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Mitochondrial Morphological and Functional Defects in Yeast Caused by *yme1* Are Suppressed by Mutation of a 26S Protease Subunit Homologue

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The absence of functional Yme1p, a putative ATP and zinc-dependent protease localized to mitochondria of yeast, results in abnormal mitochondrial function and morphology. Yeast lacking Yme1p lose DNA from mitochondria at an accelerated rate, fail to grow on nonfermentable carbon sources at 37°C, and have severely deficient growth if mitochondrial DNA suffers large deletions or is completely lost. In place of the normal reticulated mitochondrial network, strains lacking Yme1p have punctate mitochondria with some grossly swollen compartments. The growth phenotypes and morphological alterations evident in these mutant yeast can be compensated by a mutation in *YNT1*, an essential gene in yeast. The sequence of the *YNT1* gene product indicates that it is one of a number of related regulatory subunits of the 26S protease. This proteolytic activity is necessary for progression through the cell cycle and has been implicated in the regulation of transcription. Ynt1p is more distantly related to Yme1p.

INTRODUCTION

In the budding yeast *Saccharomyces cerevisiae*, mitochondrial morphology and function vary according to carbon source and cell-cycle stage (Stevens, 1977, 1981). Using genetic analyses, a number of genes involved in these dynamic processes have been isolated (McConnell *et al.*, 1990; McConnell and Yaffe, 1992; Thorsness and Fox, 1993). One such gene is *YME1*, which encodes a protein necessary for maintaining the integrity of the mitochondrial compartment (Thorsness *et al.*, 1993).

The nuclear-encoded *YME1* gene encodes a mitochondrially localized protein with a predicted size of 82 kDa (Thorsness *et al.*, 1993). According to sequence analysis, Yme1p contains sequence elements suggestive of ATPase and zinc protease activities. The gene encoding Yme1p was originally isolated in a genetic screen that identified mutations that cause an increased rate of DNA escape from the mitochondria to the nucleus in yeast (Thorsness and Fox, 1993).

The absence of functional Yme1p, whether because of the original mutant allele, *yme1-1* or the deletion allele *yme1-Δ1::URA3*, (Thorsness *et al.*, 1993), results in four phenotypes in yeast: an increased rate of DNA escape from mitochondria with subsequent migration to the nucleus, an inability to utilize nonfermentable carbon sources such as ethanol or glycerol at 37°C, slow growth on complete glucose media at 14°C, and severely compromised growth when mitochondrial DNA is deleted or lost entirely (Thorsness and Fox, 1993; Thorsness *et al.*, 1993) (Figure 1).

To further characterize Yme1p, we used suppressor analysis to isolate possible interacting gene products. Suppressing mutations that bypassed the requirement for Yme1p were isolated in a *yme1-Δ1::URA3* strain by selecting for revertants that grew on nonfermentable carbon sources at 37°C. One such reverting mutation, *ynt1-1*, suppressed all four phenotypes associated with *yme1-Δ1::URA3*. In addition, the mitochondrial morphological abnormalities of a *yme1* strain are partially reversed by this mutation. Here, we describe the isolation and characterization of *YNT1* in yeast. *YNT1* was identified by virtue of the mutant allele *ynt1-1*, a recessive bypass suppressor with an intrinsic cold-sensitive growth phenotype on nonfermentable carbon sources. Cloning and sequencing revealed that *YNT1* is identical to a previously sequenced gene, *YTA2*, and exhibits se-

quence homology to genes that may be involved in cell-cycle regulation.

MATERIALS AND METHODS

Strains

The *Escherichia 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 for 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 (10 g bactotryptone, 10 g NaCl, 5 g yeast extract/l) plus 125 μg/ml ampicillin. Yeast were grown in YPD (20 g glucose, 20 g bactopeptone, 10 g yeast extract, and 40 mg of tryptophan), YPEG (30 ml glycerol, 30 ml of ethanol, 20 g of bactopeptone, 10 g of yeast extract, and 40 mg of tryptophan), or SD + nutrients (6.7 g of yeast nitrogen base without amino acids, 20 g of glucose, and the appropriate nutrients). Nutrients included uracil at 40 mg/l, adenine at 40 mg/l, tryptophan at 40 mg/l, lysine at 60 mg/l, and leucine at 100 mg/l. For agar plates, bactoagar was added at 20 g/l. Ethidium bromide containing medium is SD + nutrients supplemented with 25 μg/ml ethidium bromide. One liter of sporulation media contained 10 g potassium acetate, 1 g yeast extract, 0.5 g glucose, and 20 g bactoagar. Bactoagar, bactopeptone, bactotryptone, yeast extract, and yeast nitrogen base without amino acids were obtained from Difco (Detroit, MI). Ampicillin and nutrients were obtained from Sigma (St. Louis, MO).

Growth Assays

The escape of DNA from mitochondria was assayed as described (Thorsness and Fox 1993). Briefly, yeast containing a copy of *TRP1* integrated into the mitochondrial genome are grown on rich media (YPD or YPEG) agar plates and then replica plated to minimal media (SD + nutrients) lacking tryptophan. Those cells in which DNA-containing *TRP1* sequences have escaped mitochondria and migrated to the nucleus give rise to colonies by virtue of complementation of the *trp1-Δ1* allele in the nucleus. Strains in which DNA escapes mitochondria at a higher rate than in wild-type yeast have an increased amount of papillation to tryptophan prototrophy on the minimal media plates after incubating for 72 h at 30°C.

The ability of yeast strains to grow in the absence of mitochondrial DNA was assayed by culturing the cells on minimal media agar plates containing 25 μg/ml of ethidium bromide. Cells are streaked to single colonies on the plates and incubated for 72 h at 30°C. Then a single colony is restreaked a second time on the same media. These plates are then scored after a second 72-h incubation at 30°C. We have found that single colonies arising on these ethidium bromide–containing plates lack mitochondrial DNA.

The cold-sensitive phenotype of yeast strains was scored after incubation of cells at 14° for 10–14 d. The temperature-sensitive phenotype of yeast strains was scored after incubation of cells at 37°C for 48 h.

Microscopy

For confocal microscopy, strains were cultured in YPEG or enriched galactose at 30 or 37°C and harvested in log phase. $DiOC_6$ (3,3'-dihexyloxacarbocyanine iodide) was used at 1 μg/ml concentrations, a mitochondrially specific concentration. Methods and equipment were used as per Koning *et al.* (1993). Strains were also observed using DAPSMI, 2-(4-dimethylaminostyryl)-N-methylpyridinium iodide, on a Nikon epifluorescence microscope (Garden City, NY) with similar results. For transmission electron microscopy strains were cultured in ethanol/glycerol at 30°C and fixed according to methods described by Byers and Goetch (1990).

Identification and Cloning of YNT1

Approximately 10^8 cells of the yeast strain PTY52 (*yme1-Δ1::URA3*) were plated onto YPEG and cultured at 37°C. Revertant cells capable of respiratory growth at 37°C were picked after 1 wk of incubation and recultured under the restrictive conditions. Multiple rounds of subculturing eventually produced three independent revertants capable of growth on nonfermentable carbon sources at the restrictive temperature, one of which, NTY1, is characterized in this work. NTY1 was backcrossed to PTY33 (wild-type) and to PTY60 (*yme1-Δ1::URA3*), the diploids sporulated, and tetrads dissected. Each colony arising from a spore was scored for nutritional markers, mating type, and the ability to grow on nonfermentable carbon sources at 14, 30, and 37°C.

Yeast strains bearing the *ynt1-1* allele grow poorly on nonfermentable carbon sources at 14°C. This phenotype was used as a basis for cloning *YNT1*. NTY4 (*ura3-52 ynt1-1*) was transformed with 100 μg of DNA prepared from a multicopy, 2 μ-based *S. cerevisiae* genomic DNA bank (a gift from Jasper Rine). Approximately 24 000 transformants were screened for complementation of the cold-sensitive respiratory deficient (Pet-cs) phenotype. Nine transformants complemented the Pet-cs phenotype. Plasmid DNA was prepared from these nine transformants and used to transform *E. coli*. Restriction mapping of the rescued plasmids showed that eight of the nine plasmids were identical. The insert DNA from one of these multicopy plasmids (pNT18) was subcloned into a *CEN* vector (pRS316) (Sikorski and Hieter 1989), and the ability of this low copy plasmid, pNT30, to rescue the Pet-cs phenotype was assayed.

Nucleic Acid Manipulations

Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Labs (Gaithersburg, MD) and New England Biolabs (Beverly, MA). 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).

Plasmid pNT30 was subcloned, and a minimal complementing insert in a low-copy plasmid was determined. pNT53 contains a 2 kilobase (kb) *Stu* I/*Xba* I insert of yeast genomic DNA in pRS315, a *CEN* plasmid (Sikorski and Hieter 1989), and is able to rescue the *pet*-cs phenotype of *ynt1-1* mutant yeast strains. An integrating plasmid containing the minimal complementing region was constructed by cloning the genomic fragment from pNT53 into pRS306, creating pNT54. pNT54 was targeted to integrate at the chromosomal locus corresponding to the insert by digestion with *Sph* I, which cuts at a unique site just 5' of the *YNT1* open reading frame.

A *ynt1-Δ1::URA3* mutation was constructed as follows. The 2-kb *Stu* I/*Xba* I fragment containing *YNT1* from pNT53 was inserted into the *Sma* I/*Xho* I sites of pBluescript KS^+ (Stratagene, La Jolla, CA). This construct was then digested with *Hinc*II, removing 60% of the *YNT1* coding region (only 30 codons of *YNT1* remain 5' to the point of insertion), and a 1.5-kb *Sma* I fragment containing *URA3* was inserted. This plasmid, pNT55, containing the *ynt1-Δ1::URA3* allele was digested with *Hind*III and *Sph* I. This linear DNA was used to transform the yeast diploid PTY33 × PTY44 to uracil prototrophy. A Ura^+ transformant was sporulated and dissected.

RESULTS

Identification and Isolation of YNT1, a Bypass Suppressor of yme1

YME1 encodes a mitochondrial protein that has putative ATPase and protease activity (Figure 3A). Yeast cells

Table 1. Yeast strains

Strain	Genotype	Source
NTY1	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ynt1-1 [ρ+, TRP1]*	This study
NTY4	*MATα ura3-52 ade2-101 leu2-3,112 trp1-Δ1 ynt1-1 [ρ+, TRP1]*	This study
PTY33	*MATa ura 3-52 ade2 leu2-3,112 trp1-Δ1 [ρ+, TRP1]*	(Thorsness and Fox, 1993)
PTY44	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 [ρ+, TRP1]*	(Thorsness and Fox, 1993)
PTY52	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 [ρ+, TRP1]*	(Thorsness *et al.*, 1993)
PTY60	*MATa ura3-52 ade2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 [ρ+, TRP1]*	(Thorsness *et al.*, 1993)
PTY61	*MATa ura3-52 ade2-101 leu2-3,112 trp1-Δ1 yme1-1 [ρ+, TRP1]*	(Thorsness and Fox, 1993)
PTY106	*MATα ura 3-52 lys2 leu2-3,112 trp1-Δ1 yme1-1 ynt1-1 [ρ+, TRP1]*	This study

lacking Yme1p have a number of phenotypes that indicate abnormal mitochondrial activity (Figures 1 and 2) (Thorsness *et al.*, 1993). To learn more about the function of this protein, a genetic approach was taken to identify other proteins or processes that could compensate for mutations in *YME1*. Initially, attempts were made to find second-site revertants of a point mutation in *YME1*, but only tightly linked (presumably intragenic) revertants were found. Therefore, spontaneous bypass revertants of a *yme1-Δ1::URA3* strain, PTY52, were isolated by culturing under the restrictive growth condition, 37°C on ethanol/glycerol medium (Thorsness and Fox, 1993; Thorsness *et al.*, 1993). One isolate, designated NTY1 and containing the nuclearly encoded suppressor *ynt1-1* (see below), suppressed all four growth phenotypes associated with *yme1* (Figure 1). Although the reverting mutation suppressed the temperature-sensitive growth phenotype (Pet-ts), growth was still less than that of wild-type under this condition (Figure 1A). Also, cold sensitivity in enriched media containing glucose or galactose was completely reversed. In addition, the suppressing mutation in NTY1 delayed onset of mitochondrial DNA (mtDNA) escape (Figure 1B), resulting in reduced papillations of colonies from Trp^- to Trp^+. The suppressor also reversed the growth deficiency of *yme1* (Figure 1C) in the presence of a partially or completely lost mitochondrial genome, as induced by growth on media containing ethidium bromide.

In backcrosses of NTY1 to the parental strain of opposite mating type (PTY60, *yme1-Δ1::URA3*), the suppression of the Pet-ts phenotype segregated 2ts::2wt in all ten tetrads dissected, indicating a single nuclear locus was responsible for the suppressor activity. This suppressor was also determined to be recessive, as evidenced by the inability of the NTY1 × PTY60 diploid to grow on nonfermentable carbon sources at 37°C. *ynt1-1* also suppressed the phenotypes of strains bearing *yme1-1*, a point mutation allele of *YME1* (Thorsness *et al.*, 1993). Backcrossing of NTY1 to PTY33 (wild-type) and sporulation allowed the recovery of Pet-ts spores (tetratype and nonparental ditype tetrads), which indicated the reverting mutation was not linked to *yme1-Δ1::URA3*. In both crosses, a Pet-cs phenotype segregated 2cs::2wt. This inability to utilize nonfermentable carbon sources at 14°C cosegregated with the suppressor locus (Figure 1A). This genetic locus was designated *YNT1* and the mutant allele *ynt1-1*.

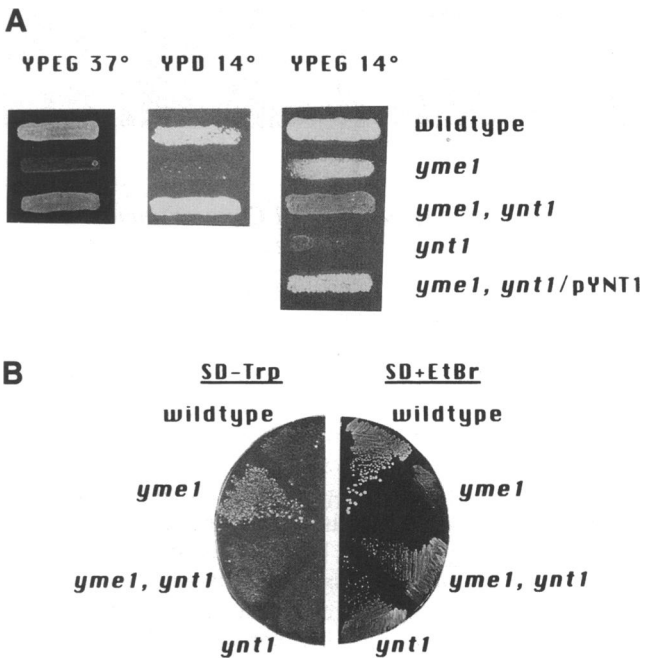

Figure 1. *ynt1-1* suppresses functional defects in mitochondria of *yme1* yeast strains. (A) Yeast with the indicated mutations were inoculated at equivalent concentrations onto YPEG or YPD and incubated at the indicated temperatures (Thorsness and Fox, 1993). A plasmid bearing *YNT1* (pYNT1) restored growth to a *yme1, ynt1-1* yeast strain on YPEG at 14°C. (B) Escape of DNA containing the *TRP1* gene from mitochondria and subsequent migration to the nucleus allows complementation of the nuclear *trp1-Δ1* mutation. The increased rate of DNA escape from mitochondria in a *yme1* strain was suppressed by *ynt1-1*. (C) Complete loss of mitochondrial DNA can be induced by culturing yeast in the presence of 25 μg/ml ethidium bromide (Thorsness *et al.*, 1993). The severe growth deficiency of *yme1* strains without mitochondrial DNA was suppressed by *ynt1-1*. Strains used in this figure are as follows: wild-type PTY44, *yme1* PTY52, *yme1 ynt1* NTY1, *ynt1* NTY4, and *yme1 ynt1*/p*YNT1* PTY106 containing pNT53.

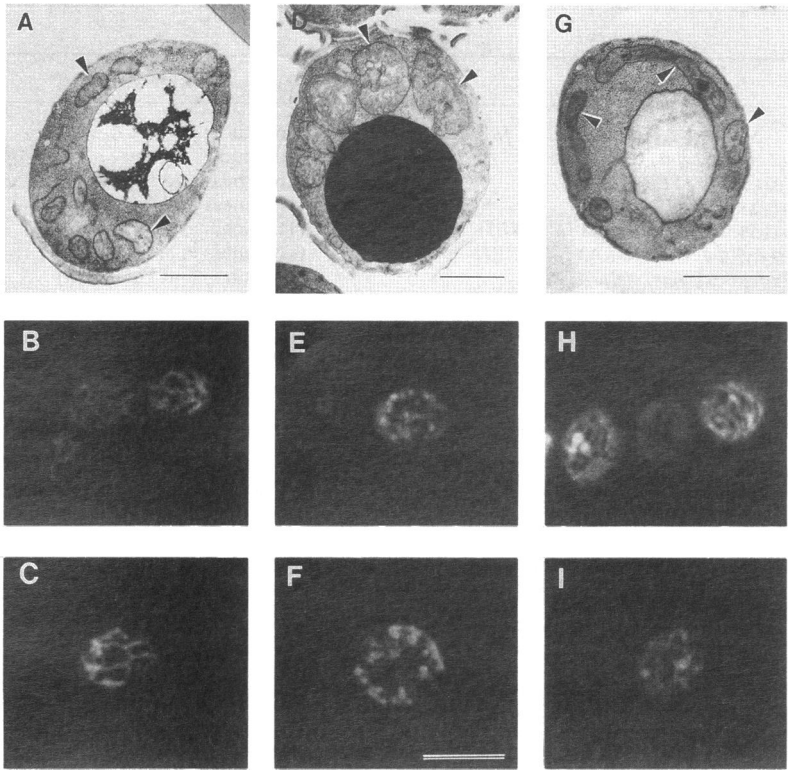

Figure 2. Morphological defects of mitochondria in a *yme1* mutant yeast strain are suppressed by the *ynt1-1* mutation. Transmission electron micrographs and confocal fluorescence images of wildtype (A-C), *yme1* (D–F), and *yme1 ynt1-1* (G–I) yeast strains revealed altered mitochondrial profiles. For the electron micrographs (A, D, and G) yeast were grown in YPEG at 30°C. Mitochondria are indicated by arrows. Bar, 1 μm. Vacuoles containing osmium-dense material (D) are occasionally detected in both wild-type and mutant yeast. For the confocal fluorescence images (B, C, E, F, H, and I), yeast were grown either in YPEG at 30°C (B, E, and H) or on rich galactose media at 37°C (C, F, and I), and mitochondria were stained with $DiOC_6$ (3,3'-dihexyloxacarbocyanine iodide). Confocal images are constructed of two to three optical sections (0.5 μm each) of a Z-series scan. Bar, 5 μm. Strains used in this figure are as follows: wild-type PTY44, *yme1* PTY52, *yme1 ynt1-1* NTY1.

Morphology of Mitochondrial Compartments in Wild-Type, yme1, and yme1/ynt1-1 Yeast

Analysis of mitochondrial compartment super-structure using confocal fluorescence and electron microscopy revealed significant morphological alterations in yeast lacking Yme1p (Figure 2). Under nonrepressing conditions in log phase, wild-type yeast have a complex reticulated network of mitochondrial compartments (Figure 2, A–C). In contrast, yeast bearing *yme1-Δ1::URA3* have abbreviated globular mitochondria with a subpopulation of the cells containing grossly swollen compartments (Figure 2, D–F). Altered mitochondrial morphology is not necessarily a consequence of mutations inhibiting oxidative phosphorylation, because several strains containing unrelated mutations affecting this process retain normal mitochondrial profiles. Also, this abnormal morphology is not merely characteristic of growth under conditions requiring respiratory growth, because punctate mitochondrial forms are evident even in a fermentable medium such as galactose (Figure 2, C, F, and I). The morphological abnormalities in a *yme1-Δ1::URA3* strain are partially reversed by the *ynt1-1* suppressor gene (Figure 2, G–I). However, revertants display shorter branching mitochondrial segments than do wild-type yeast.

The distended organelles caused by *yme1* were counted using DAPSMI staining and epifluorescence microscopy. Grossly swollen mitochondrial profiles were observed in 12% (chi square test, $p < 0.001$) of PTY52 cells observed, compared to <3% ($p < 0.001$) of the revertant strain NTY1. *ynt1-1*, when present in a wild-type *YME1* background, has no apparent affect on the mitochondrial superstructure as evidenced by epifluorescence microscopy.

Cloning and Sequencing YNT1

The cold-sensitive phenotype of *ynt1-1* strains was used to isolate the wild-type gene. *YNT1* was originally cloned from a 2-μ (multicopy) plasmid bank by complementing the cold-sensitive growth on nonfermentable carbon sources associated with *ynt1* in a plasmid-dependent fashion. However, the gene also complemented this phenotype when in a low-copy *CEN* plasmid (Figure 1A). To confirm that the isolated genomic DNA corresponded to the *ynt1-1* locus, the minimal complementing genomic fragment was cloned into an integrating *URA3* plasmid. This plasmid was targeted to integrate into the yeast genome by digesting it with the restriction endonuclease *Sph* I, which cuts just 5′ to the *YNT1* open reading frame (described below). This linear DNA was transformed into PTY61, a *yme1-1* strain. The resulting transformant was then mated to PTY106 (*yme1-1, ynt1-1*). The resulting diploid was sporulated, and tetrads were dissected. The integrated *URA3* marker segregated in opposition to the suppressor activity in 19 out of 20 tetrads examined,

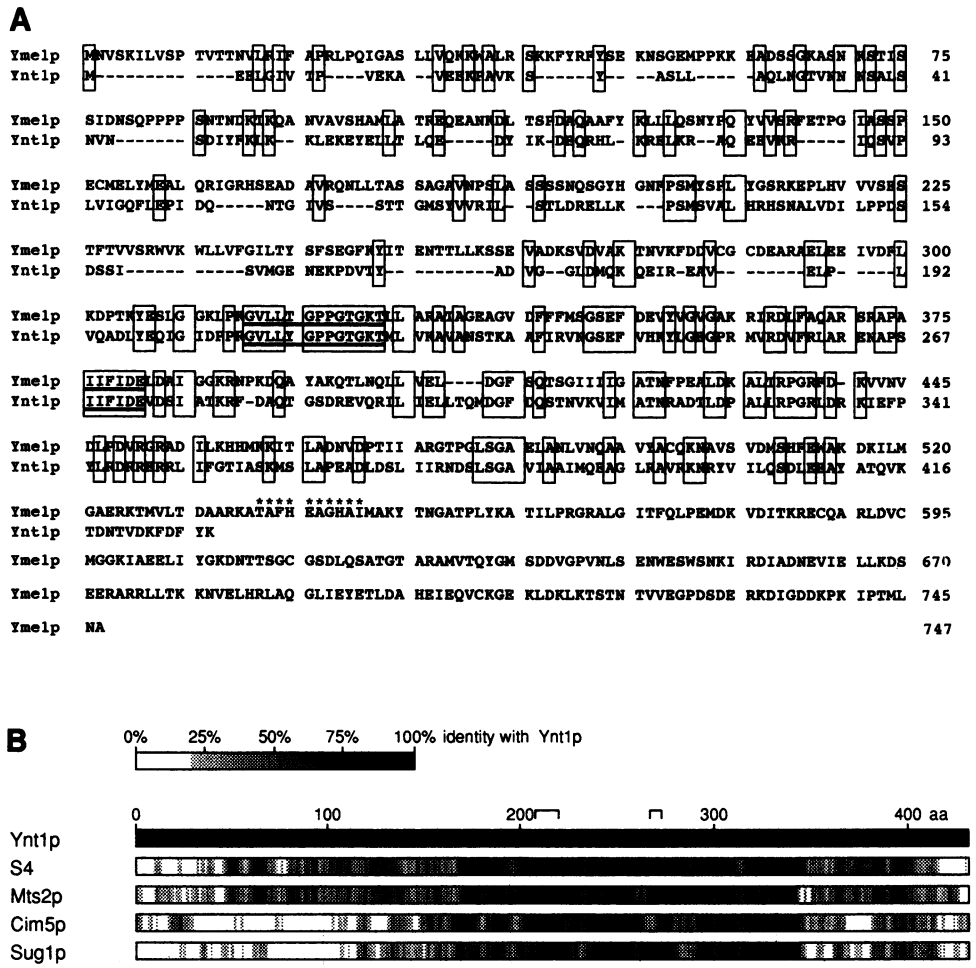

Figure 3. Ynt1p belongs to an extended putative ATPase family. (A) Alignment of Yme1p and Ynt1p amino acid sequences. Identical amino acids are boxed, the nucleotide binding motifs (Chin *et al.*, 1988) are underlined, and the putative neutral zinc protease motif is marked by *. The Ynt1p and Yme1p sequences were aligned with the aid of the computer program GeneWorks 2.2 from IntelliGenetics (Mountain View, CA); the zinc protease motif was identified by the Blocks sequence analysis program (Henikoff and Henikoff, 1991). (B) Ynt1p belongs to a subfamily of ATPase proteins that may be regulatory subunits of the 26S protease. The degree of similarity is displayed using a grayscale (Frölich, 1994). Percentage amino acid identity to Ynt1p is calculated for subsets of seven amino acids, each subset being displaced from the next by one residue, producing overlapping values of percentage identity along each sequence. Values <20% identity are displayed by white, values equal to 100% are black, and between these thresholds, gray values equivalent to the proportion of identity are used. The ATPase sequence elements are indicated by brackets.

which confirmed that isolated DNA did in fact contain the *YNT1* locus. Presumably, the one tetrad that showed 3ts::1wt had undergone gene conversion.

Sequence analysis of *YNT1* revealed that it is identical to a previously identified yeast gene of unassigned function, *YTA2*, GenBank accession number X73570 (Schnall *et al.*, 1994). The *YNT1* nucleic acid sequence has been assigned GenBank accession number U06229.

Ynt1p is homologous to an extended family of proteins, including Yme1p (Figure 3A), that are related by a 200–amino acid region containing a consensus ATPase element. Proteins in this family have been implicated in divergent processes, ranging from vesicle fusion (Diaz *et al.*, 1989) to transcription (Ohana *et al.*, 1993). It remains unclear whether sequence homologies among these family members correspond to functional similarities.

Ynt1p has a high degree of sequence homology to several genes that have been identified as regulatory subunits of the 26S protease (Figure 3B): subunit 4 of the 26S protease from humans (Dubiel *et al.*, 1992); Mts2p, a 26S protease subunit necessary for the completion of mitosis in *Schizosaccharomyces pombe* (Gordon *et al.*, 1993); and Cim5p and Sug1p, 26S protease subunits necessary for degradation of ubiquitinated substrates and for anaphase chromosome separation in *S. cerevisiae* (Ghislain *et al.*, 1993).

YNT1 Is an Essential Gene

One chromosomal copy of *YNT1* was deleted in the diploid strain PTY33 × PTY44. The deleted locus was marked with *URA3* DNA (see MATERIALS AND METHODS) and verified by DNA-blot hybridization. This diploid was sporulated and dissected, and only two viable spores were recovered from each of the 20 tetrads dissected. None of these viable spores were Ura$^+$, indicating that a functional copy of *YNT1* is necessary for cell viability.

DISCUSSION

We have demonstrated that Yme1p is necessary for proper mitochondrial function and structure in yeast.

Yme1p sequence elements suggest possible ATP-dependent neutral zinc protease activity (Thorsness *et al.*, 1993) (Figure 3). Yme1p is a mitochondrial protein (Thorsness, 1993, #595). More recent work in our laboratory has demonstrated that Yme1p is tightly associated with the inner mitochondrial membrane (Weber and Thorsness, unpublished data). A closely related protein from bacteria containing the same sequence elements, the FtsH protein, was identified in a screen for cell division mutants in *E. coli* (Begg *et al.*, 1992; Tomoyasu *et al.*, 1993). This homology, considered with the mitochondrial morphology abnormalities and deficient mitochondrial function in a *yme1-Δ1::URA3* strain, suggests Yme1p plays a role in rearranging or maintaining the proper mitochondrial superstructure. If Yme1p functions as a protease, the phenotypic consequences because of loss of Yme1p activity may result from a general reduction of mitochondrial protein turnover. The punctate and distended mitochondrial compartments in a *yme1* mutant strain may result directly from an excess of abnormal mitochondrial proteins. Alternatively, Yme1p may have a maturase activity for a subset of mitochondrial proteins. Such unprocessed proteins may lead to poorly assembled protein complexes, resulting in structurally and functionally impaired mitochondrial compartments.

An altered form of Ynt1p partially compensates for the absence of Yme1p. *ynt1-1* may mediate this suppression of *yme1-Δ1::URA3* in two different ways. First, Ynt1p may have been altered in such a way as to gain Yme1p-like function. Typically such gain-of-function mutations are dominant; however, *ynt1-1* displays a recessive phenotype. In a second model, Yme1p and Ynt1p may function in competing pathways. The loss of Yme1p may result in unbalanced pathways; the balance may be partially restored by a mutation that attenuates Ynt1p activity.

In either case, sequence homology and the suppressor activity of *ynt1* suggest that these two proteins have similar activities. However, in a sequence lineup (Figure 3A), the putative zinc protease domain of Yme1p begins immediately after the C-terminus of Ynt1p. If these proteins do have similar activities, Ynt1p may be associated in a complex with another protein having proteolytic function. Such an association has been proposed for a Ynt1p homologue, subunit 4 of the 26S protease (Dubiel *et al.*, 1992; Rechsteiner *et al.*, 1993). Rechsteiner *et al.* have proposed that subunit 4 of the 26 S proteosome, as well as homologous proteins, are involved in substrate selection for associated protease activities (Rechsteiner *et al.*, 1993).

If Ynt1p is a 26S protease subunit, could mitochondrially targeted proteins be substrates for its activity? There is only limited evidence to support this hypothesis. For instance, ubiquitination may be associated with insertion of proteins into the mitochondrial outer membrane (Zhaung and McCauley, 1989; Magnani *et al.*, 1991). A role for ubiquitination of proteins in organelle formation has been proposed based upon the observation that *PAS2* in *S. cerevisiae*, a gene necessary for peroxisome biogenesis and proliferation, encodes a member of the ubiquitin-conjugating protein family (Wiebel and Kunau, 1992). The 26S protease is known to act on both ubiquinated and nonubiquinated substrates (Hershko and Ciechanover, 1992; Murakami *et al.*, 1992). If Ynt1p, in concert with a 26S protease complex, acts on a subset of mitochondrial outer membrane proteins or proteins destined for the interior of mitochondria, perhaps the *ynt1-1* mutation alters the substrate specificity of the complex to include substrates normally acted on by Yme1p. If so, the pleiotropic growth phenotypes associated with *yme1* may merely demonstrate the results of a lack of maturation of multiple substrates. A mutated Ynt1p may partially compensate across the range of *yme1* phenotypes by targeting substrates with a lowered efficiency compared to that of Yme1p. Alternatively, the mutant Ynt1p may recognize only a subset of Yme1p substrates.

This work has provided intriguing genetic and microscopic evidence for the possible association of the 26S protease with mitochondrial function and morphology. Localization of Ynt1p within the yeast cell will be required for further elucidation of function.

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REFERENCES

Begg, K.J., Tomoyasu, T., Donachie, W.D., Khattar, M., Niki, H., Yamanaka, K., Hiraga, S., and Ogura, T. (1992). *Escherichia coli* mutant Y16 is a double mutant carrying thermosensitive *ftsH* and *ftsI* mutations. J. Bacteriol. *174*, 2416–2417.

Byers, B., and Goetsch, L. (1990). Preparation of yeast cells for thin-section electron microscopy. Methods Enzymol. *194*, 602–608.

Chin, D.T., Goff, S.A., Webster, T., Smith T., and Goldberg, A.L. (1988). Sequence of the *lon* gene in *Escherichia coli*: a heat shock gene which encodes the ATP-dependent protease La. J. Biol. Chem. *263*, 11718–11728.

Diaz, R., Mayorga, L.S., Weidman, P.J., Rothman, J.E., and Stahl, P.D. (1989). Vesicle fusion following receptor-mediated endocytosis requires a protein active in Golgi transport. Nature *339*, 398–400.

Dubiel, W., Ferrell, K., Pratt, G., and Rechsteiner, M. (1992). Subunit 4 of the 26 S protease is a member of a novel eukaryotic ATPase family. J. Biol. Chem. *267*, 22699–22702.

Frölich, K.-U. (1994). Sequence similarity presenter: a tool for the graphical display of similarities of long sequences for use in presentations. Comp. Appl. Biosci. *10*, 179–183.

Ghislain, M., Udvardy, A., and Mann, C. (1993). *S. cerevisiae* 26S protease mutants arrest cell division in G2/metaphase. Nature *366*, 358–361.

Gordon, C., McGurk, G., Dillon, P., Rosen, C., and Hastie, N.D. (1993). Defective mitosis due to a mutation in the gene for a fission yeast 26S protease subunit. Nature *366*, 355–357.

Henikoff, S., and Henikoff, J.G. (1991). Automated assembly of protein blocks for database searching. Nucleic Acids Res. *19*, 6565–6572.

Hershko, A., and Ciechanover, A. (1992). The ubiquitin system for protein degradation. Annu. Rev. Biochem. *61*, 761–807.

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

Magnani, M., Serafini, G., Antonelli, A., Malatesta, M., and Gazzanelli, G. (1991). Evidence for a particulate location of ubiquitin conjugates and ubiquitin-conjugating enzymes in rabbit brain. J. Biol. Chem. *266*, 21018–21024.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

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

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

Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S.-I., Igarashi, K., Tamura, T., Tanaka, K., and Ichihara, A. (1992). Ornithine decarboxylase is degraded by the 26S proteosome without ubiquitination. Nature *360*, 597–599.

Ohana, B., Moore, P.A., Ruben, S.M., Southgate, C.D., Green, M.R., and Rosen, C.A. (1993). The type 1 human immunodeficiency virus Tat binding protein is a transcriptional activator belonging to an additional family of evolutionarily conserved genes. Proc. Natl. Acad. Sci. USA *90*, 138–142.

Rechsteiner, M., Hoffman, L., and Dubiel, W. (1993). The multicatalytic 26 S proteases. J. Biol. Chem. *268*, 6065–6068.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Schnall, R., Mannhaupt, G., Stucka, R., Tauer, R., Ehnle, S., Schwarzlose, C., Vetter, I., and Feldmann, H. (1994). Identification of a set of yeast genes coding for a novel family of putative ATPases with high similarity to constituents of the 26S protease complex. Yeast *10*, 1141–1155.

Sherman, F., Fink, G.R., and Hicks, J.B. (1986). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Stevens, B.J. (1977). Variation in number and volume of the mitochondria in yeast according to growth conditions. A study based on serial sectioning and computer graphics reconstitution. Biol. Cellulaire. *28*, 37–56.

Stevens, B. (1981). Mitochondrial structure. In: The Molecular Biology of the Yeast *Saccharomyces*, Life Cycle and Inheritance, eds. J.N. Strathern, E.W. Jones, and J.R. Broach, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 471–504.

Thorsness, P.E., and Fox, T.D. (1993). Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. Genetics *134*, 21–28.

Thorsness, P.E., White, K.H., and Fox, T.D. (1993). Inactivation of *YME1*, a gene coding a member of the *SEC18, PAS1, CDC48* family of putative ATPases, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *13*, 5418–5426.

Tomoyasu, T., Yuki, T., Morimura, S., Mori, H., Yamanaka, K., Niki, H., Hiraga, S., and Ogura, T. (1993). The *Escherichia coli* FtsH protein is a prokaryotic member of a protein family of putative ATPases involved in membrane functions, cell cycle control, and gene expression. J. Bacteriol. *175*, 1344–1351.

Wiebel, F.F., and Kunau, W.-H. (1992). The Pas2 protein essential for peroxisome biogenesis is related to ubiquitin-conjugating enzymes. Nature *359*, 73–76.

Zhaung, Z., and McCauley, R. (1989). Ubiquitin is involved in the *in vitro* insertion of monoamine oxidase B into mitochondrial outer membranes. J. Biol. Chem. *264*, 14594–14596.