Epistatic interactions of deletion mutants in the genes encoding the F₁-ATPase in yeast *Saccharomyces cerevisiae*

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The F₁-ATPase is a multimeric enzyme ($\alpha_3\beta_3\gamma\delta\epsilon$) primarily responsible for the synthesis of ATP under aerobic conditions. The entire coding region of each of the genes was deleted separately in yeast, providing five null mutant strains. Strains with a deletion in the genes encoding α -, β -, γ - or δ -subunits were unable to grow, while the strain with a null mutation in ϵ was able to grow slowly on medium containing glycerol as the carbon source. In addition, strains with a null mutation in γ or δ became 100% ρ^0/ρ^- and the strain with the null mutation in γ grew much more slowly on medium containing glucose. These additional phenotypes were not observed in strains with the double mutations: $\Delta\alpha\Delta\gamma$, $\Delta\beta\Delta\gamma$, $\Delta atp11\Delta\gamma$, $\Delta\alpha\Delta\delta$, $\Delta\beta\Delta\delta$ or $\Delta atp11\Delta\delta$. These results indicate that ϵ is not an essential component of the ATP synthase and that mutations in the genes encoding the α - and β -subunits and in *ATP11* are epistatic to null mutations in the genes encoding the γ - and δ -subunits. These data suggest that the propensity to form ρ^0/ρ^- mutations in the γ and δ null deletion mutant strains and the slow growing phenotypes of the null γ mutant strain are due to the assembly of F₁ deficient in the corresponding subunit. These results have profound implications for the physiology of normal cells.

Keywords: ATP synthase/epistasis/mitochondrial DNA/*Saccharomyces cerevisiae*

Introduction

The mitochondrial (mt) ATP synthase is a multimeric enzyme composed of a water-soluble portion, F₁, and a membrane portion, F₀. F₁ is composed of five subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ (Todd *et al.*, 1980; Walker *et al.*, 1985). The catalytic site is largely within the β -subunit, with some participation by the α -subunit, as demonstrated by biochemical and genetic studies (for a review, see Duncan and Cross, 1992) and shown in the crystal structure of bovine F₁ (Abrahams *et al.*, 1994). As such, there are three catalytic sites formed by three α/β pairs arranged like segments of an orange. In addition, two gene products, Atp11p and Atp12p, are required for the assembly of the F₁-ATPase (Ackerman and Tzagoloff, 1990). The function of these proteins is not known, but they are not subunits of the enzyme and may provide a chaperone-like activity specific for the F₁-ATPase.

The mechanism of ATP synthesis by the ATP synthase proceeds by the alternating or binding site mechanism initially proposed by Boyer *et al.* (1973). In this mechanism, the phosphorylation of ADP is isoenergetic and occurs at one of the three catalytic sites, which have a high affinity for ATP. The flow of protons alters the conformation of the active site, lowering its affinity for ATP and thereby allowing the release of newly formed ATP from the enzyme. This conformational change is thought to occur largely by interactions of the γ -subunit with the α - and β -subunits of the enzyme (Abrahams *et al.*, 1994). Indeed, γ is in the center of F₁ and makes critical and unique contacts with each of the catalytic domains in F₁. Furthermore, γ rotates in the center of F₁ in an ATP-dependent fashion (Noji *et al.*, 1997). As such, the evidence indicates that F₁ is a molecular motor, which is driven by protons to produce ATP. The crystal structure of bovine F₁ did not show the structure or position of the δ - and ϵ -subunits. The roles of these subunits are less certain, but studies suggest they are involved in the coupling of ATP synthesis to proton translocation, with δ being part of the rotor (Jounouchi, 1992; Guelin *et al.*, 1993; Zhang *et al.*, 1994; Capaldi *et al.*, 1996; Aggeler *et al.*, 1997; Schulenberg *et al.*, 1997; Häse *et al.*, 1998).

This study uses yeast *Saccharomyces cerevisiae* to study the assembly, function and mechanism of the ATP synthase. Null mutants have been made in each of the five genes encoding the subunits of the F₁-ATPase. Mutations in all but the gene encoding the ϵ -subunit provide yeast unable to grow on a non-fermentable carbon source, indicative of cells defective in oxidative phosphorylation. Furthermore, strains with null mutations in the genes encoding γ , δ and ϵ provide additional phenotypes which allow epistatic interactions to be explored. One of these additional phenotypes is the cell's increased tendency to lose mtDNA or the ρ factor. Historically, the ρ factor was determined to be a cytoplasmically inherited genetic unit, which was later determined to be the mtDNA (Ephrussi *et al.*, 1949). Cells with ρ^- mutations, or cytoplasmic petite mutations, have large deletions in the mtDNA, while the ρ^0 mutant strain is devoid of its mtDNA (Slonimski and Ephrussi, 1949). Epistasis means 'standing above' and, in this study, mutations in the gene encoding the α - and β -subunit of the ATPase and in *ATP11* are epistatic to the mutations in the genes encoding the γ - and δ -subunits of the ATPase. The results of these experiments indicate that the ATP synthase can be assembled into complexes devoid of γ , δ or ϵ and these subunit-deficient complexes are responsible for the secondary phenotypes. This conclusion provides important implications for the physiology of normal cells.

Results

Null mutations in the genes encoding the α -, β -, γ -, δ - and ϵ -subunits of F₁-ATPase were made by homologous

Fig. 1. Growth phenotypes of yeast with null mutations in the genes encoding the α -, β - and ϵ -subunits. Growth of the strains on minimal glucose (SD) and complete medium containing glycerol as the carbon source (YPG) is shown. Strains with a null mutation in the gene encoding the α - (A and B), β - (C and D) or ϵ -subunits (E) were transformed with the yeast vector (column 1, V) and the yeast vector containing the corresponding yeast gene (column 2, pY). The cells were grown at 30°C on SD and YPG media for 4 days and at 18°C on YPG medium for 7 days.

recombination with the KanMX resistance module flanked by 40 bp of the target sequence (Guldener *et al.*, 1996). The sites of recombination were targeted to delete the entire coding regions for the mature subunits of the ATPase. In addition, the *lox* recombination sites flanked the KanMX module, which allowed the subsequent curing of the KanMX module by subsequent expression of the *cre* recombinase (Guldener *et al.*, 1996). This strategy allowed the sequential deletion of multiple genes without using additional markers for selecting cells for the recombination event.

The correct integration events were confirmed by PCR using primers that flanked the sites of recombination in the genome and paired with primers homologous to the KanMX module. Two PCRs were performed which accessed the 5' and 3' junctions of the recombination sites. The sizes of the PCR products were consistent with the sizes predicted if the correct integration event had occurred in the genome (data not shown). The deletion mutations were all made initially in the haploid strain, W303-1A. However, as is shown below, since deletion of the genes encoding the γ - and δ -subunits caused the cells to produce ρ^0/ρ^- mutations (loss of mtDNA, see Introduction) and since deletion of γ provided a slow-growing phenotype, these mutations were made in the diploid strain, W303.

Haploid cells with a null deletion mutation in the gene encoding either α or β are unable to grow on complete medium containing glycerol as the carbon source (YPG), indicating that the cells are defective in oxidative phosphorylation (Figure 1). Neither mutation has a large detrimental effect on growth on medium containing glucose. This result is in contrast to a report that suggests that a null mutation in α inhibits cell growth due to its role as a chaperone-like molecule (Yuan and Douglas, 1992). Haploid cells with a mutation in ϵ grew slowly on YPG medium, indicating that ϵ is not essential for oxidative phosphorylation (Figure 1). This result is also contrary to a report that indicated that yeast with a null mutation in ϵ is unable to grow on YPG medium (Guelin *et al.*, 1993). However, consistent with the prior report, cells devoid of ϵ have a strong tendency to produce ρ^0/ρ^- mutations (Table I).

To demonstrate clearly the phenotypes in the strains with mutations in γ and δ , the corresponding null mutations were made in the diploid strain, W303, and the cells were

Table I. Percentage of ρ^0/ρ^- formed from strains carrying single and double mutations in the genes encoding subunits of the ATPase

Genotype	Percentage ρ^0/ρ^-
W303-1A	9
$\Delta\alpha$	<1
$\Delta\beta$	<1
$\Delta\gamma$	100
$\Delta\delta$	100
$\Delta\epsilon$	60
$\Delta ATP11$	1
$\Delta\gamma\Delta\alpha$	<1
$\Delta\gamma\Delta\beta$	<1
$\Delta\gamma\Delta\epsilon$	100
$\Delta\gamma\Delta ATP11$	<1
$\Delta\gamma\Delta\beta$, β -E222K.URA3	<1
$\Delta\delta\Delta\alpha$	<1
$\Delta\delta\Delta\beta$	<1
$\Delta\delta\Delta\gamma$	100
$\Delta\delta\Delta\epsilon$	99
$\Delta\delta\Delta ATP11$	1
$\Delta\delta\Delta\beta$, β -E222K.URA3	<5

The percentages of ρ^0/ρ^- were determined by mating the cells to ρ^0 tester strains and by color development due to the *ade2* mutation. Each value is the average of two or more determinations.

sporulated and subjected to tetrad analysis. Tetrad analysis (not shown) showed that the ρ^0/ρ^- (Table I) and the slow-growing phenotypes (as seen in Figure 2), segregate 2:2, and co-segregate with the null mutations in γ and δ .

The tendency to form ρ^0/ρ^- mutations and the slow-growing phenotypes in strains with a null mutation in the gene encoding the δ -subunit (Giraud and Velours, 1994, 1997), but not the γ -subunit (Paul *et al.*, 1994), were reported earlier. These phenotypes do not occur in yeast with mutations in genes encoding the α - or β -subunits. Thus, the cause of these phenotypes was investigated.

The hypothesis tested was that the absence of neither oxidative phosphorylation nor F_1 -ATPase was responsible for the slow growing and ρ^0/ρ^- phenotypes. Instead, these phenotypes were postulated to be due to the assembly of an ATP synthase that lacked γ or δ . To test this, pairs of heterozygous mutations were generated in the diploid strain, W303, with mutations in genes encoding γ/β and δ/β . Since β is essential to form F_1 , the F_1 -ATPase in strains with both γ and β deleted would not be assembled. To obtain these strains, two sequential disruptions were performed using the KanMX module. The first mutation was made in the gene encoding either γ or δ . The KanMX module was then cured from the chromosome using the *cre* recombinase (Guldener *et al.*, 1996). The second mutation was then made in the gene encoding β using the KanMX module. The resulting diploid strains were thus heterozygous for mutations in γ/β or δ/β . These diploids were sporulated and the tetrads were separated to generate the resulting haploid strains.

Representative tetrad analysis of the heterozygous diploid strains is shown in Figure 2. Four tetrads are shown for each of the two strains. The standard nomenclature is to refer to the progeny from a single diploid strain with an arabic numeral and to the daughter cells with the upper case letters A–D. Since the genes are not linked, the mutations would be expected to segregate randomly.

The genotype of the cells was determined by PCR and

Fig. 2. Growth phenotypes of yeast with mutations in the genes encoding the δ - and γ -subunits. Double mutations were made in the diploid strain, W303, in the genes encoding the γ - and β -subunits (A) or δ - and β -subunits (B). The strains were sporulated and the tetrads dissected on rich glucose medium. The genotypes at the δ and γ loci were determined by PCR using primers that spanned the genes. The genotype at the β locus (*ATP2*) was determined by the cell's ability to grow on medium containing G418: the KanMX module replaced the *ATP2* gene. The genotypes of the spores are shown in the table at the bottom of the figure.

by growth on medium containing G418. Since the genes for γ and δ were completely deleted and the KanMX marker subsequently was removed from these loci, the presence of the wild-type or mutant allele of γ and δ was determined by whole-cell PCR using primers that flanked the genes encoding γ or δ . Thus, the mutant allele gives a PCR product that is smaller than that of the wild-type

allele since the KanMX module had been removed from that locus. The presence of the mutant β allele was marked by the presence of the KanMX module, and thus cells with the mutant β allele were able to grow on media containing G418.

The small colonies correspond to the cells with a sole null mutation in γ or δ , as is evident by their sensitivity to G418 in the medium, the marker for the β wild-type gene, and the smaller PCR product, the marker for the null mutation in γ or δ . Furthermore, those same cells are unable to grow on glycerol medium, indicating a defect in oxidative phosphorylation (not shown). Cells with a sole mutation in γ or δ were also white, while the wild-type cells were red. The red color is a result of the *ade2* mutation in the cells. However, *ade2* cells do not turn red if they are also ρ^0/ρ^- . This was confirmed by genetic analysis showing that the cells with mutations in γ and δ were ρ^0/ρ^- (Table I). In this analysis, cells from at least three different colonies from spores from the tetrad analysis were plated onto YPD medium, the cells were mated to ρ^0 tester strains, and the diploids were tested for growth on YPG medium. Finally, cells with a sole mutation in δ grew more slowly on rich medium containing glucose than wild-type cells, and cells with a sole null mutant in γ grew much more slowly on YPD medium, even when compared with the $\Delta\delta$ cells. In contrast, cells with mutations in β (or α) did not grow appreciably more slowly than wild-type cells, did not lose mtDNA at a high frequency and were tan in color. Thus, despite coding for a subunit of the same enzyme, deletion of the gene encoding the γ - or δ -subunit provided cells with phenotypes different from each other and different from those of cells with a deletion in the gene encoding the β - (or α -) subunit.

The null mutation in γ or δ provided cells that were ρ^0/ρ^- and colonies that were smaller than those of the corresponding wild-type strain. However, cells with double mutations in δ/β or γ/β provided phenotypes that were apparently identical to those of cells with a mutation in only β (Figure 2). These cells formed colonies that were similar in size to that of cells with a mutation only in β , did not form ρ^0/ρ^- at a high rate and were tan in color (Table I). Therefore, the mutation in β 'stands above' or is epistatic to the γ and δ mutations. These results are consistent with the hypothesis that a complex, which requires β , forms in the absence of γ or δ and causes the slow growing and ρ^0/ρ^- phenotypes.

If the hypothesis is correct, i.e. the assembled F_1 -ATPase complex devoid of γ or δ is responsible for the ρ^0/ρ^- and slow growing phenotypes, then mutations in any gene necessary for the assembly of F_1 would be epistatic to mutations in γ and δ . To test this, double deletion mutant strains were made pairing the single null mutation in γ or δ with a null mutation in the gene encoding α or ϵ , or in *ATP11*. *ATP11* encodes a product that is necessary for the assembly of F_1 , but is not part of the F_1 -ATPase complex (Ackerman and Tzagoloff, 1990). In addition, the $\Delta\gamma\Delta\delta$ strain was made. Tetrad analysis was performed (not shown) and the percentage ρ^0/ρ^- was determined (Table I). Cells with mutations in γ or δ , when paired with a mutation in either α or *ATP11*, had phenotypes nearly identical to the phenotypes of cells with a mutation only in either α , β or *ATP11*. These results indicate that mutations in α , β and *ATP11* are

epistatic to mutations in γ and δ . Furthermore, based on the slow-growing phenotype of the γ null strain, mutations in γ are epistatic to mutations in δ and ϵ .

These results are consistent with the hypothesis that the ρ^0/ρ^- and slow growing phenotypes are due to assembly of a complex that is devoid of either γ or δ . Upon deletion of the genes encoding γ or δ , the cell makes a defective F_1 complex that causes the cells to produce ρ^0/ρ^- mutations, while for cells with a mutation in γ , the γ -less F_1 complex also causes the cells to grow very slowly on YPD medium.

While it is clear that a null mutation in β was epistatic to the γ and δ mutations, it was not clear if this would be true with a missense mutation that altered the activity but not the assembly of the F_1 -ATPase. In other words, do the ρ^0/ρ^- and slow-growing phenotypes require a functional active site, or just an F_1 that is assembled correctly, but devoid of γ or δ ? To test this, the mutation β -E222K was integrated into the chromosome of diploid cells that contained the $\Delta\gamma\Delta\beta$ and $\Delta\delta\Delta\beta$ mutations. Residue β -E222 is in the active site and possibly acts as a general base catalyst in the reaction mechanism (Duncan and Cross, 1992). The β -E222K mutation was shown to inactivate the F_1 -ATPase, yet still allow the assembly of the F_1 complex (Liang and Ackerman, 1996). The results of this experiment are shown in Figure 3. The segregates with the desired genotype, i.e. 3A and 3B for $\Delta\delta\Delta\beta$, β -E222K, and 1B, 2B and 3D for $\Delta\gamma\Delta\beta$, β -E222K, had identical phenotypes as compared with the corresponding double null mutant strain with regard to growth and ρ^0/ρ^- formation (Table I). Thus, apparently, a functional active site is required for the γ -less or δ -less F_1 -ATPase complex to cause the slow growing and ρ^0/ρ^- phenotypes.

Discussion

The results of this study are quite surprising and have far reaching implications. First, deletion of the ϵ -encoding gene resulted in a strain of yeast that was still able to make ATP via oxidative phosphorylation as judged by its ability to grow on YPG medium. Thus, ϵ is not an essential component of the ATP synthase. This was a surprising result since in a prior study, the null mutant was reported to be unable to grow on YPG medium (Guelin *et al.*, 1993). There are at least two reasons for this discrepancy. It is possible that the differences in the construction of the null mutations could be responsible. This is unlikely since both null mutations completely removed the coding sequence from the genome. The second possibility is that strain differences are responsible. This is the favored explanation since the previous report indicated that the null mutation greatly reduced but did not eliminate the level of oligomycin-sensitive ATPase (Guelin *et al.*, 1993). Since just a small fraction of the normal level of the ATP synthase is necessary to support growth on YPG medium (Mukhopadhyay *et al.*, 1994), strain variations in the wild-type level of this enzyme could provide different phenotypes for the ϵ null mutant strain.

The second surprising result was that the null mutation in γ provided a very slow-growing phenotype and resulted in cells that were 100% cytoplasmic petite mutant strains, i.e. ρ^0/ρ^- (see Introduction for a discussion on ρ^0/ρ^-). In a prior report, the null mutation was reported to produce

Fig. 3. Growth phenotypes of yeast with a mutation in the genes encoding the δ - or γ -subunits and paired with the β -E222K active site mutation. This figure is as described in Figure 2 except that the β -E222K mutation was integrated into the $URA3$ locus using the integrating vector pRS306. In this case, the β -E222K mutation was followed by the prototrophy on SD-Ura medium. The genotypes of the spores are shown in the table at the bottom of the figure.

Fig. 4. Model to explain the phenotypes of the single and double mutants. See text for explanation.

just 20% ρ^0/ρ^- mutations (Paul *et al.*, 1994). In this case, the cause for the differences in the phenotypes cannot be ascribed to a difference in the strain, since the parent strain, W303, was used in both studies. The slow-growing phenotype and the γ null mutation co-segregated 100% in >35 tetrads analyzed using a variety of independent null mutant stains. Rather, the difference is probably due to the differences in how the null mutations were constructed. In the prior study, the null mutant was made by inserting the *HIS3* gene into the *BclI* sites of the yeast *ATP3* gene (Paul *et al.*, 1994). This construct results in a fusion protein, with the first 134 amino acids being from the yeast γ -subunit. It is possible that this protein would be able to interfere with the activity responsible for the high ρ^0/ρ^- formation and the slow-growing phenotype of the $\Delta\gamma$ mutants.

Our studies indicate that the high rate of ρ^0/ρ^- mutations formed in the γ and δ mutant strains and the slow growing phenotype of the γ deletion mutant strain are due to the formation of an ATP synthase complex devoid of the corresponding subunit. The genetic data are quite clear on this conclusion. These phenotypes are not due to the absence of the ATP synthase since they are not observed in strains with null mutations in α or β . Nor are these phenotypes due to the absence of either γ or δ *per se* since, when paired with a mutation in β , the phenotype disappears. This epistatic effect is not limited to a mutation in β , but can also be seen with mutations in α and the *ATP11* genes. Thus, a complex containing the α - and β -subunits is formed, which requires Atp11p apparently as an assembly factor, and this complex is required to cause the ρ^0/ρ^- -forming and slow-growing phenotypes. In addition, a functional active site is required, since the active site mutation β -E222K is also epistatic to the null mutations in γ and δ .

A model to explain these results is shown in Figure 4. Normally, proton flow through the ATP synthase is coupled to the synthesis of ATP. However, in a strain with a null mutation in δ or γ , an ATP synthase complex lacking γ or δ is made which is defective in coupling proton transport to ATP synthesis. This is consistent with the genetic and biochemical studies in *Escherichia coli* which implicate the subunit that corresponds to δ in coupling of proton

transport to ATP synthesis in the ATP synthase (Dunn and Futai, 1980; Jounouchi *et al.*, 1992; Zhang *et al.*, 1994). Likewise, missense mutations in *E.coli* γ have been shown to alter the coupling capacity of the enzyme (Shin *et al.*, 1992). Furthermore, an F_1 -ATP synthase that was assembled, but lacking γ , would be predicted to be uncoupled since γ acts as the rotor in the molecular motor, ATP synthase (Abrahams *et al.*, 1994; Noji *et al.*, 1997).

This model also suggests that passive proton flow through F_0 does not occur without a functional complex. As such, mutations that disrupt F_1 assembly do not provide a functional proton pore. This is consistent with results using *E.coli* that indicate that F_1 is necessary for proton conductance through F_0 (Pati *et al.*, 1991). More surprisingly, since the missense mutation β -E222K was epistatic to mutations in γ and δ , it appears that passive proton flow requires a functional active site. However, this result needs to be studied in more detail since it is possible that the missense mutation produced an enzyme that, though assembled, had an altered conformation.

Why are ρ^0/ρ^- mutations produced? Given, that the mutations in δ and γ result in passive proton flow through the F_0 portion of the enzyme, this should result in a decrease or elimination of the protonmotive force across the mitochondrial membrane. Since mitochondrial biogenesis requires a $\Delta\Psi$ across the membrane, these mutations should be lethal to the cell (Neupert, 1997). To circumvent this problem, the yeast eliminate the proton pore by eliminating the mtDNAs which encode subunits of the proton pore, subunits 6, 9 and 10 of the ATP synthase.

There is also a correlation between the formation of ρ^0/ρ^- mutants and coupling of the ATP synthase. Mutations in *ATP5* (Uh *et al.*, 1990) or the ϵ -encoding gene (Guelin *et al.*, 1993) also have the effect of uncoupling the ATP synthase and result in cells that lose their mtDNA, while cells with mutations in *ATP11*, *ATP12*, α or β do not make ρ^0/ρ^- mutants. Thus, the available data suggest that deleterious effects on a cell caused by mutations that uncouple the ATP synthase are at least partially compensated for by secondary ρ^0/ρ^- mutations.

The slow-growing phenotype of cells with a γ null mutation is suggested to occur from the hydrolysis of ATP, which decreases the intracellular ATP level. Since hydrolysis of ATP is normally controlled by the IF1, the inhibitor protein of the ATP synthase (Hashimoto *et al.*, 1990), the model suggests that IF1 is not effective on the complex that is lacking γ . It is possible that IF1 works by blocking γ rotation. This is an appealing hypothesis since it would provide a mechanical explanation of how IF1 could block the hydrolysis but not the synthesis of ATP. Like a ratchet of a wrench, IF1 may allow the unidirectional rotation of γ in the ATP synthase. This is consistent with the placement of the binding site of IF1 near the C-terminal end of the β -subunit, away from the catalytic site (Jackson and Harris, 1988).

The suggestion that an active complex is formed in the absence of γ appears to be contradictory to a prior study where the γ -less F_1 ATPase could not be detected in a yeast strain with a null mutation in the gene encoding γ (Paul *et al.*, 1994). However, γ is important for forming a stable enzyme complex (reviewed in Gromet-Elhanan, 1992), and the isolation procedure may have disrupted

any complex originally present. Additionally, *in vitro*, γ (and δ) is not essential for forming an active enzyme (Gromet-Elhanan, 1992) and the crystal structure of the bovine enzyme indicates that γ has no direct involvement in forming the active site. As such, a γ -less ATPase complex could form in the mutant cell and this complex may both be active for ATP hydrolysis and activate passive proton translocation through F_0 .

This and other studies (Jounouchi, *et al.*, 1992; Guelin *et al.*, 1993; Zhang *et al.*, 1994; Capaldi *et al.*, 1996; Aggeler *et al.*, 1997; Schulenberg *et al.*, 1997; Häslér *et al.*, 1998) suggest that δ and ϵ are involved in the coupling of the ATP synthase. In the framework of F_1 as a molecular motor, it is appealing to suggest that δ and ϵ act as a molecular clutch in the ATP synthase. This is consistent with cross-linking studies which indicate that cross-linking the equivalent of δ to γ does not inhibit the enzyme activity, and other studies, which place it in contact with F_1 and F_0 (Guelin *et al.*, 1993; Capaldi *et al.*, 1996; Aggeler *et al.*, 1997; Schulenberg *et al.*, 1997; Häslér *et al.*, 1998). As such, the coupling capacity of the ATP synthase could be controlled or altered by changes in the level of the δ -, ϵ - or even the γ -subunit. Possibly, the coupling capacity of the ATP synthase in mammals is variable and this variability might partially account for phenotypic differences, for example, in the tendency to obesity.

Materials and methods

Yeast strains

The yeast *S.cerevisiae* strains, W303-1A (a, *ade2-1*, *his3-1,15*, *leu2-3,112*, *trp1-1*, *ura3-1*) (obtained from B.Trumpower) and W303 (a/ α *ade2-1/ade2-1*, *his3-1,15/his3-1,14*, *leu2-3,112/leu2-3,112*, *trp1-1/trp1-1*, *ura3-1/ura3-1*) (obtained from S.Lindquist) were used throughout this study as the parents of the mutant strains. The percentage of ρ^0/ρ^- cells was determined for the mutant strains by mating colonies to ρ^0 tester strains, K289-3A ρ^0 and K338-8D ρ^0 (Klapholz and Esposito, 1982), followed by testing for growth on YPG plates.

Media

The yeast media are standard recipes as described previously (Sherman, 1991): YPD, 2% peptone, 1% yeast extract, 2% glucose; YPG, 2% peptone, 1% yeast extract, 3% glycerol; and YPAD, 2% peptone, 1% yeast extract, 2% glucose and 20 mg/l adenine sulfate. Minimal medium (SD) contained 2% glucose and was supplemented with adenine, histidine, arginine, methionine, tyrosine, lysine, leucine, isoleucine and tryptophan (Trp) or uracil (Ura) at 20 mg/l.

Genetic analysis

Tetrad analysis was performed by standard methods (Sherman and Hicks, 1991). The cells were grown on pre-spore media for 1 day, transferred to sporulation media for 4–6 days, and dissected on YPD medium.

Yeast transformation was performed by the lithium acetate method after growth in YPAD medium (Gietz and Schiestl, 1995). For selection of G418-resistant cells, the transformants were allowed to recover for 6–8 h at 30°C or placed at 4°C for 12–48 h and plated on YPD containing 0.2 mg/ml G418 (Gietz and Schiestl, 1995). The null mutants were made by homologous recombination of PCR products using the KanMX resistance module (Güldener *et al.*, 1996). The strains were checked by whole-cell PCR (Niedenthal *et al.*, 1996) to confirm the correct integration event in the chromosome at the 5' and 3' junctions.

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Data discussed as 'data not shown' as well as other pertinent materials can be found at <http://www.finchcms.edu/biochem/mueller/1999EMBOJ.html>.