Characterization of Mutations in the β Subunit of the Mitochondrial F<sub>1</sub>-ATPase That Produce Defects in Enzyme Catalysis and Assembly*

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The ATP2 gene, coding for the β subunit of the mitochondrial F<sub>1</sub>-ATPase, was cloned from nine independent isolates of chemically mutagenized yeast. Seven different mutant alleles were identified. In one case the mutation occurs in the mitochondrial targeting sequence (M11). The remaining six mutations map to the mature part of the β subunit protein and alter amino acids that are conserved in the bovine heart mitochondrial and Escherichia coli β subunit proteins. Biochemical analysis of the yeast ATP2 mutants identified two different phenotypes. The G133D, P179L, and G227D mutations correlate with an assembly-defective phenotype that is characterized by the accumulation of the F<sub>1</sub> and F<sub>0</sub> subunits in large protein aggregates. Strains harboring the A192V, E222K, or R293K mutations assemble an F<sub>1</sub>, of normal size that is none-the-less catalytically inactive. The effect of the ATP2 mutations was also analyzed in diploids formed by crossing the mutants to wild type. Hybrid enzymes formed with β subunits containing either the G133D, E222K, or R293K mutations are compromised for steady-state ATPase activity. The display of partial dominance confirms the importance of Gly<sup>133</sup> for structural stability and of Gly<sup>222</sup> and Arg<sup>293</sup> for catalytic cooperativity.

The F<sub>1</sub>-F<sub>0</sub> ATP synthase (ATPase) of mitochondrial, chloroplast, and bacterial membranes catalyzes ATP synthesis coupled to respiration (1, 2). The enzyme is composed of a catalytic moiety, F<sub>1</sub>, attached peripherally to an integral membrane component, F<sub>0</sub>. Three types of subunits (designated α, β, and γ in bacteria (3, 4)) constitute an evolutionarily conserved core F<sub>0</sub> structure, and there is variation among species as to whether or not additional F<sub>0</sub> subunits are present (5). F<sub>1</sub> shows a remarkable degree of structural conservation, and in most organisms studied, exists as an oligomer of five different subunits with the stoichiometry α<sub>3</sub>β<sub>3</sub>γ<sub>2</sub>ε<sub>ε</sub> (1, 2).

The x-ray structure of bovine mitochondrial F<sub>1</sub> shows the α and β subunits alternating in a hexagonal ring, with the amino and carboxyl termini of the γ subunit located in the central core of the ring (6). Six adenine nucleotide binding sites are located at the αβ interfaces. Three of these sites are catalytic and reside primarily in the β subunit; the other three sites are noncatalytic and are contributed mostly by residues of the α subunits (6). The three catalytic sites interact cooperatively during enzyme turnover; this process is probably mediated by conformational changes in the F<sub>1</sub> subunits (1, 2). A principal focus in F<sub>1</sub> research is to understand the mechanistic details that underlie catalytic cooperativity. The study of mutants with defects in the F<sub>1</sub> subunits offers a means to test the catalytic function of amino acids and to identify the residues that propagate conformational changes in the enzyme. Several excellent reviews are available that document studies performed with Escherichia coli mutants to probe the structure/function relationships in F<sub>1</sub> (7–9). The mitochondrial enzyme from Saccharomyces cerevisiae is equally amenable to genetic manipulation. Moreover, the analysis of yeast F<sub>1</sub> mutants is of particular value now that an atomic structure of the mitochondrial enzyme is available (6).

Genetic studies with yeast F<sub>1</sub> have centered on the β subunit, which is coded for by the ATP2 gene (10) in chromosome X (11). The work reported to date has used site-directed mutagenesis to effect changes in amino acids that were previously shown to react with chemical labels (12, 13) and also to change residues that constitute a sequence element conserved in several nucleotide binding proteins (the P-loop) (13–15). The present study constitutes the first report in which mutations induced chemically in the yeast β subunit gene were characterized both biochemically and genetically. Two classes of β subunit mutations were identified: those that alter catalysis without affecting the enzyme structure and those that produce defects in F<sub>1</sub> assembly.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Media—The genotypes and sources of the mutant and wild type yeast strains used in the present study are listed in Table I. Chemically induced mutants were obtained as described (17). E. coli RR1 (proA leuB lacY galK xyl-5 mtl-1 ara-14 rpsL supE hsdS λ-) was the host bacterial strain for the recombinant plasmid constructions. Yeast was grown in the following media: YPD (2% glucose, 2% peptone, 1% yeast extract), YPGal (2% galactose, 2% peptone, 1% yeast extract), YEFG (3% glycerol, 2% ethanol, 2% peptone, 1% yeast extract), WO (2% glucose, 0.67% yeast nitrogen base without amino acids (Difco)). Amino acids and other growth requirements were added at a final concentration of 20 μg/ml. The solid media included 2% agar.

Preparation of Yeast Mitochondria—Yeasts were grown aerobically in liquid YPGal at 30 °C to early stationary phase. The method of Faye et al. (18) was used to prepare mitochondria with the exception that zymolyase, instead of Glusulase, was used to digest the cell wall. Phenylmethylsulfonyl fluoride was added to 10 μg/ml final concentration during the cell-breaking step to minimize proteolysis.

Extraction of F<sub>1</sub>-F<sub>0</sub> Proteins from Mitochondria—Mitochondria were suspended at 6.6 mg/ml in 10 mm Tris-HCl, pH 8.0, 2 mm EDTA, 4 mm ATP (TEA buffer) and incubated for 15 min with 0.25% Triton X-100 to extract the F<sub>1</sub>-F<sub>0</sub> complex. The suspensions were centrifuged at 50,000 rpm in a Beckman 70.1TI rotor for 30 min, and the resultant soluble and particulate fractions were assayed for F<sub>1</sub> proteins by Western analysis.

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Sucrose Gradient Centrifugation Analysis of F$_1$-ATPase Subunits—Step sucrose gradients were used to analyze the properties of F$_1$ subunits in cases where the enzyme was found to be resistant to Triton X-100 extraction. Mitochondria (5 mg/ml) were permeabilized in TEA buffer by a brief exposure to sonic irradiation, and 0.2 ml samples were overlaid on a 4.8 ml discontinuous gradient of Tris-Cl, pH 7.5, buffered (20–80%, w/v). The preparation of the step gradients and the centrifugation protocol were as described (19). Linear sucrose gradients were used to evaluate the size of the F$_1$, F$_0$ complex in mitochondrial supernatants following Triton X-100 extraction. Solubilized protein (0.6 ml) was loaded onto a 4.4 ml 6–20% sucrose gradient prepared in 0.1% TritonX-100-supplemented TEA buffer, and the gradients were centrifuged at room temperature for 1.5 h at 55,000 rpm in a Beckman SW55 Ti rotor. For all experiments, sucrose gradient fractions were collected from the bottom of the tube.

 Colony Hybridization—The mutant atp2 genes were cloned by the method of colony hybridization (20). Following digestion of yeast genomic DNA with SphI, 7-kilobase fragments were purified from a 1% agarose gel and subcloned into a SphI-cut yeast/E. coli shuttle plasmid (either pRS316, CEN (21) or Yep352, 2 μ (22)). The mini-SphI libraries were used to transform E. coli, and the colonies were hybridized at 65 °C with a nick-translated DNA fragment containing the entire ATP2 gene.

 Construction of atp2 Null Strains—The one-step gene replacement method (23) was used to disrupt the chromosomal copy of ATP2 in the respiratory competent haploid yeasts W303–1A and W303–1B. The disruption allele (atp2::LEU2) was constructed by inserting a 2.8-kilobase DNA fragment, carrying the entire LEU2 gene, into the unique BomH I site of the ATP2 reading frame. Wild type W303 strains were transformed with atp2::LEU2 on a linear DNA fragment and the transformants were screened for the stable acquisition of leucine prototrophy that correlated with the loss of respiratory function. Respiratory-deficient, Leu$^+$ tester strains indicated that the respiratory defect of the disruption strains (W303×G1) was due solely to a nuclear mutation and Southern analysis verified that the gene disruption occurred at the ATP2 locus (data not shown). Genetic crosses also showed allelism between the disrupted gene in the W303 strains and atp2 present in the chemically mutagenized yeast strains.

 Assays—Protein concentrations were estimated by the method of Lowry et al. (24). ATPase activity was measured by determining the amount of inorganic phosphate released as described previously (25), in the absence and presence of 10 μg of oligomycin.

 Miscellaneous Procedures—Restriction endonuclease digestion of DNA, purification and ligation of DNA fragments, E. coli transformation and preparation of bacterial plasmids, and nick translation followed standard protocols (26). The dideoxy method (27) was used to sequence the mutant atp2 genes; both strands of the gene were sequenced using overlapping oligonucleotide primers spaced ~180–200 nucleotides apart. Yeast transformations were done with the LiAc procedure (28). For Western analysis, proteins were separated on 12% polyacrylamide gels run in the electrophoretic system of Laemmli (29), modified as described (25) to optimize resolution of the F$_1$ α and β subunit proteins. The procedure of Schmidt et al. (30) was used for Western blotting. Antibodies against F$_1$ α, F$_1$ β, and cytochrome c were used at dilutions of 1:2000, 1:3000, and 1:100 respectively.

### RESULTS

Genetic Analysis of Yeast atp2 Mutants—Chemically mutagenized yeast strains belonging to complementation group G1 (17) carry mutations in ATP2, the nuclear gene that codes for the β subunit of the F$_1$F$_0$ ATP synthase. The mutant atp2 genes were cloned from nine G1 strains, and the mutations were sequenced (see “Experimental Procedures”). The position of these mutations in the nucleotide and protein sequences is shown in Table II. A single missense mutation was found in each of the mutants, which alters the sequence of ATP2 relative to the wild type copy present in the parental strain D273–10B/A1 (GenBank™ accession number U46215). Two of the mutations were found in more than one strain: the P179L substitution occurs in the strains C103 and E210, and the mutation E222K is present in the strains E430 and E144. The mutation in strain E700 converts the initiator methionine to isoleucine. Since this mutation does not provide information on residues involved in F$_1$ biogenesis or catalysis, it will not be discussed further.

Mitochondrial ATPase Activity and F$_1$F$_0$ Assembly in Haploid atp2 Mutant Strains—The amount of oligomycin-sensitive mitochondrial ATPase activity present in the mutant haploid strains is shown in Table III. All of the mutations produce a near complete loss (≥93%) of ATPase activity. Western blots (Fig. 1) were used to determine the amount of F$_1$ β subunit in each of the atp2 mutants; these values are also reported in Table III. The level of F$_1$ β subunit protein ranges from 44 to 85% the control level and thus does not account for the reduc-

### Table I

| Strain | Genotype | Sources |
|--------|----------|---------|
| D273–10B/A1 | amet6 | Ref. 16 |
| W303–1A | ade2–1 his3–1,15 leu2–3,112 ura3–1 trpl–1 | This study |
| W303–1B | ade2–1 his3–1,15 leu2–3,112 ura3–1 trpl–1 | This study |
| C103 | amet6 atp2–1 | Ref. 17 |
| E210 | amet6 atp2–1 | This study |
| E144 | amet6 atp2–2 | This study |
| E430 | amet6 atp2–2 | This study |
| E700 | amet6 atp2–3 | This study |
| E588 | amet6 atp2–4 | This study |
| P159 | amet6 atp2–5 | This study |
| P133 | amet6 atp2–6 | This study |
| N123 | amet6 atp2–7 | This study |
| aW303FG1 | ade2–1 his3–1,15 leu2–3,112 ura3–1 trpl–1 atp2::LEU2 | This study |
| aW303FG1 | ade2–1 his3–1,15 leu2–3,112 ura3–1 trpl–1 atp2::LEU2 | This study |

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### Table II

| atp2 mutant | Nucleotide | Codon change | Amino acid change |
|-------------|------------|--------------|------------------|
| E700 | 3 | ATG → ATA | M11 |
| P133 | 398 | GGT → GAT | G133D |
| E210 | 536 | CCT → CTT | P179L |
| C103 | 536 | CCT → CTT | P179L |
| P159 | 575 | GCA → GCT | A192V |
| E430 | 644 | GAA → AAA | E222K |
| E144 | 644 | GAA → AAA | E222K |
| N123 | 680 | GGT → GAT | G227D |
| E588 | 878 | AGA → AAA | R293K |

The biochemical characteristics are described for the mutants E210 and E430 as the strains representative of the P179L and E222K mutations, respectively.
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**TABLE III**

**ATPase activity of haploid yeast atp2 mutants**

| Strain (mutation) | Amount β subunitb | Mitochondrial ATPase activity |
|-------------------|-------------------|------------------------------|
|                   | % control level   | μmol Pi/min/mg               | μmol Pi/min/mg | % control level |
| D273              | 100               | 4.30 (4.10)                  | 0.34 (0.46)    | 3.80           | 100            |
| P159 (A192V)      | 67                | 0.08 (0.20)                  | 0.04 (0.14)    | 0.05           | 1.3            |
| E430 (E222K)      | 85                | 0.13 (0.12)                  | 0.08 (0.04)    | 0.06           | 1.6            |
| E588 (G229K)      | 61                | 0.15 (0.12)                  | 0.03 (0.05)    | 0.09           | 2.4            |
| P133 (G133D)      | 49                | 0.16 (0.17)                  | 0.08 (0.06)    | 0.09           | 2.4            |
| E210 (P179L)      | 51                | 0.24 (0.22)                  | 0.10 (0.15)    | 0.10           | 2.6            |
| N123 (G227D)      | 44                | 0.14 (0.15)                  | 0.04 (0.03)    | 0.11           | 2.9            |

a The amount of F1 β subunit protein was determined using an AMBIS 4000 imaging system to scan Western blots of total mitochondrial protein. The mean values from the analysis of two separate Western blots are shown; one set of values is given in parentheses.

b Results from the assay of two separate mitochondrial preparations are shown; one set of values is given in parentheses.

c The mean values are reported.

**Fig. 1. Western analysis showing level of F₁ α and β protein in total mitochondrial protein.** Mitochondrial samples (20 μg), prepared from the yeast strains shown, were loaded onto a 12% SDS-polyacrylamide gel. Following transfer to nitrocellulose, the blot was exposed to antibodies against the F₁ α and β subunits as described under “Experimental Procedures.” The position of the α and β subunit bands are indicated in the left-hand margin.

**Fig. 2. Western analysis showing F₁,F₉ extraction from mitochondria.** Mitochondrial samples, prepared from the yeast strains shown, were extracted with 0.25% Triton X-100 and centrifuged as described under “Experimental Procedures.” Aliquots of total mitochondrial protein (M) and equivalent volumes of the particulate (P) and soluble (S) fractions were analyzed by Western as described in the legend to Fig. 1. The amount of mitochondrial protein loaded was 20 μg for D273 (wild type (WT)) and 30 μg for the mutants E210, P159, E430, and E588. The type of atp2 mutation is given in parenthesis under the name of the mutant strain. The position of the α and β subunit bands are indicated in the left-hand margin.
Interestingly, control amounts of F1−F0 ATPase is that measured with the double wild type diploid, assembled F1−F0 that is solubilized should be the same in each under identical extraction conditions, the percentage of mitochondrial ATPase activity by the percent solubilized that of the F1−F0 ATP synthase. This result suggests that expression of ATP2 toachondrial ATPase activity by the percent solubilized to give the values reported in the last column of Table IV. Support for using these calculations to determine the level of F1−F0 in the yeast diploids from the fact that nonassembled F1 is not extracted with Triton X-100 (19) and that under identical extraction conditions, the percentage of assembled F1−F0 that is solubilized should be the same in each of the samples. The reference value for F1−F0 catalytic activity is that measured with the double wild type diploid, W303×D273. Interestingly, control amounts of F1−F0 ATPase activity were measured in W303 VG1×D273, which is a diploid strain that harbors a single gene for the β subunit (Table IV). This result suggests that expression of ATP2 from either one or two wild type loci provides the diploid cell with the similar complement of the F1−F0 ATP synthase.

Assuming that random interactions occur among the F1 sub-units from both parental cells, there is the potential to form four types of enzyme oligomers in the wild type/mutant diploids: a 3βwt, a 3βmut, and two types of hybrids (2βwt1βmut and 1βwt2βmut). All four enzyme types are expected to form in diploids if the resident mutant β subunit does not affect F1 assembly. With respect to the strains shown in Table IV, this situation applies to W303×P159, W303×E430, and W303×E588. In such strains, the 3βwt and 3βmut enzymes are each expected to comprise 12.5% of the total F1 oligomer population; the hybrid forms constitute, cumulatively, the remaining 75% of enzyme (35). Since the P159, E430, and E588 haploid strains are devoid of ATPase activity (Table III), it is assumed that the 3βmut F1 formed in the derivative diploid cells is inactive. In cases where the 3βmut F1 is the only inactive form of the enzyme, the maximal level of ATPase activity expected for the diploid strain is 87.5% (cumulative activity from the 3βwt and hybrid enzymes). This value is within 5% error of the ATPase activity observed for W303×P159 (Table IV). Therefore, it would appear that this particular diploid forms fully active F1 hybrids in addition to the 3βwt enzyme. The other two members of this diploid class exhibit 31 and 74% the control level of F1−F0 ATPase activity. Both values are in between the maximum (87.5%) and minimum (12.5%) levels of enzyme activity expected in this analysis, which suggests the presence of partially active F1 hybrids in addition to the fully wild type enzyme. The reduction in the activity of the hybrid enzymes is most striking for W303×E430, suggesting that the E222K mutation displays significant dominance over the wild type ATP2 allele present in the diploid cell.

The other three wild type/mutant diploid strains shown in Table IV (W303×P133, W303×E210, W303×N123) synthesize mutant β subunits that do not assemble into an F1 structure in the respective haploid strains (Figs. 2 and 4). Therefore, it is assumed that the 3βmut enzyme does not form in these particular diploids. It is also likely that mutations, which affect the ability of the β subunit to assemble homogeneous mutant enzymes, will also prevent the formation of hybrid structures. In such cases the presence of the mutant atp2 gene would be no worse than having the null allele, since the pool of wild type subunits would not be used to form F1 hybrids. As long as the 3βwt enzyme would be synthesized in the diploid cell, one should see levels of F1−F0 ATPase activity comparable with that of the W303VG1×D273 strain (Table IV). This result is observed with W303×E210 and W303×N123 (Table IV), suggesting that the mutant β subunits synthesized in these strains do not interact.
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**FIG. 5.** Western analysis of Triton X-100 extracts from mitochondria of yeast diploid strains. Mitochondria, prepared from the yeast diploids shown, were extracted with 0.25% Triton X-100 and centrifuged as described under “Experimental Procedures.” Equal volumes of each supernatant were analyzed by Western analysis to detect the β subunit protein. The volume of the supernatant loaded onto the protein gel was equivalent to 20 μg of the starting mitochondrial protein.

![Western analysis of Triton X-100 extracts from mitochondria of yeast diploid strains.](image)

**TABLE IV**

| Strain (mutation) | Amount β subunit (soluble) | ATPase activity | Oligomycin-sensitive activity |
|-------------------|-----------------------------|-----------------|-----------------------------|
|                   | % control level | μmol P_i/min/mg | +Oligomycin | −Oligomycin | Mitochondrial | F_1-F_0 |
| W303×D273         | 100             | 3.60 (3.40)     | 0.75 (0.90) | 2.67       | 100           |
| W303VG1×D273 (null)| 83              | 2.60 (2.50)     | 0.40 (0.50) | 2.10       | 94.4          |
| W303×P159          | 80              | 2.10 (2.20)     | 0.20 (0.15) | 1.97       | 92.1          |
| W303×E430 (E222K)  | 88              | 1.20 (1.00)     | 0.50 (0.24) | 0.73       | 30.9          |
| W303×E588 (R293K)  | 81              | 1.80 (1.95)     | 0.25 (0.30) | 1.60       | 73.7          |
| W303×P133 (G133D)  | 65              | 1.50 (1.50)     | 0.40 (0.20) | 1.20       | 68.9          |
| W303×E210 (P179L)  | 89              | 2.80 (2.50)     | 0.60 (0.27) | 2.21       | 92.9          |
| W303×N123 (G217D)  | 85              | 2.50 (2.60)     | 0.23 (0.32) | 2.27       | 99.9          |

* The amount of soluble F_1 β subunit protein was determined using an AMBIS 4000 imaging system to scan Western blots of Triton X-100 mitochondrial extracts. The mean values from the analysis of two separate Western blots are reported; one of the blots used is shown in Fig. 5.

* Results from the assay of two separate mitochondrial preparations are shown; one set of values is given in parentheses.

* Values were calculated by dividing the mean percent mitochondrial ATPase activity by the mean percent F_1 β subunit protein solubilized with Triton X-100.

* The mean values are reported.

DISCUSSION

With the exception of the initiator methionine change to isoleucine, the atp2 mutations reported in this paper are located in the central αβ domain of the protein. This portion of the β subunit encompasses the adenine nucleotide binding site and contains the P-loop region, which is a sequence element found in many nucleotide-binding proteins, including adenylate kinase and p21ras (36). The phenotype described for the bacterial A151V mutant (38) is similar to what we have determined for the yeast strain, P159; the F_1-F_0 assemblies correctly, yet is deficient in membrane-bound ATPase activity (Table III, Fig. 3). Our results are also in accord with Shen et al. (15) who screened mutations in the yeast β subunit P-loop region and found that mutants with the A192V change were respiratory-deficient. The position occupied by alanine in the P-loop of the F_1 β subunit is a glycine residue in p21ras; notably, a mutant Ras protein with diminished GTPase activity was isolated in which this glycine residue was changed to valine (39). Apparently, substitution of valine at the 3rd position of the conserved P-loop (GXXXXGKTS) has a global effect on the catalytic activity of NTP hydrolyzing enzymes that contain this consensus sequence. In E. coli F_1, the A151V mutation produces a dramatic decrease in promotion from unisite to multisite catalysis (38). Our results with the W303×P159 diploid (Table IV) indicate that hybrid forms of F_1, which contain either one or two β subunits with the corresponding A192V mutation, are fully active. Therefore, it would appear that the P-loop Ala → Val mutation only blocks F_1 catalysis in enzyme oligomers homogenous for the mutant subunit.

**E222K**—The phenotype of yeast harboring the chemically induced E222K mutation (strains E430 and E144, Table II) can be compared with the E. coli E181Q β subunit mutant derived from site-specific mutagenesis (40). Mutation of this glutamate residue to either lysine or glutamine does not interfere with F_1-F_0 assembly, but lowers the steady-state level of membrane-bound ATPase activity to 2% the wild type level in haploid strains (Fig. 3, Table III (40, 41). The fact that mutagenesis studies in yeast (present work) and in bacteria (41) give importance to this glutamate residue in the F_1 catalytic mechanism is in accord with structural information obtained with the bovine enzyme (6). The equivalent glutamate residue in the bovine β subunit (Glu^{188}) contributes to ATP binding in the catalytic site, and there is electron density in the map that could represent a water molecule hydrogen bonded to its carboxylate group (6). These observations have led the authors to suggest that Glu^{188} activates the water, promoting an in-line nucleophilic attack on the γ-phosphate of ATP during F_1 turn-
over (6). The dramatic effect on F$_1$ catalytic activity that occurs when this glutamate residue is mutated is also readily apparent in diploid yeast (Table IV) and in E. coli transformants (41, 42) that synthesize hybrid forms of the enzyme. Our results with the W303×E430 strain (Table IV) suggest that F$_1$ catalysis is severely inhibited in F$_1$ hybrids containing the mutant subunit, which supports the idea that the affected glutamate residue is important for cooperative interactions in the enzyme.

R293K—The yeast R293K β subunit mutation produces a near complete loss of ATP hydrolytic activity in the haploid strain E588 (Table III). Two different mutations of the corresponding arginine residue in the E. coli β subunit (R246C (43) and R246H (34)) were obtained by chemical mutagenesis. The corresponding arginine residue in the β subunit (Arg$^{260}$) is located at the interface with one of the other F$_1$ subunits, in close proximity to the nonexchangeable adenine nucleotide binding moiety of ATP in the catalytic site (6). In the diploid that harbors one copy of the mutant allele (Table IV), the mutation R293K reduces the maximum steady-state level of ATPase activity by 17%. This result implies that there is only a modest effect on catalytic cooperativity in hybrid enzymes that contain this particular mutant β subunit.

The other three β subunit mutations (G133D, P179L, and P227D) reported in the present work are novel in that they alter amino acids different from those studied previously in yeast or bacteria. The overall phenotype of the haploid mutants (P133, E210, and N123) is defective F$_1$ assembly with the accumulation of the α and β subunits in large aggregates inside mitochondria (Fig. 4). However, analysis of the wild type/mutant diploid strains (Table IV) indicates two subphenotypes, which may explain why a mutation of this amino acid does not preclude the mutant subunit from interacting with the other enzyme subunits. However, inspection of the x-ray structure of bovine F$_1$ shows that the G133D mutation of strain P133 is likely to disturb the three-dimensional structure of the β subunit, since the space in the vicinity of the glycine residue is too small to accommodate the mutation to aspartic acid. The presumed structural perturbation might be responsible for the reduced level of F$_1$-$F_0$ specific activity and for the smaller amount of soluble β subunit detected in W303×P133 with respect to the other diploid strains (Table IV). Thus, in addition to assembling catalytically impaired hybrid enzymes, the P133 mutant β subunit probably engages in nonproductive interactions that lower the steady-state level of assembled (soluble) F$_1$-$F_0$.

The type of F$_1$ assembly defect observed in the atp2 mutants described here is also seen in yeast mutants where the genetic lesion affects the Atp11p and Atp12p F$_1$ assembly factor proteins (19). The proposed function of the F$_1$-specific “chaperone-type” proteins (Atp11p and Atp12p) is to ensure that productive associations between F$_1$ α and β subunits prevail over the formation of nonproductive α$_n$ and β$_n$ complexes (44). In light of evidence for direct binding between Atp11p and the F$_1$ β subunit (45), the atp2 mutations that correlate with an F$_1$ aggregation$^-$ phenotype are of particular interest, since they may affect amino acids that interact with the assembly factor.

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