Published in final edited form as:
Mitochondrion. 2011 July ; 11(4): 587–600. doi:10.1016/j.mito.2011.03.008.

Hsp90 and mitochondrial proteases Yme1 and Yta10/12 participate in ATP synthase assembly in *Saccharomyces cerevisiae*

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Abstract

Hsc82 and Hsp82, the Hsp90 family proteins of yeast, are both required for fermentative growth at 37°C. Inactivation of either of the mitochondrial AAA proteases, Yme1 or Yta10/12, allows fermentative growth of *hsc82*Δ or *hsp82*Δ strains at 37°C. Genetic evidence indicates interaction of Hsc82/Hsp82 with the Yme1 and Yta10/Yta12 complexes in promoting F_1F_o-ATPase activity, with Hsc82 specifically required for F_1-ATPase assembly. A previously reported mutation in Rpt3, one of the six ATPases of the proteasome, suppresses *yme1*Δ phenotypes and increases transcription of *HSC82* but not *HSP82*. These genetic interactions describe a functional role for Hsp90 proteins in mitochondrial biogenesis.

Keywords

Hsp90; F_1F_o-ATPase; Assembly; Yeast; AAA protease; Yme1; Yta10; Yta12

1. Introduction

Chaperones in mitochondria are associated with numerous activities, including uptake of nuclear-encoded proteins, FeS cluster synthesis, protein folding, and prevention of protein aggregation (Voos and Rottgers, 2002). Hsp90 proteins have an established function in late-stage protein folding and early-stage unfolding, but an emerging theme is that they also function in assembly and disassembly of oligomeric protein complexes (Mayer et al., 2009). In mammalian cells one Hsp90 isoform, Trap1, is localized to the mitochondria (Leskovar et al., 2008). Hsp90 is also present in mitochondria of *Tetrahymena thermophila* (Smith et al., 2007). Hsc82 (constitutively expressed) and Hsp82 (heat inducible) are the Hsp90 equivalent in *Saccharomyces cerevisiae*, and they are localized primarily in the yeast cytoplasm and nucleus (Borkovich et al., 1989). Neither has been reported to enter yeast mitochondria, nor do they have typical mitochondrial targeting sequences. Hsc82 and Hsp82 are 97% identical in their amino acid sequences and have many overlapping functions. Deletion of just one of them yields a viable cell, but deletion of both does not (Borkovich et al., 1989). However, different responses of *hsp82*Δ/*hsp82*Δ and *hsc82*Δ/*hsc82*Δ to the inhibitor macbecin II and improved efficiency of cell cycle progression during thermal stress in the presence of Hsp82 compared to Hsc82 suggest that they are not functionally

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equivalent *in vivo* (McClellan et al., 2007; Morano et al., 1999). Genetic interactions between Atp1 and Hsc82 or Hsp82 (McClellan et al., 2007) and physical interactions between Atp2 and Hsc82 or Hsp82, and between Atp3 and Hsc82 (Krogan et al., 2006) suggest a possible role for these heat shock proteins in F_1F_o-ATPase assembly. In human cancer cells, F_1F_o-ATPase functions as a co-chaperone of Hsp90-substrate protein complexes (Papathanassiu et al., 2006).

Genetic and physical interactions of Hsp90 proteins with mitochondrial ATP synthase subunits support a role for these chaperones in ATP synthase assembly, however their precise functions in this complex process are unknown. ATP synthase, also known as F_1F_o-ATPase, is assembled from three subcomplexes: F_1 containing the central stalk proteins (Atp3, Atp15, and Atp16) surrounded by a trimer of Atp1-Atp2 dimers $(Atp1Atp2)_3$, the peripheral or stator stalk of F_o (Atp4, Atp5, Atp7 and Atp14) and the rotor component of F_o containing oligomeric Atp9 (for recent review, see (Rak et al., 2009). F_1F_o-ATPase assembly occurs in a stepwise process. First the Atp9 oligomer and F_1 associate to form F_1-Atp9, which then associates with the peripheral stalk. Incorporation of Atp6, the proton channel component of F_o, is believed to be the last step in assembly of F_1F_o-ATPase (Goyon et al., 2008). Assembly of F_o involves complex regulation and integration of mitochondrial- and nuclear-encoded subunits. Atp9 oligomerization is assisted by Atp25 (Zeng et al., 2008). Specific chaperones, Atp11, Atp12, and Fmc1, are required for formation of $(Atp1Atp2)_3$ in the assembly of F_1 (Ackerman and Tzagoloff, 2005), and two other chaperones, Atp10 and Atp23, are involved in incorporation of Atp6 into the complex (Osman et al., 2007; Tzagoloff et al., 2004). Strains lacking Fmc1 do not respire at elevated temperature due to compromised in ATP synthase assembly. This defect is rescued by increased substrate level ATP synthesis by the citric acid cycle (Schwimmer et al., 2005).

The AAA (*A*TPases *a*ssociated with various cellular *a*ctivities) proteases of mitochondria, Yme1 and Yta10/Yta12, have also been implicated in determining the activity of ATP synthase. Yme1 and Yta10/Yta12 complexes have chaperone and proteolytic functions and behave as quality control agents that ensure correct formation of multienzyme complexes (Koppen and Langer, 2007; Langer et al., 2001; Leidhold and Voos, 2007). Both are localized to the mitochondrial inner membrane. Yme1 probably acts as a hexameric complex similar to bacterial FtsH, with ATPase and protease domains in the intermembrane space (IMS) and N-terminal domains in the matrix. The closely related subunits of the Yta10/Yta12 complex have ATPase and protease domains in the matrix and extended regions between their two transmembrane domains in the IMS. Deletion of either Yta10 or Yta12 inactivates the complex and leads to respiratory defects that are attributed to decreased assembly of electron transport and oxidative phosphorylation complexes (Guélin et al., 1994; Paul and Tzagoloff, 1995; Tauer et al., 1994; Tzagoloff et al., 1994). Yta10/Yta12 functions as both a chaperone and protease in the assembly of the oligomeric Atp9 complex (Arlt et al., 1996) and has a role in F_1-ATPase assembly that does not overlap that of Atp11 or Atp12 (Paul and Tzagoloff, 1995). The functions of Yme1 are still poorly understood. Only a few endogenous substrates for proteolysis have been clearly identified (Phb1, Yme2, and Cox2) (Kambacheld et al., 2005; Leonhard et al., 2000; Nakai et al., 1995; Pearce and Sherman, 1995; Weber et al., 1996). Atp4 may be another substrate for Yme1 because its level increases in *yme1*Δ strains (Jia et al., 2007; Kominsky et al., 2002; Lemaire et al., 2000). In contrast, Atp4 and Atp7 are at near normal concentrations in the absence of Yta10/Yta12 (Paul and Tzagoloff, 1995). Yme1 also directs nuclear-encoded proteins to the intermembrane space (Rainey et al., 2006).

Deletion of Yme1 leads to several phenotypes: slow growth on YPEG at 37°C, slow growth on YPD at 16°C slow growth in the absence of mitochondrial DNA (mtDNA), increased escape of mtDNA to the nucleus (Thorsness and Fox, 1993; Thorsness et al., 1993), altered

mitochondrial morphology (Campbell and Thorsness, 1998), decreased chronological life span and decreased long term spore viability (Francis et al., 2007). Loss of Yme1 also increases the level of phosphatidylethanolamine by reducing turnover of phosphatidylserine decarboxylase, Psd1 (Nebauer et al., 2007). A previously isolated suppressor of the growth defects of *yme1*Δ carries a mutation in the proteasomal ATPase subunit, Rpt3 (also known as Ynt1) (Campbell et al., 1994). Disruption of the Yta10/Yta12 complex is sometimes more detrimental to growth than disruption of the Yme1 complex. For example, cells lacking either Yta10 or Yta12 lose respiratory competence whereas respiration in the absence of Yme1 is only impaired at 37°C (Tauer et al., 1994; Thorsness and Fox, 1993; Tzagoloff et al., 1994), but growth by fermentation at 16° and in the absence of mtDNA is more detrimental in the absence of Yme1 (Thorsness and Fox, 1993).

Because Yme1 genetically interacts with Hsp82 (Zhao et al., 2005) we hypothesized that Yme1 may interact with Hsc82 and Hsp82 in mitochondria, perhaps during assembly and disassembly of protein complexes, particularly under stressful conditions, and that this interaction may be a primary function of Yme1. In support of this proposition, Yme1 has recently been shown to be synthetically lethal with the chaperone Atp12 that is involved in F_1-ATPase assembly (Costanzo et al., 2010). In this study we show that *hsc82*Δ and *hsp82*Δ yeast containing intact mtDNA are inviable or very slow-growing when grown by fermentation at 37°C, and that *yme1*Δ or *yta10*Δ suppress this phenotype. Suppression of *hsc82*Δ or *hsp82*Δ by *yme1*Δ is eliminated by introduction of *atp1*Δ, *atp2*Δ, *atp4*Δ, or by growth in the absence of mtDNA, indicating a role for Yme1 and these heat shock proteins in assembly of F_1F_o-ATPase. The observations reported here, combined with previous studies, support a model for assembly of F_1F_o-ATPase at 37°C that involves participation of Hsc82, Hsp82, Yme1, and Yta10/12.

2. Materials and methods

2.1. Strains, media and DNA techniques

Yeast strains used in this study are listed in Table 1. Standard genetic techniques were used to construct and analyze the various strains (Sherman et al., 1986). Yeast were grown in complete glucose medium (YPD) containing 2% glucose, 2% bacto peptone, 1% yeast extract, 40 mg/l tryptophan, 40 mg/l adenine; complete ethanol-glycerol medium (YPEG) containing 3% glycerol, 3% ethanol, 2% bacto peptone, 1% yeast extract, 40 mg/l tryptophan, 40 mg/l adenine; minimal glucose medium (SD) containing 2% glucose, 6.7 g/l yeast nitrogen base without amino acids (Difco), supplemented with the appropriate nutrients. The complete set of nutrients included: uracil at 40 mg/l, adenine at 40 mg/l, tryptophan at 40 mg/l, lysine at 60 mg/l, leucine at 100 mg/l, histidine at 20 mg/l, isoleucine at 30 mg/l, and valine at 150 mg/l. For plates, bacteriological agar (US Biological) was added at 16 g/l. Where indicated, geneticin was added at 300 μg/ml (US Biological), and ethidium bromide (EtBr) at 25 ug/ml. Null alleles for particular genes were generated by polymerase chain reaction (PCR) amplification of sequences from a deletion library (Open Biosystems) in which individual genes have been replaced by the kanomycin resistance gene, followed by transformation of strains in the D273-10 genetic background with the PCR products and selection of geneticin resistance, and subsequent isolation of transformants by streaking three times to single colonies on geneticin plates. Knockout strains were verified by PCR. The PCR primers used in this study are shown in Table 2.

Escherichia coli strain XL-1 Blue (Stratagene) was used for preparation and manipulation of recombinant DNA. Plasmid-containing *E. coli* were grown in LB broth (10g bactotryptone, 10g NaCl, 5g yeast extract per liter) supplemented with 125 μg/ml of ampicillin (US Biological). All manipulations of DNA were performed using standard techniques (Sambrook et al., 1989). Plasmid DNA was prepared by the boiling lysis method (Maniatis

et al., 1982). Column-purified (Qiagen) plasmids or PCR products were sequenced by the Davis Sequencing Facility (University of California, Davis). Primers used for sequencing Rpt3 are shown in Table 2.

2.2. Dilution analysis for growth and measurement of the percentage of cells containing mtDNA

Dilution assays were performed on strains grown overnight in YPD. Using the approximation that $OD^{600} = 10^7$ cells/ml, 5 μl aliquots containing 5×10^4, 5×10^3, 5×10^2 or 50 cells of each strain assayed were plated on YPD, YPEG, or SD-EtBr. Approximately 50 cells of each strain were spread on two YPD plates. After 2 days at 30°C colonies were counted and transferred to YPD plates spread with PYT33 lacking mtDNA. After one day at 30°C, cells were transferred to YPEG plates and incubated at 30°C for one additional day and the number of growing colonies recorded. The percentage of yeast cultured on the original media that contained mtDNA was calculated by dividing the number of colonies growing on YPEG by the number of colonies that grew on the YPD plates.

2.3. Isolation of mitochondria and immuno-detection of mitochondrial proteins.

Cells were grown in 1 liter of YPD or YPEG media at 30°C to an OD^{600} of approximately 1.5. Mitochondrial isolation was performed essentially as described (Yaffe, 1991). Western blots of mitochondrial proteins were performed as previously described (Kominsky et al., 2002). Antibodies against Por1 (a gift from C. Koehler), mtHsp70 (a gift from T. Lithgow), and Tom40 (a gift from C. Koehler) were used for immuno-blotting. Levels of protein in the lanes of immuno- blots were normalized using Ponceau Red staining, and relative levels of protein in individual bands in the blots were calculated after exposure of blots treated with a chemiluminescent detection reagent (GE Healthcare) to film. Standard error of the mean for each protein was derived from at least three independent densitometric measurements.

2.4. Real time PCR

For semi-quantitative RT-PCR analysis of *HSC82* and *HSP82* gene expression, individual colonies were picked from YPD plates and grown overnight at 30°C in 5 ml YPD and then for 6 hr at 37°C. Cultures were centrifuged and cell pellets frozen at −80°C. To isolate RNA, cells were resuspended in five volumes of Trizol (Invitrogen, Carlsbad, California) per volume of cell pellet. The suspension was thawed and frozen 10 times using a 40°C water bath and liquid nitrogen, then vortexed 5 times for 30 sec separated by 30 sec incubations at room temperature, extracted with 0.2 ml chloroform per ml Trizol, incubated at room temperature for 3 min and centrifuged at 4°C for 15 minutes. 0.5 ml isopropanol per ml Trizol was added to the recovered aqueous phase, incubated for 10 minutes at room temperature, and centrifuged for 15 min at 4°C. The nucleic acid pellets were washed with 75% ethanol, air dried, and dissolved in 50 μl RNase-free water. RNA was purified using the RNeasy protocol (Qiagen Inc, Sanata Clara, California) with on-column DNase I removal of contaminating genomic DNA. RNA was eluted in 30μl RNase-free water and quantitated using a NanoDrop spectrophotometer. To generate cDNA, 2 μg RNA was mixed with 4 μL of reverse transcription buffer and 1 μL of IScript reverse transcriptase (Bio-Rad Laboratories) in a total volume of 20 μL. The mixture was incubated in a thermocycler for 5 min at 25°C, 30 min at 42°C, 5 min at 85°C, then held at 4°C. The resulting cDNA was diluted with 100 μL of nuclease-free water and stored at −20°C.

Diluted cDNA (10 μL) was used as a template for RT-PCR amplification using SYBR Green Supermix (Bio-Rad). Primers for PCR amplification of internal fragments of Hsc82, Hsp82, and Act1 mRNAs were designed using Primer 3 software (http://frodo.wi.mit.edu) to generate amplicons of approximately 150 bases (Table 2). RT-PCR was performed by incubating at 95°C for 3 min, followed by 95°C for 10 s, and 60°C for 30 s for 40 cycles.

Following RT-PCR amplification, melting curve analysis was performed to ensure the quality of amplification by incubating for 10s at each step with an increase in temperature of 0.5°C in each cycle over a range of 55°C to 95°C. The resulting *Hsc82* and *Hsp82* mRNA expression levels were reported relative to Act1 as described by (Livak and Schmittgen, 2001).

3. Results

3.1. hsc82Δ and hsp82Δ impair growth by fermentation at 37°C.

We created *hsp82*Δ and *hsc82*Δ in wild type and *yme1*Δ yeast and determined how these deletions affected yeast cell growth. Growth was assessed on both fermentation media (complete glucose; YPD) and respiration media (complete ethanol/glycerol; YPEG) at three temperatures (16°C, 30°C, 37°C). As reported previously (Borkovich et al., 1989), respiratory growth of *hsc82*Δ and *hsp82*Δ yeast was similar to wild type at all temperatures, as was fermentative growth at 16°C and 30°C (Fig. 1 and data not shown). However, *hsc82*Δ and *hsp82*Δ mutants did not grow by fermentation at 37°C (Fig. 1). In addition, of about 600 *hsp82*Δ cells plated on YPD for 2 days at 37°C, 2% produced colonies. Of a similar number of *hsc82*Δ cells, 0 or 1 colony grew. When these plates were transferred to 30°C for 2 days, 75% of the *hsp82*Δ colonies and 5% of the *hsc82*Δ colonies grew. Therefore, *hsc82*Δ was more deleterious to growth at 37°C than *hsp82*Δ. Suppressors arise quickly in *hsc82*Δ and *hsp82*Δ strains grown at 37°C on YPD and the growth of these strains observed in Figure 1 was due to colonies bearing suppressors.

3.2. Inactivation of Yme1 or Yta10 suppresses slow growth of hsc82Δ *and hsp82Δ by fermentation at 37°C.*

The extremely poor growth of *hsc82*Δ and *hsp82*Δ by fermentation at 37°C was suppressed by *yme1*Δ (Fig 1). The *yme1*Δ proved to be epistatic to either *hsc82*Δ or *hsp82*Δ as *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ grew like *yme1*Δ under all conditions (Fig. 1 and data not shown). *yme1Δ* also suppressed the reduced viability of *hsc82Δ* and *hsp82Δ* yeast. When *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ cells were spread on YPD plates and incubated for 2 days at 37°C, slow growing colonies were visible, and after transfer to 30°C 95% of the cells produced colonies. The inviability of haploid spores bearing both *hsc82*Δ and *hsp82*Δ (i.e. no Hsp90 proteins) was not suppressed by inactivation of *yme1*Δ.

Like *yme1*Δ, *yta10*Δ suppressed slow growth of *hsc82*Δ or *hsp82*Δ by fermentation at 37°C (Fig. 1). *yta10*Δ grew progressively slower by fermentation compared to wild type as the temperature was reduced from 37°C to 16°C as did the *hsc82*Δ *yta10*Δ and *hsp82*Δ *yta10*Δ double mutants (Fig. 1). All *yta10*Δ strains were unable to grow by respiration (Fig. 1 and data not shown). When *hsc82*Δ *yta10*Δ or *hsp82*Δ *yta10*Δ cells were plated to YPD and incubated for 2 days at 37°C, no colonies were visible. After transfer to 30°C for 2 days, more than 95% of both double mutants produced colonies. *yta10*Δ thus improved viability of *hsc82*Δ and *hsp82*Δ yeast grown by fermentation at 37°C.

Simultaneous inactivation of both Yme1 and Yta10 is highly deleterious to yeast cell growth (Dunn et al., 2006). The *yme1*Δ *yta10*Δ double mutant as well as the *hsc82*Δ *yme1*Δ *yta10*Δ and *hsp82*Δ *yme1*Δ *yta10*Δ triple mutants grew only as microcolonies from isolated germinating spores (data not shown).

3.3. yme1Δ-directed suppression of slow growth of hsc82Δ and hsp82Δ by fermentation at 37°C requires a functional F_1F_o-ATPase.

There is significant interplay between Yme1 and the F_1F_o-ATPase, with mutations in one affecting phenotypes or activities of the other (Francis et al., 2007; Kominsky et al., 2002;

Weber et al., 1995). This observation together with the established function of Hsp90 proteins in assembly of multisubunit complexes (Mayer et al., 2009), suggested that the growth defect of *hsc82*Δ and *hsp82*Δ could reflect a role for these proteins in assembly of the F_1F_o-ATPase during fermentation at 37°C. In *yme1*Δ yeast, this growth defect might be mitigated by alterations in assembly or activity of the F_1F_o-ATPase. In this model, suppression of *hsc82*Δ or *hsp82*Δ by *yme1*Δ would require functional F_1F_o-ATPase. As the only membrane-embedded component of the peripheral stalk, Atp4 appears to play a central role in stalk assembly and the stalk's interaction with F_1-ATPase and other F_0 subunits (Rak et al., 2009). Atp5 is a component of the peripheral stalk that binds to an Atp1 subunit of F_1-ATPase (Carbajo et al., 2007; Walker and Dickson, 2006). Atp1 and Atp2 are components of F_1-ATPase. To test whether *yme1*Δ suppression required a functional F_1F_o-ATPase, triple mutants combining *hsp82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ with either *atp4*Δ, *atp5*Δ, *atp1*Δ, *or atp2*Δ were isolated and their phenotypes determined. Growth of each triple mutant was clearly slower than the corresponding *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ strains (Figs. 1 and 2). Therefore, suppression of *hsc82*Δ and *hsp82*Δ by *yme1*Δ was reduced in the absence of peripheral stalk or F_1-ATPase subunits.

Interestingly, deletion of some F_1F_o-ATPase subunits acted to suppress *hsc82*Δ or *hsp82*Δ, allowing for growth of these mutants by fermentation at 37°C. Specifically, *atp4*Δ, *atp5*Δ, *atp1*Δ and *atp2*Δ suppressed *hsp82*Δ slow growth, while *atp1*Δ and *atp2*Δ also suppressed *hsc82*Δ (Fig. 1 and 2). Suppression of *hsc82*Δ or *hsp82*Δ by deletion of F_1-ATPase subunits was dependent on Yme1 because suppression by *atp1*Δ, and *atp2*Δ was reduced in triple mutants with *yme1*Δ. Suppression of *hsp82*Δ by *atp4*Δ and *atp5*Δ was also reduced in the presence of *yme1*Δ but *hsc82*Δ *atp4*Δ and *hsc82*Δ *atp5*Δ were largely unaffected by *yme1*Δ. (Fig. 2). Thus, there was an interdependence of Yme1 and F_1F_o-ATPase subunits in their suppression of *hsc82*Δ and *hsp82*Δ growth defects.

One possible explanation for the poor growth of *hsc82*Δ and *hsp82*Δ strains on YPD at 37° was because mtDNA became unstable, and the suppressive effects brought by the loss of F_1F_o-ATPase subunits or Yme1 was due to increasing the stability of mtDNA. However, no significant destabilization of mtDNA was observed for *hsc82*Δ and *hsp82*Δ strains (Table 3), and suppressors could either have no effect on mtDNA stability (compare *hsc82*Δ to *hsc82*Δ *yme1*Δ) or actually destabilized mtDNA (compare *hsc82*Δ to *hsc82*Δ *atp4*Δ). Consequently, we concluded that changes in mtDNA stability were not the basis for suppression of growth defects of *hsc82*Δ and *hsp82*Δ strains. Strains that contained *atp4*Δ or *atp5*Δ did have unstable mtDNA, but when either of these mutations were combined with *yme1*Δ or *yme1*Δ plus *hsc82*Δ or *hsp82*Δ, the percentage of cells retaining their mtDNA increased to greater than 90%, showing that the tendency of *atp4*Δ or *atp5*Δ strains to lose mtDNA was dependent on the presence of Yme1.

To determine whether removal of a subunit from a different inner membrane complex affected growth of *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ by fermentation at 37°C similar to removing subunits of F_1F_o-ATPase, *hsc82*Δ, *hsp82*Δ, and the corresponding double mutants with *yme1*Δ were crossed with a strain lacking subunit 4 of cytochrome *c* oxidase (*cox4*Δ) to generate triple mutants. *cox4*Δ had no effect on *hsc82*Δ or *hsp82*Δ growth at 37°C and did not alter suppression of *hsc82*Δ or *hsp82*Δ by *yme1*Δ at 37°C (data not shown).

3.4. Hsc82 is more critical than Hsp82 for growth at 37°C in the absence of mitochondrial DNA.

To assess the importance of mtDNA in growth of unsuppressed and suppressed *hsc82*Δ and *hsp82*Δ yeast, strains were grown on media containing ethidium bromide to induce loss of the mitochondrial genome (SD-EtBr). *hsc82*Δ ρ^0 and *hsp82*Δ ρ^0 grew similar to wild type ρ^0 at 30°C and 16°C, but at 37°C their growth was slowed relative to wild type ρ^0 (Fig. 3A).

The 37°C growth defect was more severe for *hsc82*Δ ρ^0 than *hsp82*Δ ρ^0. Survival at elevated temperature was also more dependent on Hsc82 than Hsp82. When plated on SD-EtBr at 37°C for 4 days, no *hsc82*Δ ρ^0 cells grew into colonies, whereas 65% of *hsp82*Δ ρ^0 cells produced colonies. After transfer to 30°C for 2 days, 3% of *hsc82*Δ ρ^0 and 75% of *hsp82*Δ ρ^0 cells produced colonies. Interestingly, while the effects of *hsc82*Δ were similar in ρ^+ and ρ^0, *hsp82*Δ growth and viability were less impaired in ρ^0 than in ρ^+.

3.5. yta10Δ but not yme1Δ suppresses the inviability of hsc82Δ ρ⁰ at 37°C.

As shown in previous studies (Thorsness et al., 1993), *yme1*Δ grew very slowly as a ρ^0 strain at 30°C and 37°C, and in a ρ^0 background *yme1*Δ *hsc82*Δ or *yme1*Δ *hsp82*Δ did not grow (Fig. 3A). No colonies of *yme1*Δ ρ^0, *hsc82*Δ *yme1*Δ ρ^0, and *hsp82*Δ *yme1*Δ ρ^0 grew on SD-EtBr after 4 days at 37°C, or when these plates were subsequently transferred to 30°C, indicating that the absence of Yme1 is lethal for ρ^0 cells at 37°C, which is in contrast to yeast with the same genotypes but containing mtDNA.

Like *yta10*Δ ρ^+, growth of *yta10*Δ ρ^0 was temperature dependent, with faster growth relative to wild type ρ^0 at 37°C than at 30°C or 16°C (Fig. 3A). Unlike their ρ^+ counterparts, *hsc82*Δ ρ^0 and *hsp82*Δ ρ^0 strains bearing *yta10*Δ grew more slowly than *yta10*Δ ρ^0 at 37°C with *hsc82*Δ being clearly more deleterious to growth of *yta10*Δ ρ^0 than *hsp82*Δ. Nonetheless, *yta10*Δ suppressed the slow growth of *hsc82*Δ ρ^0 yeast and *hsp82*Δ ρ^0 yeast (Fig. 3A). Of approximately 600 plated cells on SD-EtBr, less than 0.1% of *hsc82*Δ *yta10*Δ ρ^0 cells and greater than 95% of *hsp82*Δ *yta10*Δ ρ^0 cells produced visible colonies after 4 days at 37°C. Following transfer to 30°C for 2 days, greater than 95% of *hsc82*Δ *yta10*Δ ρ^0 cells produced colonies. *hsc82*Δ *yta10*Δ ρ^0 cells did not grow but did not die at 37°C. *yta10*Δ prevented death of both ρ^+ and ρ^0 *hsc82*Δ cells.

3.6. Loss of central or peripheral stalk proteins does not affect ρ⁰ growth at 37°C.

ATP3 encodes a subunit of the F_1F_0-ATPase central stalk. *atp3*Δ yeast grow only in the absence of mtDNA, and grow more slowly than wild type ρ^0 at 30°C. As was found for *yta10*Δ strains, growth of *atp3*Δ yeast was temperature-dependent, with growth increasing relative to wild type as the temperature increased from 16°C and 37°C (Fig. 3A). Loss of the peripheral stalk proteins, Atp4 or Atp5, also showed little difference from wild type in ρ^0 growth at 37°C, whereas loss of Atp1 or Atp2 substantially, but not completely, reduced growth (Fig. 3B).

3.7. Hsc82 is required for suppression of yme1Δ by rpt3-215.

In addition to the well-characterized function of the six ATPases in the proteasome, the proteasomal ATPase subcomplex by itself has a separate function in transcription, including transcription of heat shock proteins (Gonzalez et al., 2002; Kodadek, 2010; Sulahian et al., 2006). Interestingly, a mutant proteasomal ATPase subunit, Rpt3-215, was previously isolated as a suppressor of *yme1*Δ phenotypes (Campbell et al., 1994). Therefore, we examined whether the growth-altering effects of *rpt3-215* were influenced by the presence of *hsc82*Δ or *hsp82*Δ. *hsc82*Δ *yme1*Δ *rpt3-215* grew more slowly than *hsp82*Δ *yme1*Δ *rpt3-215* on YPD and YPEG at 37°C (Fig. 4). Therefore, Hsc82 was required for suppression of *yme1*Δ by *rpt3-215*. The genetic interactions of *rpt3-215* and *HSP82/HSC82* could potentially be explained either through alteration of transcription of *HSP82/HSC82* or a direct effect on the levels of outer mitochondrial membrane proteins *via* proteasome activity.

3.8. rpt3-215 increases the level of HSC82 mRNA in strains grown at 37°C

The requirement for Hsc82 to elicit suppression of *yme1*Δ by Rpt3-215 suggested that Rpt3-215 might be altering transcription of *HSC82* but not *HSP82*. mRNA was extracted from wild type and *rpt3-215* yeast and probed by real-time PCR with primers that amplified sequences within the coding regions of *HSC82, HSP82,* and *ACT1*. The relative level of *HSC82* mRNA increased 5-fold in *rpt3-215* compared to wild type, but the level of *HSP82* mRNA did not (Fig. 5A and B). Strains bearing *hsc82*Δ or *hsp82*Δ did not produce the corresponding mRNAs, demonstrating that the primers used for amplification of mRNA gene fragments were specific for *HSC82* and *HSP82* mRNAs.

3.9. The presence of rpt3-215 correlates with increases in Por1 and Tom40 during fermentation.

We postulated that the *rpt3-215* mutation might change levels of mitochondrial outer membrane proteins through altered turnover by the proteasome (Neutzner et al., 2007). To test this hypothesis, we used immuno-blots to measure levels of Por1, a major protein involved in metabolite exchange (Crompton et al., 1999; Halestrap and Brennerb, 2003; Vyssokikh and Brdiczka, 2003), and of Tom40, a protein primarily involved in translocation of nuclear-encoded proteins into mitochondria (reviewed in Ryan et al., 2000) from ρ^+ and ρ^0 mitochondria of wild type and mutant yeast. Protein levels were normalized using Ponceau Red staining, and mtHsp70, a matrix protein involved in protein translocation into mitochondria, served as a control. The extremely slow growth of *yme1*Δ ρ^0 at 30°C by fermentation precluded its inclusion in the ρ^0 comparison, however two strains were included as partial substitutes for *yme1*Δ ρ^0. Yeast carrying the *yme1-6* point mutation have some phenotypes in common with *yme1*Δ (Francis and Thorsness, unpublished results), while *yme1*Δ *ATP1-111* yeast have a suppressor mutation in Atp1 that allows faster growth of *yme1*Δ ρ^0 (Francis et al., 2007). In ρ^+ strains, a statistically significant increase in Por1 was observed in *rpt3-215* and *yme1Δ rpt3-215* yeast grown by fermentation at 30°C as compared to wild type, *yme1*Δ, *yme1*Δ *ATP1-111* and *yme1-6* (Fig. 6A). The level of Tom40 in strains containing *rpt3-215* also increased (Fig. 6A). In contrast, the level of mtHsp70 was constant in all stains except *yme1*Δ strain, which had a reduced amount of Hsp70 (Fig. 6A). Comparison of the corresponding ρ^0 strains showed that Por1 increased in strains bearing *rpt3-215* and *yme1*Δ *rpt3-215* whereas mtHsp70 did not (Fig. 6B). In contrast, strains containing *rpt3-215* grown in YPEG at 30°C did not have increased levels of Por1, Tom40 or mtHsp70 (Fig. 6C).

Because *por1*Δ yeast are viable (Michejda et al., 1990), we were able to examine the suppressive effects of *rpt3-215* in the absence of Por1. *rpt3-215* suppressed slow growth of *yme1*Δ *por1*Δ and *yme1*Δ *por1*Δ ρ^0 (data not shown), indicating that changes in Por1 concentration is not the basis for suppression of *yme1*Δ phenotypes.

3.10. The suppressor mutation in RPT3 is in the Walker A ATP binding loop.

A mutation in *RPT3*, an essential subunit of the 26S proteasome, suppresses specific *yme1*Δ phenotypes (Campbell et al., 1994). Sequencing this suppressing allele, designated *rpt3-215*, identified a T for C mutation at position 644 that produced a leucine for proline substitution at position 215 in Rpt3. This mutation was in the conserved Walker A (or P-loop) ATP-binding sequence motif of Rtp3 (208-GVLLYGP**L**GTGKTML-222). Such a mutation might be expected to interfere with the ATPase cycle of Rpt3, thereby decreasing the activity of the proteasome and affecting growth. In accord with this expectation, *rpt3-215* and *yme1*Δ *rpt3-215* exhibited slow growth by fermentation at 30°C compared to wild type and *yme1*Δ yeast. *yme1*Δ and *yme1*Δ *rpt3-215* increased growth of yeast relative to wild type as the temperature increased from 16 to 37°C but growth of *rpt3-215* yeast decreased. In fact *yme1*Δ *rpt3-215* yeast grew faster than wild-type yeast. (Fig. 7).

3.11. rpt3-215 prevents growth by fermentation at 37°C of hsc82Δ *atp1Δ and hsc82Δ atp2Δ*, *but not hsp82Δ atp1Δ and hsp82Δ atp2Δ*.

After showing that *rpt3-215* affected growth of *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ strains differently, we attempted to determine whether *rpt3-215* produced a similar outcome when F_1-ATPase was disrupted by deletion of *ATP1, ATP2*, or *ATP3*. *hsc82*Δ *atp3*Δ *rpt3-215* and *hsp82*Δ *atp3*Δ *rpt3-215* strains, grew as microcolonies from germinating spores at 30°C, and were not further studied. *hsc82*Δ *atp1*Δ *rpt3-215* and *hsc82*Δ *atp2*Δ *rpt3-215* strains were inviable when grown by fermentation at 37°C. Slow growth of *hsp82*Δ *atp1*Δ and *hsp82*Δ *atp2*Δ strains was reduced further by the combination with *rpt3-215* (Fig. 8). Consequently, *HSC82* was also required for growth of *atp1*Δ or *atp2*Δ yeast in the presence of *rpt3-215*.

4. Discussion

4.1. Growth of strains lacking Hsc82 or Hsp82.

Although Hsp82 expression is heat inducible and Hsc82 is constitutively expressed, both *hsp82*Δ and *hsc82*Δ grow like wild type by respiration at 16, 30, and 37 °C and by fermentation at 16 and 30°C. For growth by fermentation of ρ^+ strains at 37°C there is a requirement for both of these proteins (Fig. 1A). Loss of Hsc82 is more harmful to growth at elevated temperatures than loss of Hsp82. These results support previous studies with diploid strains showing that loss of three out of four *HSC82* and *HSP82* genes results in virtually no growth (Borkovich et al., 1989). Those *hsc82*Δ and *hsp82*Δ colonies that grew at 37°C (Fig. 1A) likely bear suppressor mutations. The decreased growth of *hsc82*Δ and *hsp82*Δ strains on YPD plates at 37°C differs from the moderate growth inhibition reported for comparable homozygous diploid strains in liquid fermentation medium at 37°C after inoculation with cells grown at 25°C (Borkovich et al., 1989). This difference may be explained by the generation of spontaneous suppressors in liquid media. The fact that respiration is unimpeded at 37°C in these strains (Fig. 1B) suggests that the detrimental effects on fermentation are the result of different responses to heat shock during fermentation and respiration (Patriarca and Maresca, 1990).

Neither Hsc82 nor Hsp82 has previously been reported in mitochondria, although surveys of genetic and proteomic relationships in yeast suggest roles for Hsc82 and Hsp82 in mitochondrial membrane biogenesis (Gavin et al., 2006; Krogan et al., 2006; McClellan et al., 2007; Zhao et al., 2005). Our genetic data are best explained by the presence of these proteins in yeast mitochondria. After communicating our results with Dr. Susan Lindquist and Dr. Dan Tardiff, they confirmed by protein immunoblot that Hsc(p)82 was present in yeast mitochondrial fractions (Tardiff and Lindquist, personal communication).

4.2. Roles for Hsc82, Hsp82, Yme1, and Yta10/12 in F1F_o-ATPase assembly

Many aspects of F_1F_o-ATPase assembly have been discovered and specialized chaperones identified (Rak et al., 2009), but some steps, such as the assembly of F_1-ATPase and the assembly of the F_1-ATPase with the peripheral stalk, are not fully understood. Figure 9 shows a model for assembly of F_1F_o-ATPase that is consistent with earlier studies and the genetic evidence presented here. Although the order of events in Figure 9 has considerable support (Ackerman and Tzagoloff, 2005; Goyon et al., 2008; Kucharczyk et al., 2009; Velours and Arselin, 2000), some events, such as the order of incorporation of Atp8, must still be regarded as provisional (Rak et al., 2009).

Step A to B of Fig. 9: Formation of F_1-ATPase.—F_1-ATPase, can be assembled in the presence or absence of mtDNA. Formation of $(Atp1Atp2)_3$ requires specialized assembly factors: Atp12 and Atp11, for Atp1 and Atp2, respectively, and Fmc1 at elevated temperatures which binds to Atp12 (Ackerman, 2002; Lefebvre-Legendre et al., 2001; Smith

and Thorsness, 2005). No specialized chaperones/assembly factors are known for Atp3, or the other F_1 components of the central stalk, Atp15, or Atp16. A subcomplex containing Atp15 and Atp16 has been purified from pig heart mitochondria (Penin et al., 1990), and the stable subcomplex has been isolated when these proteins are co-expressed in *E. coli* (Orriss et al., 1996) suggesting that chaperones may not be required for this step in assembly of the central stalk. Assembly of Atp15/Atp16 with Atp3 has not been studied. The ability of $(Atp1Atp2)_3$ to be separated from the central stalk using mild detergent conditions (Wittig et al., 2008) suggests that Atp3 can be inserted into pre-formed $(Atp1Atp2)_3$. More persuasively, incorporation of Atp3 that has been C-terminally fused to GFP into active ATP synthase (Prescott et al., 2003), impaired ATP synthase assembly produced by N- and C-terminal deletions of Atp3 (Dian et al., 2008), and involvement of Atp3 in formation of $(Atp1Atp2)_3$ (Ludlam et al., 2009) argue for assembly of $(Atp1Atp2)_3$ around the central stalk.

*yta10*Δ and *yta12*Δ inhibit assembly of F_1-ATPase in a manner that does not overlap with Atp11 and Atp12 (Paul and Tzagoloff, 1995). Three lines of evidence from this study and others suggest that loss of Yta10/12 has a similar effect to loss of Atp3. First, loss of these proteins leads to destabilization of mitochondrial DNA. Strains lacking any of the three central stalk proteins are viable only as ρ^0 strains (Giraud and Velours, 1994; Lai-Zhang and Mueller, 2000; Smith and Thorsness, 2005). *yta10*Δ is also unstable with regard to loss of mtDNA (Lemaire et al., 2000; Tauer et al., 1994). Another genetic connection between *atp3*Δ and *yta10*Δ is a similar increase in growth of ρ^0 strains relative to wild type as the temperature increases from 16 to 37°C. A temperature dependent increase in growth is not observed with *atp1*Δ ρ^0 and *atp2*Δ ρ^0 showing that in these cases another ATPase does not compensate for the loss of the ATPase activity from $(Atp1Atp2)_3$. If Atp3 is required for assembly of $(Atp1Atp2)_3$ (Ludlam et al., 2009), no $(Atp1Atp2)_3$ would be present in *atp3*Δ and hence there would be no F_1-ATPase activity to assist in maintenance of membrane potential. Nonetheless, *atp3*Δ has a higher growth rate at 37°C than *atp1*Δ ρ^0 and *atp2*Δ ρ^0. Among the genes up-regulated in ρ^0 compared to ρ^+ cells are Atp11 and Atp12 (Traven et al., 2001). Fmc1 is a chaperone that is required for proper functioning of Atp12 at high temperatures (37°C) in the incorporation of Atp1 and Atp2 into F_1-ATPase (Lefebvre-Legendre et al., 2001). A possible explanation for the temperature dependence of *atp3*Δ and *yta10*Δ growth rate is that as Fmc1 becomes increasingly expressed $(Atp1Atp2)_3$ can form independently of Atp3 using Atp11 and Atp12. –Taken together, the evidence suggests a function for Yta10/12 in the step by which the central stalk becomes incorporated into F_1-ATPase, although the protein(s) that interacts with Yta10/12 has yet to be identified.

In several instances, a difference is observed between the effects of *hsc82*Δ and *hsp82*Δ on growth. For example, *hsp82*Δ ρ^0 and *hsp82*Δ *yta10*Δ ρ^0 yeast grow faster at 37°C than *hsc82*Δ ρ^0 and *hsc82*Δ *yta10*Δ ρ^0 yeast (Fig. 3A). Loss of Yta10/12 has a subtle effect on ρ^+ or ρ^0 growth of *hsc82*Δ at 37°C; it prevents *hsc82*Δ death but not its slow growth. Suppression of slow growth at 37°C of *hsp82*Δ yeast but not *hsc82*Δ yeast could be achieved by removal of peripheral stalk proteins, Atp4 or Atp5 (Fig. 2). In the case of *hsp82*Δ *atp4*Δ and *hsp82*Δ *atp5*Δ strains, a reduced requirement for Hsc82 during association of the peripheral stalk to F_1-ATPase (discussed below) may allow increased cycling of Hsc82 for F_1-ATPase assembly thereby allowing suppression. The preference of F_1-ATPase assembly for Hsc82 means that suppression is not observed with *hsc82*Δ *atp4*Δ or *hsc82*Δ *atp5*Δ. A possible explanation for the greater growth defects conferred by *hsc82*Δ yeast compared to *hsp82*Δ yeast would involve Hsc82 interacting with Atp3 (Krogan et al., 2006).

Loss of mtDNA from *yme1*Δ is more detrimental for growth than it is for either *yta10*Δ or *atp3*Δ, and *yme1*Δ strains do not lose mtDNA as readily as *atp3*Δ or *yta10*Δ. The double

mutations, *yta10*Δ *yme1*Δ and *atp3*Δ *yme1*Δ, only allow growth of yeast as micro-colonies after spore germination showing that the combination of these nulls is more severe than each of the nulls individually, and suggesting that *yme1*Δ acts separately from *atp3*Δ or *yta10*Δ. One effect of *yme1*Δ is to allow an increase in the level of Atp4 (Lemaire et al., 2000). In the absence of Atp6, Atp8, and Atp9 encoded by the mtDNA, increased Atp4 might allow greater assembly of F_1-ATPase with the peripheral stalk. However, *atp4*Δ ρ^0 and *atp5*Δ ρ^0 strains grow almost as well as wild type ρ^0 at 30°C and 37°C (Fig. 3B and data not shown) showing that F_1-ATPase binding to the peripheral stalk is not important for ρ^0 growth. In addition, *yme1*Δ *atp4*Δ ρ^0 yeast grow extremely slowly, similar to *yme1*Δ ρ^0 yeast (Kominsky et al., 2002), showing that *yme1*Δ is controlling ρ^0 growth and has an effect that is independent of *atp4*Δ. The severe effect of *yme1*Δ on ρ^0 growth is counteracted by mutations in F_1-ATPase subunits that increase ATPase activity (Francis et al., 2007). This is consistent with a role for Yme1 in F_1-ATPase assembly: a lower level of correctly assembled F_1-ATPase in *yme1*Δ ρ^0 yeast can be offset by increased F_1-ATPase activity. This suggests that Yme1, like Yta10/12, has a role in F_1-ATPase assembly that involves incorporation of the central stalk into F_1-ATPase. Combination of *yme1*Δ with *hsc82*Δ or *hsp82*Δ does not suppress ρ^0 yeast growth at 37°C, and at 16°C, 30°C, and 37°C *yme1*Δ produces a similar decrease in ρ^0 yeast growth of *hsc82*Δ and *hsp82*Δ strains (Fig. 3A). The role for Yme1 in F_1-ATPase assembly may involve an interaction with either Hsc82 or Hsp82, which in turn binds to Atp2 (Krogan et al., 2006). In summary, the proposed mechanism for this step involves assembly of Atp1 and Atp2 around the central stalk, with release of Hsc82 and Yta10/12 from the central stalk and Atp11, Atp12, and Fmc1 from Atp1 and Atp2. Yme1 remains associated with Atp2 via Hsc82/Hsp82 after assembly of F_1-ATPase prior to association with Atp9 and the peripheral stalk.

Step B to C of Fig.9: Interaction of F_1-ATPase with oligoAtp9.—Atp25 is the chaperone for assembly of the Atp9 oligomer (Zeng et al., 2008). Newly synthesized oligomeric Atp9 forms an F_1-oligoAtp9 subcomplex prior to its association with Atp6 and the peripheral stalk complex (Ackerman and Tzagoloff, 2005).

Step C to D of Fig.9: Interaction of F_1-oligoAtp9 with the peripheral stalk.—Association of F_1-oligoAtp9 with the peripheral stalk occurs with a preformed peripheral stalk complex (Rak et al., 2009). An interesting parallel exits between the effects of *yme1*Δ on *hsc82*Δ/*hsp82*Δ and *oxa1*Δ. Addition of *yme1*Δ to either *hsc82*Δ or *hsp82*Δ imparts the corresponding *yme1*Δ phenotypes and for *hsc82*Δ and *hsp82*Δ this means suppression of slow fermentative growth at 37°C (Fig. 1A). The only other reported strain for which *yme1*Δ improves growth is *oxa1*Δ (Lemaire et al., 2000). Oxa1 is required for assembly of F_1F_o-ATPase (Altamura et al., 1996), and assists in the correct assembly of the Atp9 oligomer with Atp6. In the absence of Oxa1, Atp6 and Atp4 levels in mitochondria are greatly reduced (Jia et al., 2007). *yme1*Δ restores assembly of F_1F_o-ATPase in *oxa1*Δ but does not restore respiratory growth or increase steady state levels of cytochrome *c* oxidase subunits (Lemaire et al., 2000). Apparently, *yme1*Δ suppresses the effects of *oxa1*Δ by diminishing turnover of Atp4 and Atp6 (Lemaire et al., 2000). The level of Atp4 also increases in *yme1*Δ ρ^0 (Kominsky et al., 2002). A common link for these effects of *yme1*Δ on *oxa1*Δ and *hsc82*Δ/*hsp82*Δ may be increased levels of Atp6 and peripheral stalk complex made possible by the absence of the proteolytic activity of Yme1, which in turn allows a higher level of active F_1F_o-ATPase to be assembled. We have no evidence to suggest a difference between Hsc82 and Hsp82 in this regard. *yme1*Δ does not suppress slow growth of *hsc82*Δ or *hsp82*Δ yeast in the presence of *atp1*Δ, *atp2*Δ, *atp4*Δ, or atp5Δ (Fig. 2), suggesting a role for Yme1 in sensing Atp4 through the ATPase/protease domains in the IMS, and in sensing F_1-ATPase via matrix-localized Hsc82 or Hsp82 through its N-terminal domain. Hsc82 and Hsp82 interact physically with Atp2 (Krogan et al., 2006), and have a synthetic growth

defect with Atp1 (McClellan et al., 2007). Therefore, Yme1 may assist in assembly of the F_1-Atp9 subcomplex with the peripheral stalk through an interaction with Hsc82/Hsp82 that is bound to Atp2. In this step of the model, Hsc82/Hsp82 is displaced from Atp2 during assembly of the peripheral stalk with the F_1-oligoATP9 complex. One obvious possibility is that the N-terminal domain of Yme1 is a co-chaperone for Hsc82/Hsp82. Incorporation of Atp8 into the complex may occur before or after assembly of the peripheral stalk.

A role for Yme1 and Hsc82/Hsp82 in assembly of F_1-ATPase with the peripheral stalk is supported by the changes in the percentage of ρ^+ cells of *atp4*Δ or *atp5*Δ yeast cultures in the presence of *yme1*Δ and *hsc82*Δ/*hsp82*Δ. Nearly all of the loss of mtDNA observed for *atp4*Δ and *atp5*Δ strains is prevented in the double mutants with *yme1*Δ or the triple mutants with *yme1*Δ and *hsc82*Δ or hsp82Δ (Table 3). Loss of central or peripheral stalk proteins allows a proton leak across the inner mitochondrial membrane via Atp6 that is counteracted by the loss of mtDNA (Mueller, 2000; Uh et al., 1990). In the case of *atp4*Δ and *atp5*Δ strains this leak is apparently unable to form in the absence of Yme1, presumably because the F_1-ATPase/oligoAtp9 complex is unable to form a complex with Atp6 under these conditions.

Step D to E of Fig. 9: Incorporation and processing of Atp6.—The last step in F_1F_o-ATPase assembly is incorporation of Atp6, the subunit that contains the proton channel that drives rotation of the central stalk (Goyon et al., 2008). Assembly of Atp6 involves three other proteins, Oxa1 (Jia et al., 2007), Atp10 (Tzagoloff et al., 2004), and Atp23 (Osman et al., 2007), the last of which encodes a protease that cleaves part of Atp6.

4.3. The Rpt3-215 suppressor increases transcription of Hsc82

The *rpt3-215* mutation is in the active site of an essential proteasomal subunit. The Pro215Leu mutation in the highly conserved ATP binding loop of Rpt3 may behave similarly to the oncogenic Gly12Val mutation in the sequence, 7-VVVGA**V**GVGKS-17, of the GTP binding loop of ras p21 that inhibits GTP hydrolysis and acts as a permanent ‘on’ switch (Tong et al., 1991). If so, activation of *HSC82* transcription by Rpt3-215 would require the ATP bound conformation of Rpt3.

rpt3-215 generally decreases growth consistent with an altered ATPase cycle for Rpt3-215 that decreases proteasomal function. Growth is increases when *yme1*Δ is combined with *rpt3-215*. The double mutant grows better than either of the single mutants by fermentation and respiration at 37°C. A particularly informative observation is that *rpt3-215* suppresses slow growth at 37°C of *hsp82*Δ *yme1*Δ but not *hsc82*Δ *yme1*Δ (Fig. 4). This led to the discovery that *rpt3-215* increases transcription of *HSC82* but not *HSP82*. Increased *HSC82* transcription can account for the increased growth of *hsp82*Δ *yme1*Δ *rpt3-215* compared to *hsp82*Δ *yme1*Δ at 37°C. The very slow growth of *hsc82*Δ *yme1*Δ *rpt3-215* at 37°C compared to *hsc82*Δ *yme1*Δ shows that heat induced transcription of *HSP82* cannot compensate for the loss of constitutively expressed *HSC82*. Therefore, Hsc82 supports growth in a way Hsp82 does not, consistent with a specific role for Hsc82 in F_1F_o-ATPase assembly involving Atp3. Increased *HSC82* transcription by Rpt3-215 is notably similar to induction of two other heat shock proteins, *HSP26* and *HSP104*, by two other proteasomal ATPases, Rpt6 (Sug1) and Rpt4 (Sug2), acting independently of their roles in proteolysis (Kodadek, 2010; Sulahian et al., 2006).

Another possibility is that Rpt3-215 alters proteasomal turnover of proteins in the outer mitochondrial membrane leading perhaps to increased metabolite exchange. Approximately a 2-fold increase in levels of Por1 and Tom40 is found in mitochondria isolated from *rpt3-215* and *yme1*Δ *rpt3-215* compared to those from wild type when grown by fermentation, but not when grown by respiration. However, suppression of slow growth of

*por1*Δ strains by *rpt3-215* suggests that Rpt3-215 does not act through this mechanism alone.

4.4 Growth in the absence of F_1-ATPase.

Yeast lacking Atp1 or Atp2 grow by fermentation, albeit slower than wild type. Previous studies have demonstrated that loss of either one of these subunits results in aggregation of the other subunit into mitochondrial inclusion bodies (Lefebvre-Legendre et al., 2005). Because growth requires maintenance of a membrane potential across the inner membrane, and the membrane potential during fermentation depends primarily upon ATP hydrolysis in the matrix, another mechanism for ATP hydrolysis must compensate for the absence of F_1-ATPase. Remarkably, slow growth of both *hsc82*Δ and *hsp82*Δ is suppressed by *atp1*Δ or *atp2*Δ (Fig 2). It appears that when Hsc82 or Hsp82 is not cycling through F_1F_o-ATPase assembly it becomes available to assist an alternate mechanism for ATP hydrolysis. Whatever this mechanism may be, it also requires Yme1 because *yme1*Δ eliminates the suppression by *atp1* or *atp2*Δ (Fig. 2). *rpt3-215* suppresses slow fermentative growth at 37°C of *hsp82*Δ *atp1*Δ and *hsp82*Δ *atp2*Δ but not *hsc82*Δ *atp1*Δ and *hsc82*Δ *atp2*Δ, showing that suppression can occur in the absence of F_1-ATPase. Loss of ATP synthase assembly at elevated temperature is suppressed by increased substrate level mitochondrial ATP synthesis via the citric acid cycle (Schwimmer et al., 2005), suggesting that growth by fermentation in the absence of F_1-ATPase, may be compensated by improved substrate level ATP hydrolysis in mitochondria by succinyl-CoA ligase, an enzyme of the citric acid cycle, operating in the reverse direction. Future studies may discover specific functions for Hsc82/Hsp82 in mitochondria in addition to the proposed role in F_1F_o-ATPase assembly.

Acknowledgments

Thanks to William Murdoch for assistance with statistical analyses, and Kathy Austin and Kumaran Mani for assistance with RT-PCR. Drs. Carla Koehler and Trevor Lithgow supplied antisera. We also thank Drs. Susan Lindquist and Dan Tardiff for communicating results prior to publication. This work was supported by grants from the National Institutes of Health (GM068066) and National Center for Research Resources and the Wyoming INBRE (P20RR016474).

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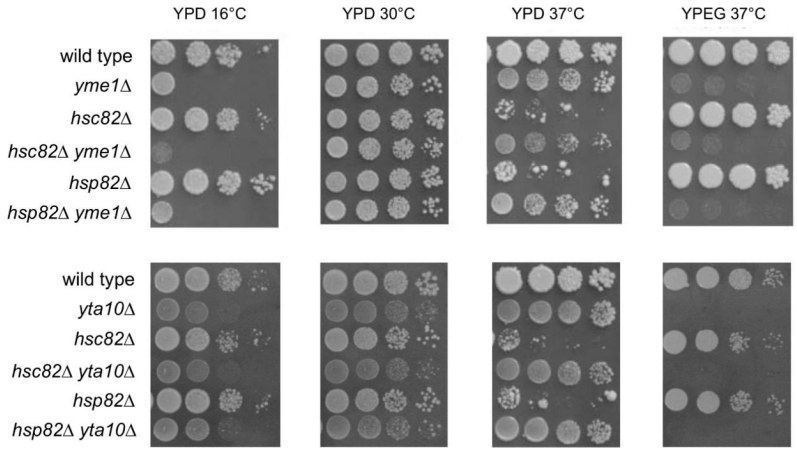

Fig. 1.

Growth of strains bearing *hsc82*Δ, *hsp82*Δ, *yme1*Δ, and *yta10*Δ on YPD at 16°C, 30°C, and 37°C, and YPEG at 37°C. Strains compared: wild type (PTY44), *yme1*Δ (PTY52), *hsc82*Δ (BFY261), *hsp82*Δ (BFY265), *hsc82*Δ *yme1*Δ (BFY263), *hsp82*Δ *yme1*Δ (BFY267), *yta10*Δ (TCY86), *hsc82*Δ *yta10*Δ (BFY350), *hsp82*Δ *yta10*Δ (BFY334).

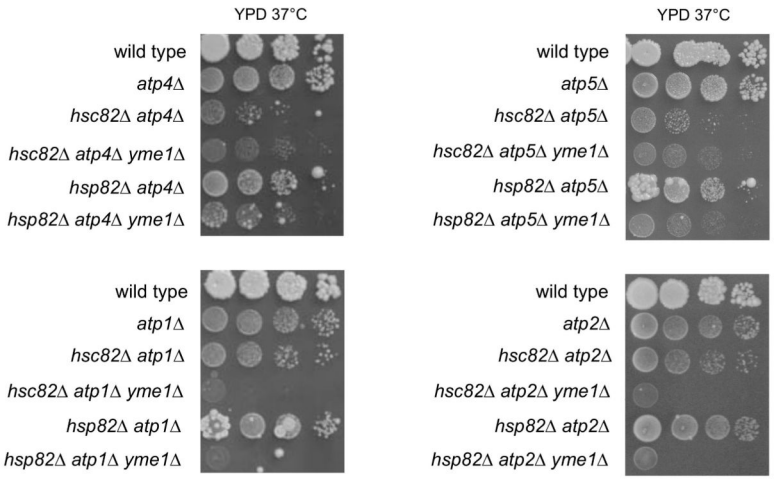

Fig. 2.

Growth at 37°C on YPD of *hsc82*Δ *yme1*Δ and *hsp82*Δ *yme1*Δ in the presence of *atp4*Δ, *atp5*Δ, *atp1*Δ, and *atp2*Δ.

Strains compared: wild type (PTY44), *atp4*Δ (DKY40), *hsc82*Δ *atp4*Δ (BFY327), *hsc82*Δ *atp4*Δ *yme1*Δ (BFY328), *hsp82*Δ *atp4*Δ (BFY330), *hsp82*Δ *atp4*Δ *yme1*Δ (BFY332), *atp5*Δ (TCY71), *hsc82*Δ *atp5*Δ (BFY351), *hsc82*Δ *atp5*Δ *yme1*Δ (BFY357), *hsp82*Δ *atp5*Δ (BFY353), *hsp82*Δ *atp5*Δ *yme1*Δ (BFY358), *atp1*Δ (TCY47), *hsc82*Δ *atp1*Δ (BFY291), *hsc82*Δ *atp1*Δ *yme1*Δ (BFY300), *hsp82*Δ *atp1*Δ (BFY296), *hsp82*Δ *atp1*Δ *yme1*Δ (BFY306), *atp2*Δ (TCY49), *hsc82*Δ *atp2*Δ (BFY293), *hsc82*Δ *atp2*Δ *yme1*Δ (BFY303), *hsp82*Δ *atp2*Δ (BFY298), *hsp82*Δ *atp2*Δ *yme1*Δ (BFY308).

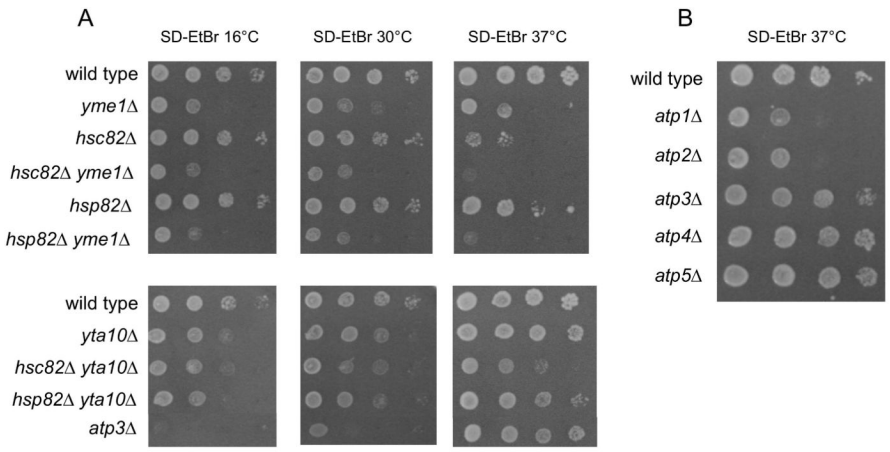

Fig. 3.

Growth of strains lacking mtDNA at 16°C, 30°C, and 37°C.

Strains compared: **A**. wild type (PTY44), *yme1*Δ (PTY52), *hsc82*Δ (BFY261), *hsp82*Δ (BFY265), *hsc82*Δ *yme1*Δ (BFY263), *hsp82*Δ *yme1*Δ (BFY267), *yta10*Δ (TCY86), *hsc82*Δ *yta10*Δ (BFY350), *hsp82*Δ *yta10*Δ (BFY334), *atp2-227* (TCY55), *atp3*Δ (JTY3), *atp3*Δ *atp2-227* (JTY6). **B**. wild type (PTY44), *atp1*Δ (TCY47), *atp2*Δ (TCY49), *atp3*Δ (JTY3), *atp4*Δ (DKY40), *atp5*Δ (TCY71).

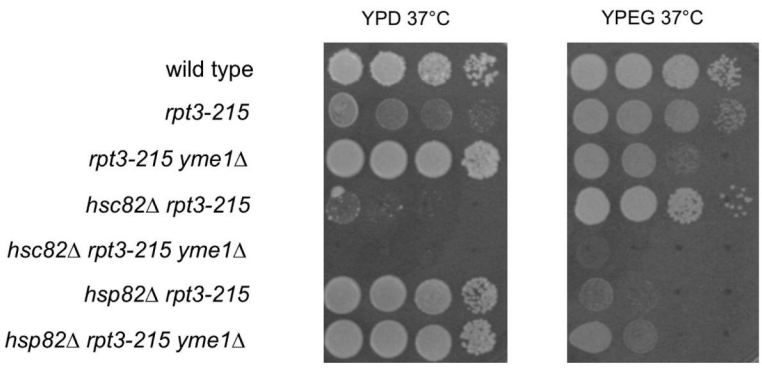

Fig. 4.

Growth of strains bearing *hsc82*Δ, *hsp82*Δ, *yme1*Δ, and *rpt3-215* on YPD at 37°C and YPEG at 37°C. Strains compared: wild type (PTY44), *rpt3-215* (BFY238), *yme1*Δ *rpt3-215* (NTY1), *hsc82*Δ *rpt3-215* (BFY310), *hsc82*Δ *yme1*Δ *rpt3-215* (BFY312), *hsp82*Δ *rpt3-215* (BFY314), *hsp82*Δ *yme1*Δ *rpt3-215* (BFY316).

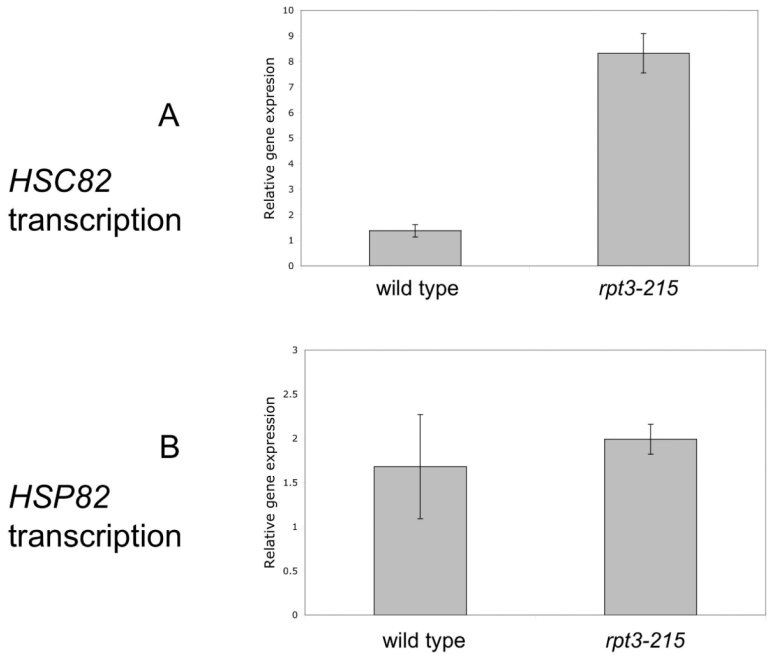

Fig. 5.

Relative gene expression of *HSC82* and *HSP82*. Analysis of *HSC82* (A) and *HSP82* (B) mRNAs in wild type (PTY44) and *rpt3-215* (BFY238).

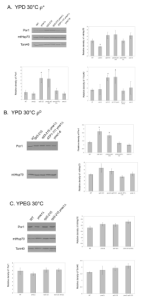

Fig. 6.

Western blots using antibodies against Por1, mtHsp70, and Tom40 of mitochondrial proteins from ρ^+ strains (A) and ρ^0 strains (B) grown in YPD at 30°C, and strains grown in YPEG at 30°C (C). * indicates significant difference. Strains compared: wild type (PTY44), *yme1*Δ (PTY52), *rpt3-215* (BFY238), *yme1*Δ *rpt3-215* (NTY1), *yme1*Δ *ATP1-111* (BFY138), *yme1-6* (PTY206).

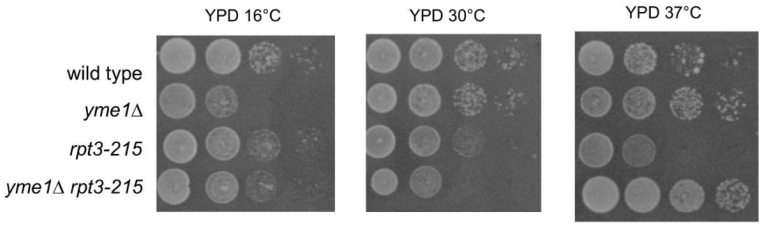

Fig. 7.
Growth of strains bearing *yme1*Δ and *rpt3-215* at 16°C, 30°C, and 37°C. Strains compared: wild type (PTY44), *yme1*Δ (PTY52), *rpt3-215* (BFY238), *yme1*Δ *rpt3-215* (NTY1).

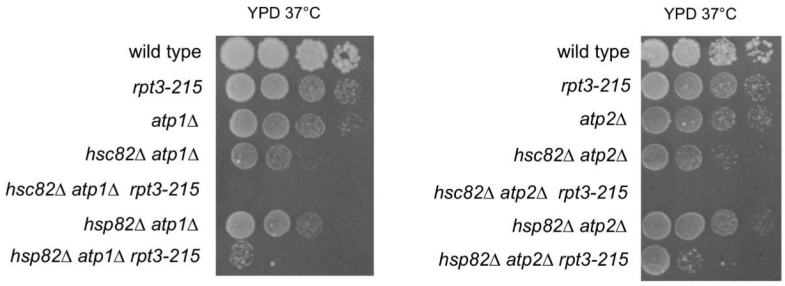

Fig. 8.

Growth on YPD at 37°C of strains bearing *hsc82*Δ, *hsp82*Δ, *rpt3-215*, *atp1*Δ, and *atp2*Δ. Strains compared: wild type (PTY44), *rpt3-215* (BFY238), *atp1*Δ (TCY47), *hsc82*Δ *atp1*Δ (BFY291), *hsc82*Δ *atp1*Δ *rpt3-215* (BFY361), *hsp82*Δ *atp1*Δ (BFY296), *hsp82*Δ *atp1*Δ *rpt3-215* (BFY365), *atp2*Δ (TCY49), *hsc82*Δ *atp2*Δ (BFY293), *hsc82*Δ *atp2*Δ *rpt3-215* (BFY366), *hsp82*Δ *atp2*Δ (BFY298), *hsp82*Δ *atp2*Δ *rpt3-215* (BFY368).

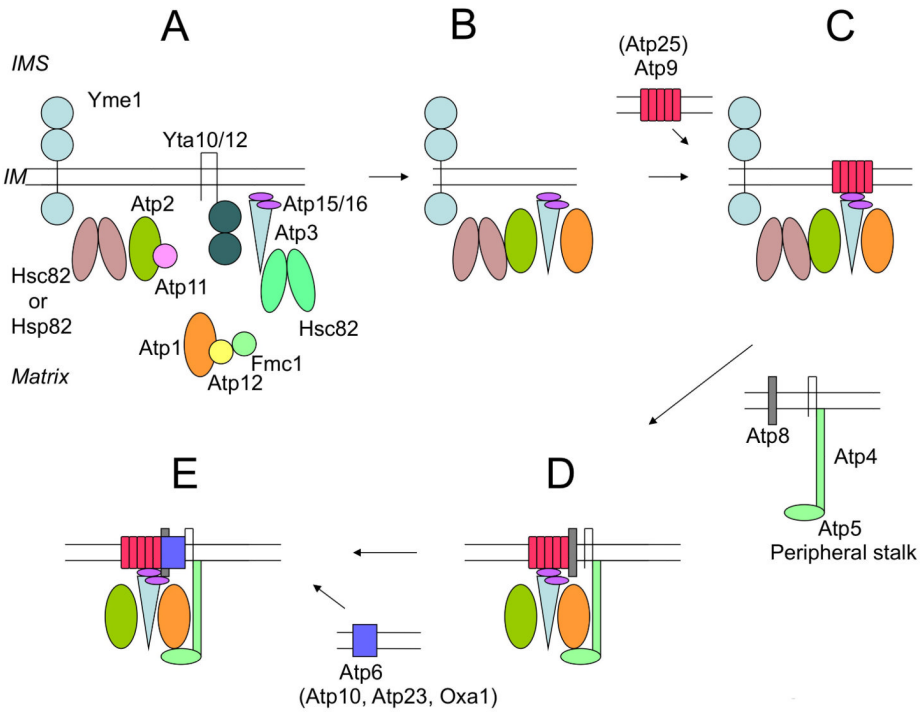

Fig. 9.

Model for assembly of F_1F_o-ATPase at 37°C. The inner membrane (IM) is shown with the intermembrane space above the membrane and the matrix below. One subunit of the Yme1 and Yta10/12 complexes is shown. **A**. Assembly of F_1. Yme1 interacts genetically with Hsc82 or Hsp82 to which Atp2 is bound with Atp11 as chaperone. Yta10/12 interacts in an unknown way with the central stalk to which only Hsc82 is bound. Atp1 is bound to chaperone Atp12, which in turn is bound to Fmc1. Assembly of Atp1 and Atp2 around Atp3 begins with interaction of Atp3 with Atp1 displacing Atp12 and Fmc1. As assembly proceeds Atp11, Atp12, and Yta10/12 are displaced and Hsc82 is displaced from Atp3 to produce **B**. The interaction of Yme1 with Atp2 via Hsc82/Hsp82 remains while F_1 associates with oligo-Atp9 that has been formed with assistance from Atp25 to form **C**. F_1-Atp9 associates with Atp8 and the peripheral stalk complex (only Atp4 and Atp5 are shown). Yme1 and Hsc82/Hsp82 are displaced to form **D**. Atp6 is incorporated with assistance from Atp10, Atp23 and Oxa1 to produce F_1F_0-ATPase, **E**.

Table 1

Strains

Strain	Genotype[a]	Source
BFY138	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 ATP1-111[ρ+ , TRP1]*	(Francis et al., 2007)
BFY145	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1Δ1::URA3 atp1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY178	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1Δ1::URA3 atp2-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY190	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1Δ1::URA3 atp5-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY222	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ cox4-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY234	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1Δ1::URA3 por1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY236	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ por1-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
BFY238	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ rpt3-215 [ρ+, TRP1]*	This study
BFY240	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 por1-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
BFY261	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY263	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 hsc82-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY265	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY267	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 hsp82-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY291	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY293	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp2-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY296	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY298	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp2-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY300	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 yme1-Δ::URA3 atp1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY303	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 yme1-Δ::URA3 atp2-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY306	*MATα ura3-52 ade2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 yme1-Δ::URA3 atp1-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY308	*MATα ura3-52 ade2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 yme1-Δ::URA3 atp2-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY310	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
BFY312	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 yme1-Δ1::URA3 rpt3-215 [ρ+, TRP1]*	This study
BFY314	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
BFY316	*MATα ura3-52 ade2 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 yme1-Δ1::URA3 rpt3-215 [ρ+, TRP1]*	This study
BFY322	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp3-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY324	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp3-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY327	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp4-Δ1::URA3 [ρ+, TRP1]*	This study
BFY328	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 yme1-Δ1::URA3 atp4-Δ1::URA3 [ρ+, TRP1]*	This study
BFY330	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp4-Δ1::URA3 [ρ+, TRP1]*	This study

Strain	Genotype[a]	Source
BFY332	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 yme1-Δ1::URA3 atp4-Δ1::URA3 [ρ+, TRP1]*	This study
BFY334	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 yta10-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY340	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 cox4-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY343	*MATα ura3-52 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 cox4-Δ1::KanMX6 yme1-Δ1::URA3 [ρ+, TRP1]*	This study
BFY344	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 cox4-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY347	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 cox4-Δ1::KanMX6 yme1-Δ1::URA3 [ρ+, TRP1]*	This study
BFY350	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 yta10-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY351	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp5-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY353	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp5-Δ1::KanMX6 [ρ+, TRP1]*	This study
BFY357	*MATα ura3-52 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp5-Δ1::KanMX6 yme1-Δ1::URA3 [ρ+, TRP1]*	This study
BFY358	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp5-Δ1::KanMX6 yme1-Δ1::URA3 [ρ+, TRP1]*	This study
BFY361	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsc82-Δ1::KanMX6 atp1-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
BFY365	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 hsp82-Δ1::KanMX6 atp1-Δ1::KanMX6 rpt3-215 [ρ+, TRP1]*	This study
DKY40	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp4-Δ1::URA3 [ρ+, TRP1]*	This study
DKY48	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 atp4-Δ1::URA3 [ρ+, TRP1]*	This study
JTY3	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp3-Δ1::KanMX6 [ρ+, TRP1]*	(Smith and Thorsness, 2005)
KWY44	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 por1Δ::KanMX6 [ρ+, TRP1]*	This study
NTY1	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 rpt3-215 [ρ+, TRP1]*	(Campbell et al., 1994)
PTY33 (Wild type)	*MAT**a** ura3-52 ade2 leu2-3,112 trp1-Δ1 [ρ+, TRP1]*	(Thorsness and Fox, 1993)
PTY44 (Wild type)	*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)
PTY206	*MATα ura3-52 ade2 leu2-3,112 trp1-Δ1 yme1-6 [ρ+, TRP1]*	This study
TCY47	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp1-Δ1::KanMX4 [ρ+, TRP1]*	This study
TCY49	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp2-Δ1::KanMX4 [ρ+, TRP1]*	This study
TCY71	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 atp5-Δ1::KanMX4 [ρ+, TRP1]*	This study
TCY86	*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 yta10-Δ1::KanMX6 [ρ+, TRP1]*	This study

[a]The mitochondrial genotype is bracketed.

Table 2

Primers used for PCR amplification of genes in the Open Biosystems Yeast deletion library:	
Hsc82F	5′-AAGCGTTGGGTAATGAGGGA
Hsc82R	5′-GGACAGCTGGTAGGACAATTT
Hsp82F	5′-TAATACCAACCAGGTCCTTCC
Hsp82R	5′-CGATTTCAGATTCTTCGCGT
Cox4F	5′-CAGATCGAACGAAACGGAAA
Cox4R	5′-AACTTCGCAACAACCTCTAGC
Primers used for PCR amplification and sequencing of *RPT3*:	
Rpt3F	5′-TGGACAAACCAATTCTGCCT
Rpt3R	5′-CGTGATAAAAAAGTGGCGTC
Rpt3seq1	5′-GGCAATCGGATAAAATTC
Rpt3seq2	5′-GAACCTATTGATCAGAAC
Rpt3seq3	5′-CCGGTAAGACGATGCTTG
Rpt3seq4	5′-GATTAGATAGAAAGATTGAG
Primers used for RT-PCR amplification of ~150 base segments of *HSC82*, *HSP82*, and *ACT1*:	
Hsc82F	CTGCCATCAGAACTGGTCAA
Hsc82R	TATCTTGTGCACCACCCTCA
Hsp82F	ACTCCAAAGCCAGAGCAAAA
Hsp82R	ACCTGAACTCTGTCGGCAAC
Act1F	GCCTTCTACGTTTCCATCCA
Act1R	GGCCAAATCGATTCTCAAAA

Table 3
Proportion of cells containing mtDNA.

Strain	% ρ^+ cells
wild type	99
yme1 Δ	99
hsc82 Δ	99
*hsc82*Δ *yme1*Δ	100
hsp82 Δ	99
*hsp82*Δ *yme1*Δ	100
atp4 Δ	0
*atp4*Δ *yme1*Δ	98
*hsc82*Δ *atp4*Δ	33
*hsc82*Δ *atp4*Δ *yme1*Δ	98
*hsp82*Δ *atp4*Δ	30
*hsp82*Δ *atp4*Δ *yme1*Δ	92
atp5 Δ	31
*atp5*Δ *yme1*Δ	100
*hsc82*Δ *atp5*Δ	30
*hsc82*Δ *atp5*Δ *yme1*Δ	99
*hsp82*Δ *atp5*Δ	10
*hsp82*Δ *atp5*Δ *yme1*Δ	100
atp1 Δ	97
*atp1*Δ *yme1*Δ	96
*hsc82*Δ *atp1*Δ	97
*hsc82*Δ *atp1*Δ *yme1*Δ	98
*hsp82*Δ *atp1*Δ	100
*hsp82*Δ *atp1*Δ *yme1*Δ	98
atp2 Δ	97
*atp2*Δ *yme1*Δ	97
*hsc82*Δ *atp2*Δ	99
*hsc82*Δ *atp2*Δ *yme1*Δ	98
*hsp82*Δ *atp2*Δ	98
*hsp82*Δ *atp2*Δ *yme1*Δ	100

Strains used: wild type, PTY44; *yme1*Δ, PTY52; *hsc82*Δ, BFY261; *hsc82*Δ *yme1*Δ, BFY263; *hsp82*Δ, BFY265; *hsp82*Δ yme1Δ, BFY267; *atp4*Δ, DKY40; *atp4*Δ *yme1*Δ, DKY48; *hsc82*Δ *atp4*Δ, BFY327; *hsc82*Δ *atp4*Δ *yme1*Δ, BFY328; *hsp82*Δ *atp4*Δ, BFY330; *hsp82*Δ *atp4*Δ *yme1*Δ, BFY332; *atp5*Δ, TCY71; *atp5*Δ *yme1*Δ, BFY190; *hsc82*Δ *atp5*Δ, BFY351; *hsc82 atp5*Δ *yme1*Δ, BFY357; *hsp82*Δ *atp5*Δ, BFY353; *hsp82*Δ *atp5*Δ *yme1*Δ, BFY358; *atp1*Δ, TCY47; *atp1*Δ *yme1*Δ, BFY145; *hsc82*Δ *atp1*Δ, BFY291; *hsc82*Δ *atp1*Δ *yme1*Δ, BFY300; *hsp82*Δ *atp1*Δ, BFY296; *hsp82*Δ *atp1*Δ *yme1*Δ, BFY306; *atp2*Δ, TCY49; *atp2*Δ *yme1*Δ, BFY178; *hsc82*Δ *atp2*Δ, BFY293; *hsc82*Δ *atp2*Δ *yme1*Δ, BFY303; *hsp82*Δ *atp2*Δ, BFY298; *hsp82*Δ *atp2*Δ *yme1*Δ, BFY308.