bromide and the inclusion of uridine and pyruvate in the culture medium (DESJARDINS *et al.* 1985; KING and ATTARDI 1989). Nuclear mutations at either of two separate loci in *Kluyveromyces lactis* or *S. pombe* allow these organisms, usually inviable in the absence of mtDNA, to grow on fermentable carbon sources with no mtDNA present (HAFFTER and FOX 1992; CHEN and CLARK-WALKER 1993). Thus it appears that a number of organisms are able to tolerate the loss of mtDNA provided certain nutrient or genetic criteria are met. Presumably, the presence of a mitochondrial compartment, with or without mtDNA, is essential for viability of all eucaryotic cells.

Although wild-type *S. cerevisiae* is able to grow with mutations of the mitochondrial genome (rho$^-$), or in the absence of mtDNA (rho^0), certain mutations impair this ability. Mutations in the nuclear gene *op1*, which encodes the ADP/ATP translocator, result in both a respiratory deficient phenotype and a rho$^-$/rho^0 lethality (KOVACOVA *et al.* 1968; KOLAROV *et al.* 1990). Recently, we have shown that mutations in the nuclear gene *YME1* cause a number of phenotypes that are indicative of dysfunctional mitochondria, including severely impaired growth in the absence of mtDNA (THORSNESS and FOX 1993; THORSNESS *et al.* 1993). In an attempt to better understand why mtDNA is so important in the *yme1* mutant, even when electron transport and oxidative phosphorylation are not required, we have carried out a suppressor analysis to identify mutations that would enable *yme1* rho^0 double mutants

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TABLE I

Yeast strains

Strain	Genotype[a,b]	Source
PTY33	*MATa ura3-52 ade2 leu2-3,112 trp1-Δ1 [rho⁺, TRP1]*	THORSNESS and FOX (1993)
PTY44	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 [rho⁺, TRP1]*	THORSNESS and FOX (1993)
PTY52	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 [rho⁺, TRP1]*	THORSNESS *et al.* (1993)
PTY62	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-1 [rho⁺, TRP1]*	THORSNESS and FOX (1993)
PTY73	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-1 [rho⁰]*	This study
PTY74	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-2 [rho⁰]*	This study
PTY75	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-3 [rho⁰]*	This study
PTY76	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-4 [rho⁰]*	This study
PTY77	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-5 [rho⁰]*	This study
PTY78	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 YNT3-1 [rho⁰]*	This study
PTY90	*MATa ura3-52 lys2 leu2-3,112 trp1-Δ1 atp3-5 [rho⁺, TRP1]*	This study
PTY93	*MATa ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 YNT3-1 [rho⁺, TRP1]*	This study
PTY100	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp3-5 [rho⁺, TRP1]*	This study
PTY109	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP3-1 [rho⁺, TRP1]*	This study
REY2	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ ATP3::URA3 [rho⁺, TRP1]*	This study
REY3	*MATa ura3-52 ade2 leu2-3,112 trp1-Δ1 atp3-Δ1::LEU2 [rho⁺, TRP1]*	This study
777-3A	*MATa ade1 op1 [rho⁺]*	THOMAS D. FOX

[a] The mitochondrial genotype is bracketed.

[b] The allele of *ATP3* in PTY77, PTY90 and PTY100 is dominant with respect to suppression of the *yme1* rho⁰ slow growth phenotype and recessive with respect to an inability of strains bearing that allele to use nonfermentable carbon sources. Hence, the "*ATP3-5*" designation is used when discussing the suppression activity of that allele and the "*atp3-5*" designation is used when discussing the inability of strains to use nonfermentable carbon sources.

to grow at a wild-type rate. We demonstrate here that mutations in the gamma subunit of the mitochondrial ATP synthase suppress the rho⁰ slow-growth phenotype associated with the *yme1* mutation.

MATERIALS AND METHODS

Strains, strain constructions and genetic methods: The *Escherichi coli* strain used for preparation and manipulation of DNA was DH5α [F-, *endA1, hsdR17(rk-mk+), supE44, thi-1, λ recA, gyrA96, relA1, Δ(argF-laczya), U169, φ80 lac ZΔM15*].

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 (SHERMAN *et al.* 1986).

Media: *E. coli* containing plasmids were grown in LB (10g bactotryptone, 10 g NaCl, 5 g yeast extract per liter) plus 125 μg/ml ampicillin. Yeast were grown in complete glucose medium (YPD), complete ethanol and glycerol medium (YPEG), or minimal glucose medium plus the indicated nutrients (SD) (THORSNESS and FOX 1993). Where indicated, ethidium bromide was included in SD media at a concentration of 25 μg/ml. Ampicillin, ethidium bromide and nutrients were obtained from Sigma.

Nucleic acid techniques and plasmid constructions: Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Labs and New England Biolabs. Standard techniques for generating recombinant DNAs and performing DNA blot hybridizations were used (MANIATIS *et al.* 1982). DNA sequence was determined on double-stranded templates using the nucleotide chain termination method (SANGER *et al.* 1977). Templates were generated from pATP3 [a 1.5-kb *XbaI/NdeI* fragment in pRS316 (SIKORSKI and HIETER 1989)] by subcloning with restriction enzymes (see below). Several gaps in the sequence were closed using synthetic oligonucleotide primers.

The polymerase chain reaction was carried out on the *ATP3* locus with Taq DNA polymerase. Chromosomal DNA from PTY44, PTY73, and PTY100 were prepared as described (SHERMAN *et al.* 1986). The oligomers used to amplify *ATP3* had the sequences: 5′-AACATTATTTTATTTAGTACATATG-TTGTCAAGAATTGTA-3′ and 5′-GTTCTACAAAAACAAC-GTC-3′. PCR products were purified from an agarose gel and ligated into pBluescript (Stratagene) and the sequence of the insert DNA determined.

Isolating suppressors of *yme1* rho⁰ lethality: Approximately 2 × 10⁸ yeast cells from six independent YPD cultures of the *S. cerevisiae* strain PTY52 (*yme1-Δ1::URA3*) were used to inoculate 500 ml of SD media containing 25 μg/ml ethidium bromide. These cultures were grown to saturation at 30° over several days. Ten milliliters of each culture were transferred to 1 liter of fresh SD media containing 25 μg/ml ethidium bromide. These cultures were also incubated at 30° with vigorous shaking until grown to saturation, which for several of the cultures required 10 days. A sterile loop was used to streak a sample of these cultures to single colonies on SD plus 25 μg/ml ethidium bromide plates. A single fast-growing colony was isolated from each culture and colony purified by restreaking on SD plus 25 μg/ml ethidium bromide plates three times. These six independent revertant strains were designated PTY73–PTY78.

Isolation of *ATP3* DNA: The *atp3–5* suppressor had a recessive collateral phenotype, an inability to grow on nonfermentable carbon sources. The yeast strain PTY100, bearing the *atp3–5* and *ura3–52* mutations was transformed with a YCp50-based genomic library (ROSE *et al.* 1987). Approximately 10,000 Ura⁺ transformants were obtained, replica plated to YPEG, and incubated at 30°. Two transformants were capable of growth on YPEG. Recovery and restriction analysis of one of the plasmids, pYNT2, identified a 6.4-kb chromosomal fragment carried on the plasmid. Subsequent subcloning and transformation of PTY100 defined a 1.5-kb fragment that complemented *atp3–5*.

RESULTS

Effects of ethidium bromide on growth of wild type and mutant yeast strains: Wild-type, *op1*, and *yme1* mu-

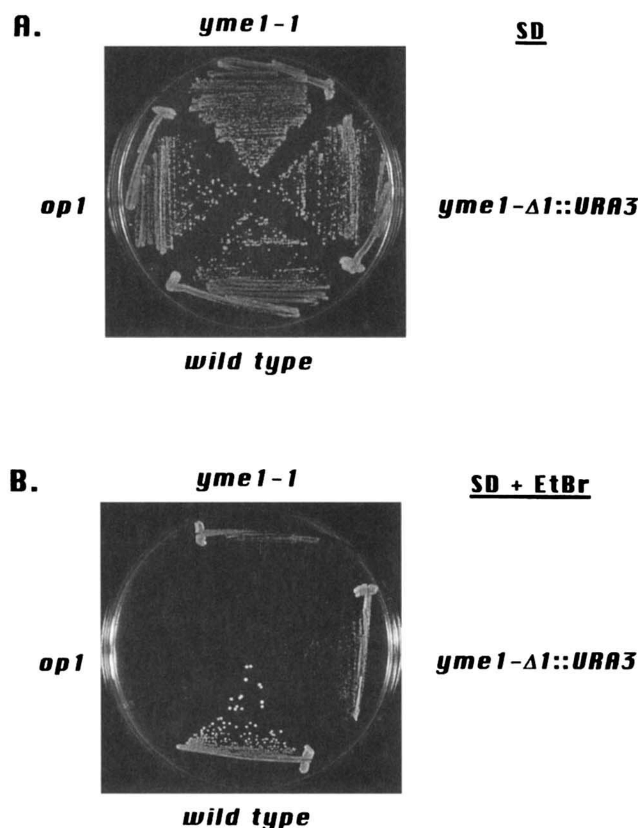

FIGURE 1.—Loss of mitochondrial DNA inhibits growth of *op1* and *yme1* mutant yeast. Yeast bearing the point mutation *yme1-1* (PTY62), the null mutation *yme1-Δ1:: URA3* (PTY52), the *op1* mutation (777-3A), and a wild-type yeast strain (PTY44) were streaked on either a synthetic glucose plate (A) and incubated for 3 days or a synthetic glucose plate containing 25 μg/ml ethidium bromide (B) and incubated at 30° for 7 days.

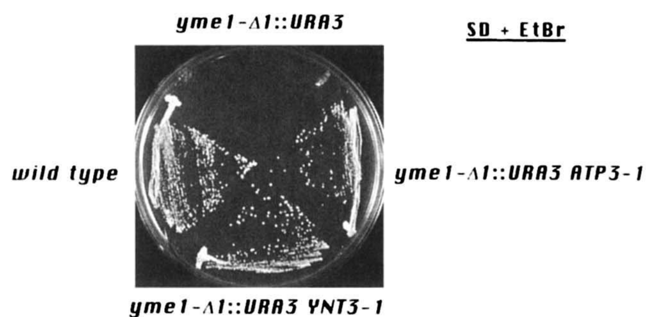

FIGURE 2.—Suppression of the *yme1* rho^0 slow-growth phenotype by *ATP3-1* and *YNT3-1*. Yeast cells were streaked onto synthetic glucose media containing 25 μg/ml ethidium bromide and incubated at 30° for 4 days. Wild type (PTY44); *yme1-Δ1:: URA3* (PTY52); *yme1-Δ1:: URA3 ATP3-1* (PTY109); *yme1-Δ1:: URA3 YNT3-1* (PTY93).

tant yeast were cultured on synthetic glucose media containing 25 μg/ml ethidium bromide (Figure 1). Growth of yeast in the presence of ethidium bromide leads to the rapid loss of mtDNA (SLONIMSKI *et al.* 1968). Previous work has demonstrated that *op1* yeast strains rapidly become inviable when mtDNA is lost (KOVACOVA *et al.* 1968). As shown in Figure 1, the loss of mtDNA as a result of culturing in the presence of ethidium bromide completely inhibits growth of *op1* yeast. Growth of *yme1* strains in the presence of ethidium bromide is also severely inhibited, although *yme1* rho^0 strains are still viable. It was previously reported that *yme1* cells became inviable if the cells became rho$^-$ or rho^0 (THORSNESS *et al.* 1993). In the original experiments, *yme1* rho^0 strains were incubated for 3 or 4 days. We have found that *yme1* rho^0 mutant strains form visible colonies on agar plates only after 7-10 days of incubation at 30°.

Isolation and genetic characterization of second-site suppressors of *yme1* rho^0 slow-growth phenotype: To identify suppressors of the *yme1* rho^0 slow-growth phenotype, a *yme1-Δ1:: URA3* mutant strain was cultured in SD media containing 25 μg/ml ethidium bromide. Six independent revertant strains were isolated that grew as well as wild-type yeast on minimal media containing glucose and ethidium bromide. Only the slow-growth phenotype found in *yme1* rho^0 strains was suppressed in these revertant strains (Figure 2). Other phenotypes associated with *yme1* mutations (THORSNESS *et al.* 1993), a high rate of DNA escape from mitochondria, temperature-sensitive growth on nonfermentable carbon sources, and cold-sensitive growth on rich glucose media, were not suppressed in these revertant strains (Figure 3). There are two possible ways to suppress the slow growth of a *yme1* mutant when cultured in the presence of ethidium bromide: a genetic change that allows *yme1* cells to grow well in the absence of mtDNA or a genetic change that allows the yeast to maintain its mtDNA despite the presence of ethidium bromide. A mutation has been reported that partially prevents loss of mtDNA when cultured in low concentrations of ethidium bromide (CHOW and KUNZ 1991), however no strains of *S. cerevisiae* have been reported that are resistant to the loss of mtDNA in the presence of 25 μg/ml ethidium bromide. Additionally, our revertants were unable to utilize nonfermentable carbon sources such as ethanol or glycerol for growth and did not form respiring diploids when mated to a rho^0 of opposite mating type. Thus, these isolates had apparently lost at least some of their mtDNA and were in fact suppressing the slow-growth phenotype associated with the *yme1* rho^0 double mutant.

Each suppressed strain was backcrossed to the isogenic parent strain (*yme1-Δ1:: URA3*) of opposite mating type. For all of the strains, the suppressing mutation allowed growth of the homozygous *yme1-Δ1:: URA3* diploid strain in the presence of ethidium bromide, indicating that the suppressor was dominant. When the suppressed strains were crossed to each other, sporulated and the tetrads dissected, two linkage groups were identified. Five of the isolates, PTY73-PYT77, were suppressed by virtue of a mutation occurring in a gene designated *ATP3* (for reasons described below). The mutation in the sixth isolate (PTY78) occurred in a gene designated *YNT3* (Figure 2). Both *ATP3* and *YNT3*

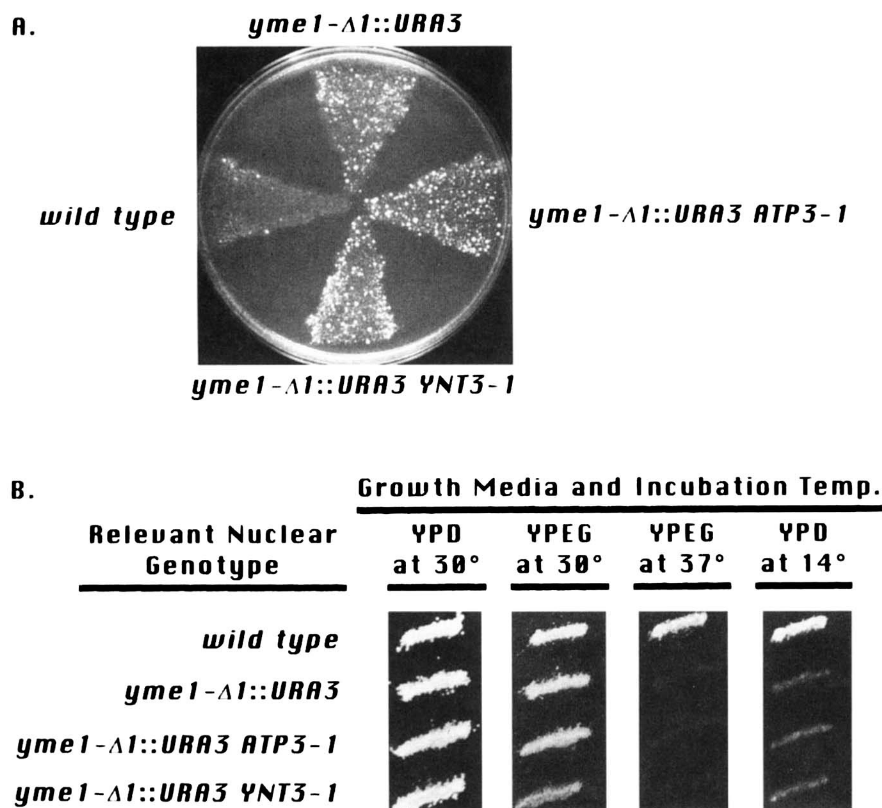

FIGURE 3.—Suppressors of the *yme1* rho^0 slow-growth phenotype do not suppress other *yme1* phenotypes. (A) Detection of DNA escape from mitochondria to the nucleus. Confluent sectors were grown on rich glucose medium, and replica plated to SD medium supplemented with adenine, uracil and lysine (the plate shown in the figure) to detect Trp$^+$ colonies resulting from the escape of *TRP1* from mitochondria. The plate was photographed after 5 days incubation at 30°. (B) Growth phenotypes associated with wild-type and *yme1* yeast. Yeast strains with the indicated phenotypes were patched onto rich glucose media (YPD) and transferred to rich glucose media or to rich ethanol/glycerol media (YPEG). The plates were then incubated for 2 days for cells grown at 30°, three days for cells grown at 37°, and for 5 days for cells grown at 14°. Wild type (PTY44); *yme1-Δ1*:: *URA3* (PTY52); *yme1-Δ1*:: *URA3 ATP3–1* (PTY109); *yme1-Δ1*:: *URA3 YNT3–1* (PTY93).

are unlinked to *YME1*, because unsuppressed *yme1-Δ1*:: *URA3* spores were recovered in a backcross to an isogenic wild type.

In addition to the dominant suppression of the *yme1* rho^0 slow-growth phenotype, some *ATP3* alleles exhibited collateral phenotypes. Yeast strains carrying the *ATP3–5* suppressor allele had an intrinsic, recessive phenotype: the inability to use nonfermentable carbon sources for growth (Figure 4). This phenotype was independent of the genotype of the *YME1* locus. Yeast

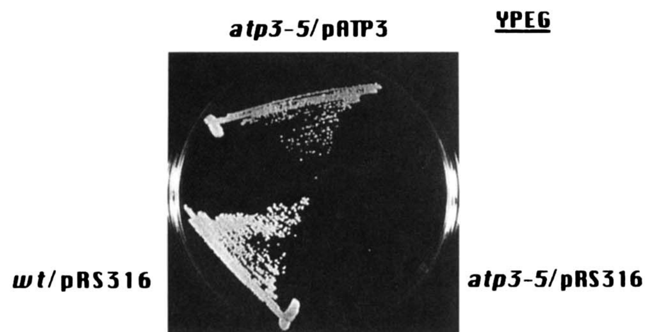

FIGURE 4.—Complementation of the intrinsic respiratory defect of *atp3–5* by the cloned *ATP3* gene. Yeast cells containing a plasmid bearing the minimal complementing fragment of the *ATP3* locus (pATP3) or the vector (pRS316) were cultured on rich media containing ethanol and glycerol as carbon sources. *atp3–5*/pATP3 is yeast strain PTY100 containing the pATP3 plasmid. *atp3–5*/pRS316 is yeast strain PTY100 containing the vector. wt/pRS316 is the wild-type yeast strain PTY44 containing the vector. The cells were incubated on rich ethanol glycerol media (YPEG) at 30° for 4 days.

strains that carried either *ATP3–3* or *ATP3–4* and *yme1* grew poorly on nonfermentable carbon sources. This recessive phenotype was linked to the *ATP3–3* and *ATP3–4* loci and was not evident if the strains had a wild-type *YME1* gene. Yeast strains carrying the *ATP3–1* and *ATP3–2* alleles had no collateral phenotypes.

To test whether the suppressors of the *yme1* rho^0 slow-growth phenotype were more general suppressors of rho^0 lethality, we tested whether *ATP3–1* could suppress the rho^0 lethality associated with mutations in *op1*. The *op1* mutation results in the loss of the mitochondrial ADP/ATP translocator (KOLAROV *et al.* 1990), and strains bearing the *op1* mutation are very sensitive to the loss of mitochondrial DNA and do not grow in the presence of ethidium bromide (Figure 1). An *op1* strain was crossed to a strain bearing the *ATP3–5* suppressor allele. The resulting diploid was sporulated and four complete tetrads displaying cosegregation of the respiratory growth defect phenotypes of the *op1* and *ATP3–5* alleles (nonparental ditype tetrads) were assayed for the ability to grow in the presence of ethidium bromide. Those spores containing both the *op1* and *ATP3–5* alleles (the nonrespiring spores in the nonparental ditype tetrads) were unable to grow in the presence of ethidium bromide. We thus concluded that *ATP3–5* did not suppress the lethal consequences of the loss of mtDNA in an *op1* strain.

Isolation and characterization of the *ATP3* gene: The *ATP3–5* suppressor had a recessive collateral phenotype, an inability to grow on nonfermentable carbon sources. This phenotype enabled us to isolate a comple-

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Atp3p (Yeast)   MLSRIVSNNA TRSVMCHQAQ VGILYKTNPV RTYATLKEVE MRLKSIKNIE   50
UncG (E. coli)                                      MAGAKEIR SKIASVQNTQ   18

Atp3p (Yeast)   KITKTMKIVA STRLSKAEKA KISAKKMDEA EQLFYKNAET KNLDVEAT--   98
UncG (E. coli)  KITKAMEMVA ASKMRKSQDR MAASRPYAET MRKVIGHLAH GNLEYKHPYL   68

Atp3p (Yeast)   ETGAPKEL-I VAITSDKGLC GSIHSQLAKA VRRHLNDQPN ADIVTIGDKI  147
UncG (E. coli)  EDRDVKRVGY LVVSTDRGLC GGLNINLFKK LLAEMKTWTD KGVQCDLAMI  118

Atp3p (Yeast)   KMQLLR-THP NNIKLSINGI GK-DAPTFQE SALIADKLLS VMKAGTYPKI  195
UncG (E. coli)  GSKGVSFFNS VGGNVVAQVT GMGDNPSLSE LIGPVKVMLQ AYDEGRLDKL  168

Atp3p (Yeast)   SIFYNDPVSS LSFEPSEKPI FNAKTIEQSP SFGK---FEI DTDAN-VPRD  241
UncG (E. coli)  YIVSNKFINT MSQVPTISQL LPLPASDDDD LKHKSWDYLY EPDPKALLDT  218

Atp3p (Yeast)   LFEYTLANQM LTAMAQGYAA EISARRNAMD NASKNAGDMI NRYSILYNRT  291
UncG (E. coli)  LLRRYVESQV YQGVVENLAS EQAARMVAMK AATDNGGSLI KELQLVYNKA  268

                     *    -
Atp3p (Yeast)   RQAVITNELV DIITGASSLG                                   311
UncG (E. coli)  RQASITQELT EIVSGAAAV                                    287
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FIGURE 5.—Alignment of Atp3p and UncG amino acid sequences. Identical and highly conserved amino acids are boxed. The conserved threonine that is changed to an alanine in strains bearing the *atp3–5* allele is marked (*). The conserved isoleucine that is changed to a threonine in strains bearing the *ATP3–1* allele is marked by (¯). The Atp3p and UncG (gamma subunit of the ATP synthase from *E. coli*) amino acid sequences were aligned using the computer program GeneWorks 2.2 from IntelliGenetics. The annotated *ATP3* sequence can be found in GenBank, accession number U08318. The UncG sequence is from (Saraste *et al.* 1981). Similarities: L = I = V; K = R; S = T.

menting plasmid that contained the wild type form of the suppressor from a yeast genomic library. The yeast strain PTY100, bearing the *atp3–5* and *ura3–52* mutations was transformed with a YCp50-based genomic library (ROSE *et al.* 1987). Approximately 10,000 Ura$^+$ transformants were obtained, replica plated to YPEG, and incubated at 30°. Two transformants were capable of growth on YPEG. Total DNA was prepared from these transformants, and their plasmids were recovered by transformation of *E. coli.* Reintroduction of these plasmids into PTY100 demonstrated that they complemented the inability to utilize nonfermentable carbon sources caused by *atp3–5*. Restriction analysis of these plasmids revealed them to be identical. Further analysis of this plasmid, pYNT2, revealed a 6.4-kb insert. Analysis of subclones identified a 1.5-kb *Xba*I/*Nde*I fragment (pATP3) complemented the *atp3–5* mutation (Figure 4). This fragment was inserted into the integrating *URA3* vector pRS306 (SIKORSKI and HIETER 1989). The resulting plasmid, pRE3, was linearized within the insert using the restriction endonuclease *Pml.* This linear DNA was used to transform the wild-type strain PTY44. A Ura$^+$ transformant, REY2, was mated to the *atp3–5* strain PTY90, and the resulting diploid was sporulated. There were no recombinants among 20 tetrads that exhibited both uracil prototrophy and an inability to grow on nonfermentable carbon sources, indicating that the cloned chromosomal DNA corresponded to the *ATP3* locus.

The 1.5-kb fragment that complemented the *atp3–5* mutation was subcloned into the plasmid pBluescript and sequenced on both strands. This sequence had a single large open reading frame of 933 nucleotides that encoded a protein of 311 amino acids with a predicted relative molecular weight of 34,000 D (Figure 5). The amino acid sequence of the predicted protein was used to search sequence data bases (BILOFSKY and BURKS 1988). Significant homology between this ORF and the gamma subunit of the mitochondrial ATP synthase from many different organisms led us to name the gene *ATP3.* A comparison of the sequence of Atp3p and the gamma subunit of ATP synthase from *E. coli* is shown in Figure 5. *ATP3* has also been independently cloned by another research group (PAUL *et al.* 1994). The yeast genome sequencing effort has revealed *ATP3* to be located on the right arm of chromosome *II* (H. FELDMANN, unpublished data).

Characterization of suppressing mutations in *ATP3*: Total genomic DNA was prepared from wild-type (PTY44), *ATP3–1* (PTY73) and *atp3–5* mutant yeast (PTY100), and the *ATP3* locus was amplified by the polymerase chain reaction (PCR). The PCR products were cloned into pBluescript and the sequence of three independent plasmids derived from the wild-type and mutant strains was determined. The only difference in sequence between the wild-type *ATP3* locus and the mutant *atp3–5* locus occurred in codon 297 of the *ATP3* open reading frame. The first position had been changed from an A to a G, resulting in a threonine codon being replaced by an alanine codon. Therefore, both the dominant suppression of the *yme1* rho^0 slow-growth phenotype and the recessive inability to utilize nonfermentable carbon sources in the *atp3–5* strain resulted from a threonine to alanine change at amino acid 297 in Atp3p. The *ATP3–1* locus was also changed at a single site in the 3′ end of the open reading frame. The change, a T to a C, occurred at the second position of codon 303 and converted a conserved isoleucine to a threonine. This change in the *ATP3–1* allele was able to suppress the *yme1* rho^0 slow-growth phenotype without having a detectable affect on the ability of strains bearing this allele to utilize nonfermentable carbons sources.

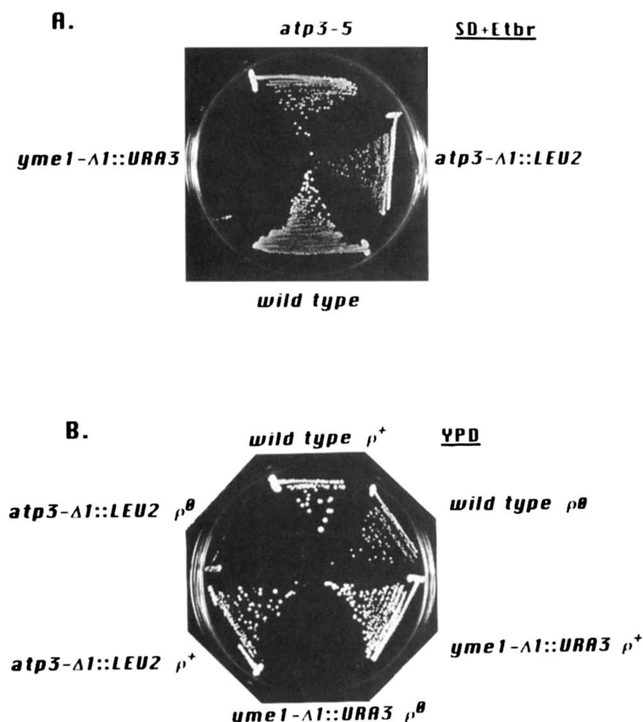

FIGURE 6.—Growth of rho^0 yeast bearing null alleles of *yme1* or *atp3*. Yeast were streaked on synthetic glucose media containing 25 μg/ml ethidium bromide and grown for 4 days at 30° and then recultured onto the same media. Single colonies arising on these plates after 7 days (A) were then picked and streaked on the YPD plates (B) and grown for 4 days at 30°. Reculturing yeast in the ethidium bromide containing media generates rho^0 strains. *atp3-5* (PTY100); *atp3-Δ1::LEU2* (REY3); *yme1-Δ1::URA3* (PTY52); wild type (PTY44).

Generation and characterization of an *atp3* null mutation: To determine the phenotypic consequences of an *ATP3* null mutation (*atp3-Δ1::LEU2*), 635 nucleotides of *ATP3* coding sequence, including the initiating methionine, were deleted and replaced by the *LEU2* gene. The resulting DNA fragment carried *LEU2* flanked by ~345 nucleotides of 5′ *ATP3* sequence, and ~525 nucleotides of 3′ *ATP3* sequence (including the C-terminal 214 codons). This DNA, in a linear form, was used to transform the wild-type strain PTY33. Several Leu$^+$ transformants were purified and analyzed. Each of the transformants displayed a respiratory deficient phenotype. Although *atp3-Δ1::LEU2* mutants were able to grow in the presence of ethidium bromide, these strains grew very poorly after this treatment, indicating that *atp3-Δ1::LEU2* mutants also displayed a rho^0 slow-growth phenotype. The *atp3-Δ1::LEU2* rho^0 slow-growth phenotype was intermediate between a wild-type strain and a *yme1* rho^0 double mutant (Figure 6). The *yme1-Δ1::URA3 atp3-Δ1::LEU2* strain grew very slowly on any media (data not shown). We have found that *atp3-Δ1::LEU2* strains rapidly become rho$^-$ or rho^0. The *yme1-Δ1::URA3 atp3-Δ1::LEU2* strains are thus likely to be slow growing as a consequence of rho$^-$/rho^0 formation in the *yme1Δ* background.

DISCUSSION

To better understand the slow-growth phenotype of *yme1* rho^0 double mutants and the general phenomenon of rho^0 lethality, we have undertaken a genetic analysis to isolate second site suppressors of the slow-growth phenotype in *yme1* rho^0 yeast. Using this approach we have isolated several strains that have a dominant suppressing mutation in the gamma subunit of the mitochondrial ATP synthase (Atp3p). Mitochondrial ATP synthase can be separated into two distinct multisubunit complexes, the soluble F_1 and the membrane-bound F_0. The gamma subunit is an important constituent of F_1, playing a role in assembly of the synthase and in the synthesis or hydrolysis of ATP by F_1 (PEDERSEN and AMZEL 1993). It has also been implicated in controlling the flow of protons through the membrane via F_0 (ZANOTTI *et al.* 1992). The mutations we identified in *ATP3* that suppress the slow-growth phenotype of *yme1* rho^0 strains do not suppress any of the other phenotypes associated with a *yme1* mutant (Figure 3), including an increased rate of DNA escape from mitochondria to the nucleus (THORSNESS and FOX 1993) and morphological changes seen in mitochondria of a *yme1* strain (CAMPBELL *et al.* 1994). Deletion of the *ATP3* locus renders a haploid yeast strain unable to utilize nonfermentable carbon sources. Yeast containing a deleted *ATP3* locus and lacking mtDNA also grow very slowly (Figure 6).

Sequence elements contained within Yme1p and similarity of Yme1p to a family of 26S protease subunits have led us to propose a model in which Yme1p is an ATP- and zinc-dependent protease (CAMPBELL *et al.* 1994). Our data is consistent with the putative Yme1p protease acting either as a processing enzyme to modify protein substrates or as a protease responsible for the turnover of excess or damaged proteins. This latter model assigns a function for Yme1p analogous to that postulated for a closely related homologue, Yta10p. This yeast protein is proposed to have a direct role in the proteolytic breakdown of membrane-associated polypeptides in mitochondria (PAJIC *et al.* 1994).

The accumulation of unprocessed, defective, or excess mitochondrial proteins in yeast lacking Yme1p could result in the pleiotropic phenotypes observed in *yme1Δ* strains. Modification of the gamma-subunit by mutation compensates for some undefined defect in mitochondrial metabolism, perhaps in the F_1-ATPase itself, that is evident when *yme1* yeast lack mtDNA. Conversion of a strictly conserved threonine residue (thr-297) to alanine or conversion of an isoleucine residue (ile-303) to threonine near the C terminus of Atp3p are two examples of *ATP3* mutations that bypass the need for Yme1p (Figure 5). Strikingly similar mutations affecting *uncG* (the gamma subunit of ATP synthase) have been isolated in *E. coli*. Conversion of threonine-273 (corresponding to the conserved threonine changed in the *ATP3-5* allele) to serine and conversion

of isoleucine-279 (corresponding to the conserved isoleucine changed in *ATP3-1*) to threonine, suppress the effects of a mutation near the amino-terminus of the *uncG* gene product (NAKAMOTO *et al.* 1993).

In *yme1* rho$^+$ strains, the biochemical defect(s) affecting the mitochondrial ATP synthase are either absent or less critical as this complex is functional: *yme1* rho$^+$ yeast can grow on nonfermentable carbon sources at 30° (Figure 3). However, in *yme1* rho^0 strains, improper processing or accumulation of some mitochondrial protein(s) is very deleterious, perhaps because the higher order structure or activity of the F_1 complex is not maintained or properly regulated. Rho^0 and rho$^-$ yeast strains lack important structural proteins for the F_0 portion of the mitochondrial ATP synthase (mitochondrial encoded genes *ATP6, 8,* and *9*). Perhaps interactions between F_0 and F_1 complexes result in a sufficiently stabilized structure or regulated activity of an F_1 complex compromised by the lack of Yme1p activity.

Biochemical and genetic analysis of bacteria has led to the conclusion that the gamma subunit of ATP synthase is important in regulating proton flow through the proton channel (ZANOTTI *et al.* 1992). Presumably, Atp3p plays a similar role in mitochondria of yeast in rho$^+$ strains. However, in rho^0 strains this is an unlikely role for Atp3p because the mitochondrial encoded subunits of the F_0 proton channel are not present, and electron transport does not occur. Recently, it has been shown that the gamma subunit of the mitochondrial ATP synthase from *S. cerevisiae* is involved in the assembly/stability of the F_1 portion of the ATP synthase (PAUL *et al.* 1994). Our data suggest that Atp3p is also important in cells that are not respiring. The observation that suppression of slow-growth in *yme1* rho^0 yeast occurs in a dominant fashion suggests an active role for Atp3p and the F_1 complex of ATP synthase in the proper functioning of mitochondria in rho^0 yeast. The observation that strains lacking Atp3p also display a slow-growth phenotype when mitochondrial DNA is absent provides additional evidence of a function for Atp3p in rho^0 yeast. The simplest explanation for these data is that the F_1 complex, even in the absence of a recognizable F_0 complex, plays a role in mitochondrial metabolism. This role presumably involves an assembled F_1 complex in the soluble fraction of the mitochondrial matrix. However, it is not clear what biochemical role a F_1 complex would play in rho^0 mitochondria. CLARKSON and POYTON (1989) have shown that perturbations in the proton gradient affect the biogenesis of cytochrome oxidase subunit II. Perhaps, in an analogous fashion, mutations affecting the structural integrity or activity of the ATP synthase have pleiotropic effects on mitochondrial function.

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