A Single Amino Acid Change in Subunit 6 of the Yeast Mitochondrial ATPase Suppresses a Null Mutation in ATP10*

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In an earlier study, the ATP10 gene of Saccharomyces cerevisiae was shown to code for an inner membrane protein required for assembly of the F0 sector of the mitochondrial ATPase complex (Ackerman, S., and Tzagoloff, A. (1990) J. Biol. Chem. 265, 9952–9959). To gain additional insights into the function of Atp10p, we have analyzed a revertant of an atp10 null mutant that displays partial recovery of oligomycin-sensitive ATPase and of respiratory competence. The suppressor mutation in the revertant has been mapped to the OLI2 locus in mitochondrial DNA and shown to be a single base change in the C-terminal coding region of the gene. The mutation results in the substitution of a valine for an alanine at residue 249 of subunit 6 of the ATPase. The ability of the subunit 6 mutation to compensate for the absence of Atp10p implies a functional interaction between the two proteins. Such an interaction is consistent with evidence indicating that the C-terminal region with the site of the mutation and the extramembrane domain of Atp10p are both on the matrix side of the inner membrane. Subunit 6 has been purified from the parental wild type strain, from the atp10 null mutant, and from the revertant. The N-terminal sequences of the three proteins indicated that they all start at Ser11, the normal processing site of the subunit 6 precursor. Mass spectral analysis of the wild type and mutants subunit 6 failed to reveal any substantive difference of the wild type and mutant proteins when the mass of the latter was corrected for Ala → Val mutation. These data argue against a role of Atp10p in post-translational modification of subunit 6. Although post-translational modification of another ATPase subunit interacting with subunit 6 cannot be excluded, a more likely function for Atp10p is that it acts as a subunit 6 chaperone during F0 assembly.

The F0 component of the proton translocating ATPase consists of a set of hydrophobic proteins that are embedded in the mitochondrial inner membrane. This important constituent of the larger F1-F0 complex catalyzes vectorial transfer of protons across the inner membrane, the direction being dependent on whether the enzyme is functioning in an ATP synthetic or hydrolytic mode (1). In baker's yeast, three subunits of F0 are encoded in mitochondrial DNA (2). The other F0 subunits are all products of nuclear genes. Most of the F0 subunits are required for binding and conferral of oligomycin sensitivity on the F1-ATPase (3, 4). The exception are three recently described subunits (5) that have been proposed to be involved in dimerization of the F1-F0 complex in the membrane. Mutations in these subunits do not appear to influence the basic ATPase activity of the complex (5).

Maintenance of functional ATPase depends not only on the expression of mitochondrial and nuclearly encoded subunits of the enzyme but also on nuclear gene products that promote essential events during ATPase assembly. Some factors such as Atp11p and Atp12p have been shown to interact with the α- and β-subunits and to render them competent to oligomerize into the F1-ATPase (6–8). Other factors are required for transcription/translation of subunit 9 of the complex (9, 10). In an earlier study we reported that the product of the ATP10 gene does not affect assembly of F1 or synthesis of subunit 9 but is essential for expression of functional F0 (11). Mutations in ATP10 resulted in a loss of oligomycin sensitivity and a more labile interaction of F1 with the membrane. Both of these properties are hallmarks of a defect in F0. Atp10p is localized in the mitochondrial inner membrane but is not a constituent of the ATPase complex. As with so many factors that have been implicated in assembly of ATPase and of respiratory chain complexes, its precise function has remained obscure.

To learn more about the role of Atp10p in F0 assembly, we have extended the analysis of the atp10 null mutant and have studied an extragenic suppressor that rescues the respiratory defect of the mutant. The suppressor has been mapped to mitochondrial DNA and identified as a single amino acid substitution in the OLI2 gene for subunit 6 of F0. These data suggest a functional interaction of Atp10p with subunit 6. The location of the suppressor mutation near the C-terminal region of subunit 6 argues against a role of Atp10p in processing of the subunit 6 precursor. This is also supported by the presence of mature subunit 6 in the atp10 mutant. Mass spectrometric analysis of subunits 6 and 9 purified from wild type and mutants have also excluded a role of Atp10p in post-translational chemical modification of these ATPase constituents. Atp10p, therefore, is more likely to be a chaperone for subunit 6.

MATERIALS AND METHODS

Yeast Strains and Growth Media—The genotypes and sources of the wild type and pet1 null mutants of Saccharomyces cerevisiae used in this study are listed in Table I. The compositions of the media for growth of yeast have been described elsewhere (13).

Preparation of Yeast Mitochondria and ATPase Assays—Mitochondria were prepared by the method of Faye et al. (14) except that Zymolyase 20,000 instead of Glusulase was used to convert cells to spheroplasts. ATPase activity was assayed by measuring release of inorganic phosphate from ATP at 37 °C in the presence and absence of oligomycin (15).

Cloning and Sequencing of the oli2 Gene—Mitochondrial DNA purified from W303-1A, w303ΔATP10, and three independent revertants 10R1, 10R2, and 10R3 (16) were used as templates for polymerase chain reaction amplification of the OLI2 gene. One of the two synthetic primers had the sequence matching the sense strand from nucleotides

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TABLE I
Genotypes and sources of Saccharomyces cerevisiae strains

| Strain     | Abbreviation | Genotype                              | Source  |
|------------|--------------|---------------------------------------|---------|
| W303–1A    | WT           | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 |         |
| W303–1B    | WT           | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 |         |
| W303p     | ΔATP10       | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | Ref 11  |
| aW303ATP10 | ΔATP10       | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | Ref 11  |
| AW303ΔATP10/R1 | 10R1     | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | This study |
| aW303ATP10/R2 | 10R2     | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | This study |
| aW303ATP10/R3 | 10R3     | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | This study |
| aW303/10R3 | 10R3/ΔATP10  | a ade2–1 his3–15 leu2–3,115 trp1–1 ura3–1 | This study |
| M28–2     | a met6 ol2   | a met6 ol2                             | Ref 12  |
|           |              |                                       |         |

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RESULTS AND DISCUSSION

Isolation and Genetic Characterization of atp10 Rever-tants—a W303ΔATP10, abbreviated as ΔATP10 in this text, is a haploid strain of yeast with an atp10 null allele (11). This mutant grows very poorly on nonfermentable carbon sources such as ethanol and/or glycerol. The compromised respiratory activity of mitochondria in the null mutant as well as in atp10 point mutants was previously attributed to a defect in the F0 component of the ATPase (11). To learn more about the biochemical lesion responsible for the F0 assembly defect, spontaneous revertants of ΔATP10 were isolated. Such revertants appear frequently (104–105) under conditions containing ethanol and glycerol as carbon sources. Three independent revertants (10R1, 10R2, and 10R3) were chosen for further study. The generation time of the revertants in liquid medium containing glycerol was estimated to be about two times longer than the parental wild type (Table II). The revertant phenotype was found to be transmitted in a stable manner after propagation of the cells on glucose or galactose.

The revertants were further characterized by crosses to E103, an atp10 mutant obtained by mutagenesis of the respiratory competent strain D273-10B/A1 with ethylmethane sulfonate (26). Diploid cells issued from the cross grew on respiratory substrates with approximately the same generation time as the haploid revertant indicating that the suppressor(s) were either nuclear dominant or mitochondrial mutations. To distinguish between these two possibilities, spontaneous ρ− derivatives were isolated from each revertant and were crossed to E103. Diploid cells formed in these crosses failed to grow on nonfermentable carbon sources, indicating extragenic mutations in mitochondrial DNA. This was confirmed by segregation tests. The revertants were crossed to E103 in glucose containing medium for 6 h. Diploid cells were prototrophically selected on minimal glucose. Following 20–30 generations they were spread for single colonies on rich glucose medium, and after 2 days of growth at 30°C were replicated on rich medium containing glycerol. Two distinct growth phenotypes were noted on the glycerol medium. In all cases 30–50% of the colonies displayed the revertant phenotype, whereas the remaining cells showed the very slow growth characteristics of the mutant. Several respiratory competent diploid cells from the first segregation were grown on glucose and tested a second time for mitotic segregation as described above. In every instance all of the segregants displayed revertant properties. The possibility that the revertant harbored a second nuclear suppressor that, together with the mitochondrial mutation, was responsible for the respiratory competent phenotype was excluded from the results of a cross of revertant 10R3 to the atp10 null mutant. Respiratory competent diploid cells produced from this cross were sporulated, and the meiotic spore progeny were analyzed by tetrad dissections. In nine complete tetrads all the spores exhibited the revertant phenotype. These data together with the results of the crosses of the ρ− derivatives of the revertants to the atp10 point mutant indicated that the suppressor is inherited as a mutation in the mitochondrial genome. The mitotic and meiotic segregation results also exclude the suppressor from being a rearrangement of mitochondrial DNA that coexists as an independently replicating ρ− genome in an otherwise ρ+ background (27, 28).

The mitochondrial suppressor was transferred to a wild type nuclear background by crossing 10R3 to a ρ+ derivative of W303-10B. The diploid cells were sporulated and Leu− meiotic progeny with the ATP10 gene were obtained (10R3/ΔATP10).
These cells grew on glycerol as well as the wild type strain at 30 °C but were partially temperature sensitive at 37 °C (Fig. 1). The temperature-sensitive phenotype was also detected in the *atp10* mutant and revertant. The normal growth of 10R3/*ATP10* on glycerol at 30 °C indicates that suppressor does not affect the ATPase in cells expressing Atp10p.

Properties of the Mitochondrial ATPase in the Revertant Strains—In earlier studies *atp10* mutants were found to have normal F$_1$-ATPase (11). The larger F$_1$-F$_0$ complex, however, had altered properties, one of which was decreased oligomycin sensitivity. Assays of mitochondrial ATPase activity from different strains indicated that sensitivity to oligomycin is partially restored in the revertants (Table III). The ATPase activities in the three revertants 10R1, 10R2, and 10R3 were inhibited 25–33% by oligomycin. In the same assay the mitochondrial ATPase of the wild type was inhibited by 75%, whereas in ∆ATP10, the ATPase was completely insensitive to the antibiotic. The partial restoration of oligomycin-sensitive ATPase in the revertants is consistent with the ability of the revertants to grow on respiratory substrates.

The absence of oligomycin sensitivity in the *atp10* mutant has previously been ascribed to the failure of F$_1$ to correctly interact with F$_0$ (11). The oligomycin sensitivity observed in the revertants therefore indicated that the suppressor permits some F$_1$ to be assembled with F$_0$. This was confirmed by sucrose gradient sedimentation analysis of detergent extracts of wild type and mutant mitochondria. In agreement with previous results (29), all the α- and β-subunits of F$_1$ in the wild type extract co-sedimented as part of the larger F$_1$-F$_0$ complex (Fig. 2). This was also true of the extract from 10R3/ATP10, which contains the suppressor in the context of the wild type ATP10 gene. Even though the F$_1$ subunits also co-sedimented in the extract from the *atp10* mutant, their slower sedimentation indicated that they were part of the F$_1$ oligomer but not of the F$_1$-F$_0$ complex (29). In the case of the revertant extract, two separate peaks were observed. Approximately 30% of the α- and β-subunits sedimented as the F$_1$-F$_0$ complex, whereas the remainder of the subunits sediments as the F$_1$ oligomer (Fig. 2). Similar results were obtained when the sedimentation analysis was extended to subunits 4 and 6 of the F$_0$ (data not shown). In this case also, only a fraction of the F$_0$ subunits in the revertant extract co-sedimented with the F$_1$-F$_0$ complex.

F$_0$ Proteolipids in *atp10* Mutants and Revertants—Subunits 6, 8, and 9 of the F$_0$ sector are encoded in mitochondrial DNA (2). These hydrophobic constituents are products of *OLI1*, *AAP1*, and *OLI2*, respectively. Two different approaches were used to estimate the levels of these proteins in the mutants and revertants. The chloroform/methanol extraction conditions of Michel and Velours (19) were used to isolate subunits 6 and 9 from mitochondria of the *atp10* null mutant ∆ATP10, from the three revertants and from the parental wild type strain. The extracts were analyzed by SDS-PAGE, and the proteolipids were visualized by silver staining. Quantitation of the stained gel revealed about 16 times less subunit 6 in the mutant than in the wild type extract (Fig. 3). The amount of subunit 6 in the revertant extracts was significantly increased in the mutant, although it was still lower than in the wild type. It is interesting that the *oli2* point mutant, which is able to grow slowly on glycerol, also has a low level of subunit 6 that can be extracted with chloroform/methanol. The decreased steady-state concentration of subunit 6 in the mutant could be because of an effect of the mutation either on synthesis or on turnover of the protein.

The synthesis of the ATPase proteolipids in the different strains was estimated by *in vivo* labeling of the mitochondrial translation products with $^{35}$SO$_4$$^2-$ in the presence of cycloheximide. Subunit 6 was found to be synthesized in all the *atp10* mutants (Fig. 4), indicating that the low level of this protein in the chloroform/methanol extract of ∆ATP10 mitochondria was not a consequence of a translational defect but rather of an increased turnover of the protein in the mutant. Similar results were obtained when the mitochondrial translation products were synthesized in isolated mitochondria (data not shown). The lability of subunit 6 is not unique to *atp10* mutants and has also been reported in other strains that are blocked in F$_0$ assembly because of mutations in the structural genes (31–33).

Significantly, subunit 6 detected in the 10R3 revertant had

### Table II

| Strain        | Doubling time (h) |
|---------------|------------------|
| W303-1A      | 210              |
| ∆ATP10       | ND               |
| 10R1         | 470              |
| 10R2         | 330              |
| 10R3         | 410              |

The different strains of yeast were inoculated at approximately the same densities into liquid medium containing 2% glycerol, 2% peptone, and 1% yeast extract. Growth was monitored by absorbance at 600 nm. ND, not determined.

### Table III

| Strain        | ATPase (µmol/min/mg) | Inhibition (%) |
|---------------|----------------------|----------------|
|               | − Oligomycin         | + Oligomycin   |

| Strain        | ATPase (µmol/min/mg) | Inhibition (%) |
|---------------|----------------------|----------------|
| W303-1A      | 5.88 ± 0.07          | 1.49 ± 0.01    |
| ∆ATP10       | 3.09 ± 0.34          | 3.02 ± 0.08    |
| 10R1         | 4.02 ± 0.03          | 2.99 ± 0.01    |
| 10R2         | 3.79 ± 0.05          | 2.90 ± 0.12    |
| 10R3         | 3.50 ± 0.20          | 2.32 ± 0.02    |

The respiratory competent parental strain W303-1A (*ATP10/OLI2*), the *atp10* null mutant ∆ATP10 (*atp10/OLI2*), the revertant 10R3 (*atp10/oli2*), and the wild type strain with the mitochondrial genome of the revertant (*ATP10/oli2*) were diluted serially and spotted, starting from 10⁶ cells, on two YPD (rich glucose) and two YEPG (rich glycerol plus ethanol) plates that were incubated for 3 days at 30 and 37 °C. No differences in growth at the two temperatures were found on the YPD medium (not shown). Only the YEPG plates are shown.

### Table II

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#### Generation time of wild type and mutant strains on rich glycerol medium

The different strains of yeast were inoculated at approximately the same densities into liquid medium containing 2% glycerol, 2% peptone, and 1% yeast extract. Growth was monitored by absorbance at 600 nm. ND, not determined.

| Strain       | Doubling time (min) |
|--------------|---------------------|
| W303-1A      | 210                 |
| ∆ATP10       | ND                  |
| 10R1         | 470                 |
| 10R2         | 330                 |
| 10R3         | 410                 |

The different strains of yeast were inoculated at approximately the same densities into liquid medium containing 2% glycerol, 2% peptone, and 1% yeast extract. Growth was monitored by absorbance at 600 nm. ND, not determined.
an altered electrophoretic mobility (Fig. 4). The slightly faster migration of subunit 6 was also discerned in the other two revertants, 10R1 and 10R2, (data not shown). The faster migration of subunit 6 from the revertant is probably due to an increased capacity of the protein to bind sodium dodecyl sulfate as a result of the C-terminal mutation (see below).

Localization of the Suppressor and Sequencing of OLI2 from the atp10 Mutant and Revertants—To map the mitochondrial suppressor, the three revertants were treated with ethidium bromide, and the resultant ρ− derivatives were collected. The ρ− clones of each library were crossed to the atp10 mutant, and the diploid cells that formed in the crosses were tested for appearance of the suppressed phenotype. The regions of mitochondrial DNA conserved in several ρ− clones that were able to suppress the respiratory defect of the atp10 mutant were determined by physical analysis of their ρ− genomes. In each case the ρ− genomes were ascertained to contain the OLI2 gene for subunit 6 of the ATPase (17).

The mitochondrial OLI2 gene was amplified from mitochondrial DNA of W303-1A, ΔATP10, and the three revertants 10R1, 10R2, and 10R3 were analyzed by polymerase chain reaction. The sequences of the genes cloned from the wild type strain and from the ΔATP10 null mutant were identical to the sequence of OLI2 previously reported for the respiratory competent strain D273-107B/1A (17). The sequences of the genes obtained from the three revertants, however, showed a single identical C → T base change at nucleotide 746. The C → T transition replaces the alanine at residue 249 near the C terminus of the protein with a valine. In view of the identical mutation in the three revertants all subsequent experiments on the revertant made use of 10R3.

An alignment of the C-terminal 16 residues of subunit 6 from fungal, plant, and animal sources shows that the Ala 249 is not a conserved amino acid (Table IV). It is also interesting that some fungi lack the C-terminal sequence corresponding to the region of the yeast protein with the mutation.

N-terminal Sequence of Subunit 6—Subunit 6 of yeast ATPase is synthesized as a precursor with a 10-amino acid extension at the N terminus (19). The mature protein starts with the serine at residue 11 of the primary translation product and extends at the N terminus (19). The mature protein starts with the serine at residue 11 of the primary translation product and ends with the aspartic acid at residue 292 (19). The sequence of subunit 6 was determined by physical analysis of the mitochondrial DNA conserved in several ρ− clones that were able to suppress the respiratory defect of the atp10 mutant (see below). The C → T transition replaces the alanine at residue 249 near the C terminus of the protein with a valine. In view of the identical mutation in the three revertants all subsequent experiments on the revertant made use of 10R3.

An alignment of the C-terminal 16 residues of subunit 6 from fungal, plant, and animal sources shows that the Ala 249 is not a conserved amino acid (Table IV). It is also interesting that some fungi lack the C-terminal sequence corresponding to the region of the yeast protein with the mutation.

N-terminal Sequence of Subunit 6—Subunit 6 of yeast ATPase is synthesized as a precursor with a 10-amino acid extension at the N terminus (19). The mature protein starts with the serine at residue 11 of the primary translation product (19). To determine whether subunit 6 is correctly processed in the mutant and the revertants, the protein was purified from the different strains by reverse-phase chromatography of chloroform/methanol extracts of mitochondria. No significant difference was noted in the elution times of the protein obtained from the wild type and the ΔATP10 mutant or revertant. The sequences of the first 10 residues indicated that the proteins purified from the mutant and revertant strains begin with Ser11 as did the wild type protein. This result indicates that Atp10p is not involved in processing of the precursor.
Localization and Topology of Atp10p—Atp10p was previously found to be associated with the mitochondrial inner membrane. It was solubilized with NaBr, suggesting that it may be an extrinsic membrane protein (11). This could not be confirmed in the present study. When submitochondrial vesicles were extracted with carbonate, the alkaline conditions failed to release Atp10p from the membrane, indicating that it is an intrinsic membrane constituent (Fig. 5A).

The ability of a single amino acid substitution in the C-terminal region of subunit 6 to partially rescue the atp10 null mutant could indicate that Atp10p interacts with the C-terminal region of subunit 6. Subunit a, the E. coli homolog of mitochondrial subunit 6, has been proposed to have five transmembrane domains with its N terminus on the periplasmic and the C terminus on the cytoplasmic side of the plasma membrane (34, 35). An alignment of the E. coli and yeast subunit 6 sequences suggests a similar number of transmembrane domains in the latter protein. Moreover, based on the E. coli model (35), the 17 C-terminal residues of the yeast protein, including the site of the mutation, are predicted to lie outside of the phospholipid bilayer in the matrix compartment.

The topology of Atp10p was probed by testing its sensitivity to proteinase K digestion. Mitochondria and mitoplasts prepared by hypotonic swelling of mitochondria were converted to submitochondrial particles by sonic irradiation. The submitochondrial particles extracted in the presence of 0.1M sodium carbonate at a final protein concentration of 10 mg/ml. After incubation on ice for 10 min the extrinsic membrane proteins were separated from the membranes by centrifugation at 400,000 × g for 30 min. Equivalent volumes of mitochondria, submitochondrial particles (SMP), sodium carbonate extract, and pellet were separated on a 12% polyacrylamide gel and transferred to nitrocellulose paper. The Western blot was reacted with antisera against Atp10p using the Super Signal detection system (Pierce). B, mitochondria were prepared by the method of Glick (36) from W303-1A. The mitochondria, at a protein concentration of 8 mg/ml, were diluted with 8 volumes of 20 mM Hepes, pH 7.5, containing 0.6 M sorbitol (Mit). Mitoplasts (Mpl) were prepared by dilution of mitochondria in 20 mM Hepes, pH 7.5, without the sorbitol. One half of each sample was treated with proteinase K (prot K) at a final concentration of 100 μg/ml and incubated for 60 min on ice. Phenylmethylsulfonyl fluoride was added to a final concentration of 2 mM to stop the proteolysis, and the mitochondria and mitoplasts were recovered by centrifugation at 20,000 g for 10 min and centrifuged, and the pellets were dissolved in Laemmli sample buffer. The samples were heated at 65 °C for 10 min and centrifuged, and the pellets were dissolved in Laemmli sample buffer (23). Total mitochondrial and mitoplast proteins (25 μg) were separated on a 12% polyacrylamide gel and transferred to nitrocellulose, and the Western blots were treated either with antisera to cytochrome b or to Atp10p. The migration of molecular mass standards are marked in the left-hand margin. Cytochrome b2 (B2) and Atp10p are identified in the right-hand margin.

**Table IV**

| C-terminal sequences of subunit 6 from fungal, plant, and animal sources |
|-------------------|-------------------|-------------------|
| **Species**       | **Sequence**       | **Sequence**       |
| S. cerevisiae     | S. cerevisiae     | S. cerevisiae     |
| Schizosaccharomyces pombe | Schizosaccharomyces pombe | Schizosaccharomyces pombe |
| Candida parapsilosis | Candida parapsilosis | Candida parapsilosis |
| Penicillium chrysogenum | Penicillium chrysogenum | Penicillium chrysogenum |
| Neurospora crassa | Neurospora crassa | Neurospora crassa |
| Agericus bitorquis | Agericus bitorquis | Agericus bitorquis |
| Sorghum bicolor | Sorghum bicolor | Sorghum bicolor |
| Chrysodidymus syrnoideus | Chrysodidymus syrnoideus | Chrysodidymus syrnoideus |
| Arabidopsis thaliana | Arabidopsis thaliana | Arabidopsis thaliana |
| Zea mays | Zea mays | Zea mays |
| Ricchetia prowazekii | Ricchetia prowazekii | Ricchetia prowazekii |
| Paracentrobus lucidus | Paracentrobus lucidus | Paracentrobus lucidus |
| Homo sapiens | Homo sapiens | Homo sapiens |
| Gyroporus cyanescens | Gyroporus cyanescens | Gyroporus cyanescens |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |
| S. cerevisiae | S. cerevisiae | S. cerevisiae |

**Table V**

| Mass determinations of subunit 6 of yeast mitochondrial ATPase |
|-------------------|-------------------|-------------------|-------------------|
| **Strain**       | **Apparent mass** | **Number of Determinations** | **Correction** | **Corrected mass** |
| W303–1A | 27,931.4 ± 4.8 | 5 | 0 | 27,931.4 ± 4.8 |
| ΔATP10 | 27,935.9 ± 4.3 | 3 | 0 | 27,935.9 ± 4.3 |
| 10R3 | 27,954.2 ± 7.3 | 3 | 28% | 27,926.2 ± 7.3 |
| 10R3/ATP10 | 27,950.1 ± 0.2 | 3 | 28% | 27,922.1 ± 0.2 |

*The experimentally determined masses of subunit 6 from the two strains containing the Ala → Val mutation were normalized to that of the wild type and atp10 null mutant by subtracting 28 daltons, the mass difference between alanine and valine. The values reported are averages with the ranges next to them.*

difference, which lies within the accuracy of the instrument, is too small to be due to a chemical modification. The mass of subunit 9 obtained from the same strains agreed well with the known sequence of the protein (data not shown), thereby excluding a role of Atp10p in chemical modification of subunit 9. Rather these data suggest the alternative explanation that
Atp10p acts as a subunit 6-specific chaperone that may confer an assembly-competent conformation on subunit 6 or facilitate its insertion into the inner membrane. Attempts to detect a complex of Atp10p and subunit 6 by cross-linking experiments have so far failed.

Atp10p could also be involved in modification of a neighboring subunit. In the absence of the modification, interaction with subunit 6 would be weakened, causing a defect in F0 assembly. The presence of a bulkier and more hydrophobic residue in subunit 6 would be weakened, causing a defect in F0 assembly. In the absence of the modification, interaction with subunit 6 could also be involved in modification of a neighbor.

REFERENCES

1. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
2. Dujon, B. (1980) in The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 505–635, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Tzagoloff, A. (1980) J. Biol. Chem. 225, 1545–1551
4. Velours, J., Spannagel, C., Chaignepain, S., Vaillier, J., Arselin, G., Graves, P. V., Velours, G., and Camougrand, N. (1998) Biochimie (Paris) 80, 783–801
5. Arnold, I., Pfeiffer, K., Neupert, W., Stuart, R. A., and Schagger, H. (1998) EMBO J. 17, 7170–7178
6. Ackerman, S., and Tzagoloff, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4986–4990
7. Wang, Z.-G., and Ackerman, S. (2000) J. Biol. Chem. 275, 5767–5772
8. Wang, Z.-G., Velours, G., and Ackerman, S. H. (2000) EMBO J. 19, 1486–1493
9. Payne, M. J., Schweizer, E., and Lukins, H. B. (1991) Curr. Genet. 19, 343–351
10. Ellis, T. P., Lukins, H. B., Nagley, P., and Corner, B. E. (1999) Genetics 151, 1353–1363
11. Ackerman, S., and Tzagoloff, A. (1990) J. Biol. Chem. 265, 9952–9959
12. Fry, F., and Tzagoloff, A. (1976) Eur. J. Biochem. 68, 113–119
13. Slonimski, P. P., and Tzagoloff, A. (1976) Eur. J. Biochem. 61, 27–41
14. Faye, G., Kujawa, C., and Fukuhara, H. (1974) J. Mol. Biol. 88, 185–203
15. Tzagoloff, A. (1978) Methods Enzymol. 55, 351–358
16. Dieckmann, C. L., and Tzagoloff, A. (1983) Methods Enzymol. 97, 361–373
17. Macino, G. and Tzagoloff, A. (1980) J. Biol. Chem. 255, 20284–20290
18. Hill, J. E., Myers, A. M., Koerner, T. J., and Tzagoloff, A. (1986) Eur. J. Biochem. 163–167
19. Michon, T., Galante, M., and Velours J. (1988) Eur. J. Biochem. 172, 621–625
20. Bizzozero, O., Besio-Moreno, M., Pasquini, J. M., Soto, E. F., and Gomez, C. J. (1983) J. Chromatogr. 227, 33–44
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 49–56
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Schmidt, R. J., Myers, A. M., Gilham, N. W., and Boynton, J. E. (1984) Mol. Biol. Evol. 1, 317–334
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
26. Tzagoloff, A., and Dieckmann, C. L. (1990) Microbiol. Rev. 54, 211–225
27. Muller, P. F., Reif, M. K., Zonghou, S., Sengstag, C., Mason, T. L., and Fux, T. D. (1984) J. Mol. Biol. 175, 431–452
28. Dieckmann, C. L., Koerner, T. J., and Tzagoloff, A. (1984) J. Biol. Chem. 259, 4722–4731
29. Tzagoloff, A., and Meagher, P. (1971) J. Biol. Chem. 246, 7325–7336
30. Douglas, M., Finkelstein, D., and Butow, R. A. (1979) Methods Enzymol. 56, 58–66
31. Paul, M. F., Velours, J., Arselin de Chateaubodeau, G., Aigle, M., and Guerin, B. (1989) Eur. J. Biochem. 185, 163–171
32. Norais, N., Prome, D., and Velours, J. (1991) J. Biol. Chem. 266, 16541–16549
33. Arselin, G., Vaillier, J., Graves, P. V., and Velours, J. (1996) J. Biol. Chem. 271, 20284–20290
34. Valiyaveetil, F. I., and Fillingame, R. H. (1998) J. Biol. Chem. 273, 16241–16247
35. Wada, T., Long, J. C., Zhang, D., and Vik, S. B. (1999) J. Biol. Chem. 274, 17353–17357
36. Glick, B. S. (1985) Methods Enzymol. 200, 224–231
37. Spannagel, C., Vaillier, J., Chaignepain, S., and Velours, J. (1998) Biochemistry 37, 615–621
38. Paumard, P., Vaillier, J., Napias, C., Arselin, G., Brethes, D., Graves, P.V., and Velours, J. (2000) Biochemistry 39, 4199–4205