Partial Uncoupling of the Mitochondrial Membrane by a Heterozygous Null Mutation in the Gene Encoding the γ- or δ-Subunit of the Yeast Mitochondrial ATPase*

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Prior genetic studies indicated that the yeast mitochondrial ATP synthase can be assembled into enzyme complexes devoid of the γ-, δ-, or ε-subunits (Lai-Zhang, J., Xiao, Y., and Mueller, D. M. (1999) EMBO J. 18, 58–64). These subunit-deficient complexes were postulated to uncouple the mitochondrial membrane thereby causing negative cellular phenotypes. This study provides biochemical and additional genetic data that support this hypothesis. The genetic data indicate that in a diploid cell, a heterozygous deletion mutation in the gene encoding the γ- or δ-subunit of the ATPase is semidominant negative due to a decrease in the gene number from 2 to 1. However, the heterozygous ATP2Δ mutation is epistatic to the heterozygous mutation in the gene encoding γ or δ, suggesting that the semidominant negative effect is because of a gain of activity in the cells. Biochemical studies using mitochondria isolated from the yeast strains that are heterozygous for a mutation in γ or δ indicate that the mitochondria are partially uncoupled. These results support the hypothesis that the negative phenotypes are caused by the formation of a γ- or δ-less ATP synthase complex that is uncoupled.

The mitochondrial (mt) ATP synthase is the major enzyme responsible for the synthesis of ATP under aerobic conditions. The ATP synthase converts the energy obtained from the oxidation of foodstuffs to the usable form of ATP. Thus, the efficiency of energy transduction is dependent on the efficiency, or coupling capacity, of ATP synthase. Mutations that alter the coupling capacity of the ATP synthase would be predicted to have profound effects on cellular metabolism.

The ATP synthase is a multimeric enzyme composed of a water-soluble portion, F1, and a membrane portion, F0 (for reviews see Refs. 1 and 2). The F1 is composed of five unique subunits with the stoichiometry, α3β3γδε, and contains the catalytic site of the enzyme (3). There are three catalytic sites formed by the α- and β-subunits, which are arranged front to back forming a near sphere (4).

The mechanism of ATP synthesis by the ATP synthase proceeds by the binding site mechanism (5). In this mechanism, the phosphorylation of ADP does not require energy and the reaction occurs at one of the three catalytic sites, which has a high affinity for ATP. The flow of protons alters the conformation of the high affinity site, decreasing its affinity for ATP, 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 active site of the enzyme (4). 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 (6–8). As such, the evidence indicates that F1 is a molecular motor that produces ATP.

The F0 portion of the enzyme is a proton pore, which apparently drives the rotation of the γ-subunit within F1, coupled to the flow of protons from the cytosol to the matrix of the mitochondrion. The role of the δ and ε subunits is not certain, but studies suggest that they are involved in the coupling of ATP synthesis to proton translocation, with δ being part of the rotor (9–15). Alternatively, and maybe more descriptively, the δ- and ε-subunits may function as a molecular clutch (9). As such, enzymes defective in the δ- and ε-subunits would be predicted to be unable to couple ATP synthesis to the flow of protons through F0.

This study uses yeast Saccharomyces cerevisiae to study the assembly and structure/function relationship of the ATP synthase. The prior study provided genetic evidence that the ATP synthase could be assembled into an enzyme complex devoid of the γ-, δ-, or ε-subunit (9). A hypothesis was proposed that the subunit-deficient enzyme complexes provided an enzyme that freely passed protons from the cytosol to the matrix of the mitochondrion, but was unable, or only poorly able to drive the synthesis of ATP, i.e. the ATP synthase was uncoupled.

The uncoupling of the mitochondrial membrane was proposed to be responsible for the cells tendency to lose their mtDNA (to become cytoplasmically petite or ρ−/ρ0). The present study provides biochemical and additional genetic evidence that supports this hypothesis. The implications of these results are profound, because if these subunit-deficient complexes occur in the human cell, either in the normal or disease state, then the efficiency for ATP synthesis would be compromised and the physiology of the organism could be largely altered.

EXPERIMENTAL PROCEDURES

Yeast Strains and Genetics—The diploid yeast S. cerevisiae strains used in this study are listed in Table I. The percentage of cytoplasmic ρ−/ρ0 cells (loss or deletion of mtDNA) was determined for the strains by taking a single colony from YPD plates, growing the cells in YPD medium for 2 days, replating the cells on YPD plates, replica plating the colonies to YPG medium, determining the percentage of colonies that cannot grow on YPG medium, and by determining the percentage of white colonies, an indicator of the ρ−/ρ0 mutation in an ade2− strain (9, 16).

Tetrad analysis was performed by standard methods (17). The cells were grown on prespore medium for 1 day, transferred to sporulation medium for 4–6 days, and dissected on YPD medium.

Yeast transformation was performed by the LiAc method after growth in YPAD medium (18). For selection of G418 resistance cells, the

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Partial Uncoupling by ATPase Null Mutations

### Table I

| Number | Diploid strain | Pertinent genotype | Description |
|--------|----------------|--------------------|-------------|
| 1      | W303          | wt*                | wt diploid strain |
| 2      | W303 Δα/α     | α−/α+              | heterozygous deletion mutation in ATP1 (codes for α-subunit) |
| 3      | W303 Δβ/β     | β−/β+              | heterozygous deletion mutation in ATP2 (codes for β-subunit) |
| 4      | W303 Δγ/γ     | γ−/γ+              | heterozygous deletion mutation in ATP3 (codes for γ-subunit) |
| 5      | W303 Δδ/δ     | δ−/δ+              | heterozygous deletion mutation in ATP6 (codes for δ-subunit) |
| 6      | W303 Δε/ε     | ε−/ε+              | heterozygous deletion mutation in ATP8 (codes for ε-subunit) |
| 7      | W303 Δγp505   | γ−/γ+              | same as 4 with vector pRS305 integrated at the LEU2 locus |
| 8      | W303 Δγp505ATP | γ−/γ+              | same as 4 with plasmid pRS305/ATP3 integrated at the LEU2 locus |
| 9      | W303 Δδp606   | δ−/δ+              | same as 5 with plasmid pRS306 integrated at the URA3 locus |
| 10     | W303 Δδp606ATP | δ−/δ+              | same as 5 with plasmid pRS306/ATP3 integrated at the URA3 locus |
| 11     | W303 Δεp3/εβ | ε−/ε+              | heterozygous deletions in ATP3 and ATP2 |
| 12     | W303 Δεp3/εβ | δ−/δ+              | heterozygous deletions in ATP6 and ATP2 |

* wt, wild type.

Transformants were allowed to recover for 6–8 h at 30 °C and plated on YPD containing 0.2 mg/ml G418 (18). The null deletion mutants were made by homologous recombination of PCR products using the KanMX resistance module (19). The deletion mutations eliminated the entire coding region of the gene with the exception of atpΔ, which deleted the entire coding region of the mature peptide. The strains were checked by whole cell polymerase chain reaction to confirm that the correct integration event occurred in the chromosome at the targeted gene.

Media—The yeast media are standard recipes as described (20): YPD, 2% glucose, 1% yeast extract, 2% peptone; YPG, 2% peptone, 1% yeast extract, 3% glycero; and YPAD, 2% glucose, 1% yeast extract, 2% peptone, and 20 mg/ml adenine sulfate. Semisynthetic medium containing 2% galactose, 0.05% glucose (21), and minimal medium (SD) with 2% glucose were supplemented with adenine, histidine, arginine, methionine, tyrosine, lysine, leucine (Leu), isoleucine, and tryptophan (Trp) or uracil (Ura) at 20 mg/ml.

Biochemical Studies—Mitochondria were isolated from yeast after growth in semisynthetic medium containing 2% galactose and 0.05% glucose (21). In this medium, the percentage of cells that are p−p0 for cells with a heterozygous mutation in either ATP3 or ATP6 is greatly diminished relative to the same cells after growth in YPD medium (Table I). The most likely reason for this difference is that the p−p0 mutant cells do not grow as well as the p+ cells in this medium and are selected against.

The concentration of soluble protein was determined by the method of Bradford using Serva Blue for soluble proteins, whereas that of membrane preparations was determined by the Lowry method (22). Bovine serum albumin was used as the standard in both assays. ATPase activity was determined by the coupled enzyme reaction using 2 mM ATP and 3 mM MgCl2, at 30 °C (23). Respiratory rates, respiratory ATPase activity was determined by the coupled enzyme reaction using membrane preparations was determined by the Lowry method (22).

Results

The prior epistatic studies on mutations in the genes encoding subunits of the ATPase provided diploid strains with heterozygous mutations in the genes encoding the γ or δ-subunit of the ATPase (9). These diploid strains were clearly phenotypically different from either the wild type strain, W303, or strains with heterozygous mutations in the genes encoding the other subunits of the ATPase. One obvious difference was that these ade2− strains were pink rather than red. Typically, ade2− strains turn red on YPD medium because of the accumulation of an intermediate in the adenine biosynthetic pathway. However, strains that are ade2− and respiratory deficient are white (16). The diploid strains with a heterozygous mutation in either the γ or δ-subunit, in addition to being pink, apparently became cytoplasmically white or p−p0 (loss or deletion of mtDNA) at a higher frequency as based on the high percentage of white colonies on YPD plates. These preliminary observations suggested that these heterozygous mutations were semi-dominant negative.

An extensive analysis was performed to understand the nature of the heterozygous mutations in either ATPδ or ATP3 (the genes that code for the δ- and γ-subunites, respectively). The percentage of cytoplasmic p−p0 cells was determined for a number of strains (Table I, Table II). In Table IIA, the percentage of p−p0 is compared between the wild type strain and strains with heterozygous mutations in the genes encoding subunits of the F1-ATPase. These results indicate that the strains with a heterozygous mutation in either ATP3 or ATP6 have a greatly increased rate of cytoplasmic p−p0 formation, increasing from 1–3% in the wild type strain to about 35% in the mutant strains. However, strains with heterozygous deletion mutations in ATP1, ATP2, or ATP8 (encoding the genes for the α, β, and ε-subunits, respectively) accumulated an equivalent percentage of p−p0 mutations as the wild type strain.

The increased rate of p−p0 formation in the heterozygous mutant strains could be due to cis-acting effects of the mutations on neighboring genes. In other words, it is possible that the deletion mutations altered the expression of neighboring genes, and this did not decrease in the gene number of ATPδ or ATP3, is responsible for the increased rate of p−p0 formation. To differentiate between these possibilities, the ATPδ or ATPδ genes were integrated at a separate locus on the chromosome and assessed for the formation of p−p0 mutations. Table IIB indicates that if ATP3 is integrated in the chromosome at the LEU2 locus in the strain that has a heterozygous mutation in ATP3, then the percentage of p−p0 drops to the wild type level. Similarly, if the ATP6 gene is integrated into the chromosome at the URA3 locus in the yeast strain that has a heterozygous mutation in ATPδ, then the percentage of p−p0 drops to the wild type level. These results indicate that the tendency to form p−p0 in the mutant strains is because of the drop in the gene number of ATP3 or ATPδ from 2 to 1 and it is not due to any cis-acting events on the chromosome due to the atpδΔ or atpδΔ mutations.

Our prior studies in the haploid strains, the mutation in ATP2 was epistatic to the mutation in either ATPδ or ATP3 (9). This epistatic relationship was also evaluated in the diploid strains with the heterozygous mutations. Table IIC shows that a heterozygous mutation in the gene encoding the β-subunit, ATP2, is epistatic to either the heterozygous mutation in ATP3 or ATP6. This was concluded because the percentage of p−p0 in an atpδΔ/Δp3 Δ double mutant or the atpδΔ/Δp3 Δ double mutant strain was the same as the level in the cells with the atpδΔ mutation alone. Thus, the atpδΔ mutation “stands above” or epistatic to either the atpδΔ or atpδΔ mutations. This result suggests that the semi-dominant negative effect of the atpδΔ or atpδΔ mutations is because of a gain of activity rather than a loss of function. This is suggested because the atpδΔ/Δp3 and atpδΔ/Δp3 yeast strains do not show the negative phenotypes and yet contain the heterozygous deletion mutation in ATP3 or ATPδ. As such, this result implies that the negative phenotype caused by the decrease in the gene dosage from 2 to 1 in ATP3.
or ATPδ is not because of a decrease in the level of an enzyme, such as the ATP synthase. Rather, it is because of the gain of an activity not normally present in wild type cells or in cells with the atp2Δ heterozygous mutation alone or paired with heterozygous atpδΔ or atpδΔ mutations. This conclusion was also reached from results using haploid cells with mutations in ATP3 or ATPδ (9).

A second phenotype that was observed for the strains with a heterozygous mutation in either the ATP3 or ATPδ genes is that they were not as effective growing in medium containing glycerol as the carbon source (Fig. 1). This phenotype was then used to address the same questions as those in Table II and identical conclusions were obtained. 1) Strains with a heterozygous mutation in either the ATP3 or ATPδ genes are defective (Fig. 1A). This is observed both in the slower approach to stationary state and the final cell density in stationary state. 2) These growth defects are because of a gene dosage effect and not because of a cis-acting effect of the mutation (Fig. 1B). This was concluded because integration of either the ATP3 or ATPδ gene at a locus away from the corresponding deletion mutation complemented growth phenotype. 3) The atp2Δ mutation was epistatic to either the atpδΔ or the atpδΔ mutation (Fig. 1C).

The heterozygous mutations in ATP3 or ATPδ have the same phenotypic consequences on the cellular phenotypes as observed for deletion mutations in the haploid cell (9) and differ only in their magnitude. Furthermore, the same epistatic relationship that occurs between the atp2Δ mutation and the atpδΔ and atpδΔ mutations in the haploid state occurs in the diploid state. Thus, it is concluded that the underlying biochemical defect responsible for the negative phenotypes in the haploid mutant cells is the same as that in the heterozygous mutant diploid cells.

The mitochondria are uncoupled in the atpδΔ or atpδΔ mutant cells. The hypothesis suggested from this and the prior study (9) is that the negative phenotypes of the strains with the deletion mutation in either ATP3 or ATPδ is because of the assembly of an ATP synthase complex that is devoid of the γ- or δ-subunit. These subunit-deficient enzyme complexes were postulated to freely pass protons into the mitochondrial matrix thereby uncoupling the mitochondria. To address this, mitochondria were isolated from the strains and the coupling of the mitochondrial membranes was determined by measuring the respiratory control ratio, the P/O ratio, and the ΔΨ across the membrane with the fluorescent probe, rhodamine 123 (Table III). The rate of oxygen consumption reflects the rate of respiration, which is indicative of the rate of electron transport along the electron transport chain. Because respiration is obligatory coupled to ATP synthesis, the rate of respiration in the absence of ADP (state 4) is indicative of the permeability of the membrane to protons. The results indicate that mitochondria isolated from the strains with heterozygous mutations in either ATP3 or ATPδ have an increased state 4 rate and thus an increased permeability to protons. However, the addition of an inhibitor of F0, DCCD, inhibits the state 4 rate of the mutant mitochondria to the level observed for the mitochondria isolated from the wild type strain. This suggests that the proton leakage is via F0 of the ATP synthase. Upon adding ADP (state 3) or the uncoupler CCCP, the rate of respiration of the same mitochondria was similar to mitochondria isolated from the

| Table II: Percentage of ρ’/ρ after growth of the cells in YPD medium |

| Diploid strain | Genotype | Percentage ρ’/ρ* |
|----------------|----------|------------------|
| A W303         | wt       | <1               |
| W303 Δαδ/αδ    | Δαδ/Δαδ  | 2                |
| W303 Δβγ/βγ    | Δβγ/Δβγ  | <1               |
| W303 Δγδ/γδ    | Δγδ/Δγδ  | 1                |
| W303 Δεε/εε    | Δεε/Δεε  | 3                |
| W303 Δγ/γ06    | γ/γ06/ATPγ, LEU2 | <1 |
| W303 Δδ/δ06    | δ/δ06/ATPδ, URA3 | 3   |
| W303 Δδ/δ06    | δ/δ06/ATPδ, URA3 | 3   |
| B W303 Δγ/γ05  | γ/γ05/ATPγ, LEU2 | <1 |
| W303 Δδ/δ05    | δ/δ05/ATPδ, URA3 | 3   |

* wt, wild type.

**Fig. 1. Growth curves of wild type and heterozygous mutant strains on medium with a nonfermentable carbon source, YPG. The cells were grown at 30 °C with shaking, and the cell growth was monitored by the absorbency at 600 nm. The curves are from a single experiment but are representative of two to three separate measurements. A, wild type (wt) cells and cells with heterozygous mutations in the genes encoding the β-, γ-, and δ-subunits are shown as marked. B, wild type cells and cells with heterozygous mutations in the genes encoding the δ- or γ-subunits with either the plasmid pRS306 (p06) or the plasmid pRS306 containing the ATPδ gene or the plasmid pRS305 containing the ATP3 gene integrated into the chromosome at either the LEU2 (pRS305) or URA3 (pRS306) locus as marked. C, wild type cells and cells with heterozygous mutations in ATP2 paired with heterozygous mutations in either ATP3 or ATPδ as marked. Table I provides a more thorough description of the cells studied here.**
Heterozygous null mutations in ATP2, ATP3, and ATP6, were made in the wild type diploid strain W303. The average respiratory measurements obtained from two independent experiments are shown. Respiratory activities (nmol of O/min/mg of protein) were measured in the presence of succinate (5 mM), ADP (0.75 mM) (state 3), DCCD (10 μg), Og (5 μg), or CCCP (15 μg), as indicated. The ΔΨ was evaluated by the quenching (arbitrary units) of rhodamine 123 fluorescence (ΔF) in buffer containing 5 mM Tris-succinate, 0.2 μM rhodamine 123, 2.7 μM rotenone at a mitochondrial protein concentration of 0.05 mg/ml. The temperature was 30 °C for all assays. The specific ATPase activity is in units of μmol/min/mg of protein. The standard deviation of all the values did not exceed 20% of the reported values except for those of ATPase with oligomycin, which did not exceed 50% of the reported value.

| Strain  | Experiment number | Respiratory state | RCR | P/O | ΔΨ Rhod. 123 (m) | ATPase SA (n) |
|---------|-------------------|------------------|-----|-----|-----------------|----------------|
| W303    | 1                 | State 4          | +DCCD | +CCCP | 3.5 | 1.75 | 46 (3) | 2.0 (3) | 0.12 (3) |
|         | 2                 | State 3          | +DCCD | +CCCP | 3.5 | 1.76 |        |        |          |
| W303δββ | 1                 | 93              | State 3 | +DCCD | 3.4 | 1.66 | 49 (3) | 2.1 (2) | 0.29 (2) |
|         | 2                 | 95              | State 3 | +DCCD | 3.3 | 1.74 |        |        |          |
| W303δγγ | 1                 | 168             | State 3 | +DCCD | 4.0 | 0.89 | 26 (3) | 0.97 (3) | 0.08 (3) |
|         | 2                 | 193             | State 3 | +DCCD | 2.2 | 1.18 |        |        |          |
| W303αβα | 1                 | 174             | State 3 | +DCCD | 2.0 | 0.96 | 24 (3) | 0.85 (3) | 0.14 (3) |
|         | 2                 | 156             | State 3 | +DCCD | 1.8 | 0.95 |        |        |          |
| W303δεε | 1                 | 84              | State 3 | +DCCD | 3.0 | 1.6  | 45 (2) | 2.2 (2) | 0.18 (2) |
|         | 2                 | 76              | State 3 | +DCCD | 3.5 | 1.76 |        |        |          |
| W303γγγ | 1                 | 71              | State 3 | +DCCD | 3.6 | 1.72 | 54 (2) | 2.2 (2) | 0.15 (2) |
|iego  ATP | 2                 | 80              | State 3 | +DCCD | 3.0 | 1.75 |        |        |          |
| W303δβδ | 1                 | 87              | State 3 | +DCCD | 3.3 | 1.78 | 55 (2) | 2.3 (2) | 0.22 (2) |
|         | 2                 | 85              | State 3 | +DCCD | 3.5 | 1.75 |        |        |          |
| W303    | 1                 | 98              | State 3 | +DCCD | 3.0 | 1.71 | 51 (2) | 2.0 (2) | 0.16 (2) |
|         | 2                 | 95              | State 3 | +DCCD | 2.95 | 1.64 |        |        |          |
| W303    | 1                 | 79              | State 3 | +DCCD | 3.0 | 1.65 | 49 (2) | 2.0 (2) | 0.15 (2) |
|         | 2                 | 97              | State 3 | +DCCD | 3.1 | 1.64 |        |        |          |

wild type strain. This is reflected in the respiratory control ratio (respiratory control ratio, state 3/state 4), which decreases from 3.5 for the wild type strain to about 2.0 for the strains with the heterozygous mutations in ATP3 or ATP6. This decrease in the respiratory control ratio indicates that the ATP synthase is partially uncoupled from respiration in the mutant strains.

The P/O ratio is a measure of the amount of ATP made/oxygen atom reduced during respiration. Typically, this value is about 2 for wild type mitochondria using succinate as the carbon source. A decrease in the P/O ratio is indicative of an uncoupling of the mitochondrial membrane. Mitochondria isolated from the wild type strain has a P/O ratio just under 2, whereas mitochondria isolated from the yeast with heterozygous mutations in either ATP3 or ATP6 is reduced to about 1.0. This result also indicates that the ATP synthase is partially uncoupled from respiration in the mutant strains.

The ΔΨ across the mitochondrial membrane was measured using the fluorescent probe, rhodamine 123 (25), whose fluorescence decreases in response to a decrease in the mitochondrial membrane. These results indicate that the ΔΨ response, and thus the ΔΨ across the membrane, is lower for the mitochondria isolated from the strains with the heterozygous mutations in ATP3 or ATP6, as compared with the wild type strain. Again, these results indicate that the mitochondrial membrane is partially uncoupled in the cells with the heterozygous mutation in either ATP3 or ATP6 as compared with the wild type.

The questions that were addressed by measuring the cellular phenotypes (Fig. 1 and Table II) were also addressed in the biochemical studies (Table III). The conclusions from the results of the biochemical measurements are consistent with those from the measurements of the cellular phenotypes. 1) The mitochondria isolated from cells with a heterozygous mutation in either ATP3 or ATP6 are impaired because they are partially uncoupled. This conclusion is based on the decreases in the respiratory control ratio, P/O ratio, and the ΔΨ across the membrane. 2) The decrease in the copy number of ATP3 or ATP6 from 2 to 1 is responsible for the partial uncoupling of the mitochondria. This is clear because when either the ATP3 or ATP6 gene is integrated at a separate location on the corresponding mutant cell chromosome, the coupling capacity of the mitochondria isolated from the cells is normal. 3) The atp2δ mutation is epistatic to the atp3δ and atp3δ mutations. This is concluded because the mitochondria isolated from the atp2δ/ atp3δ or the atp2δ/atp3δ mutant cells have a normal coupling capacity. Thus, the conclusions derived from the analysis of the biochemical phenotypes are consistent with those derived from the analysis of the growth and cellular phenotypes, and all are consistent with the initial hypothesis.

Lastly, the level of the ATP synthase was measured by determining the level of the oligomycin-sensitive ATPase activity. The relationship of the level of the oligomycin-sensitive ATPase in the individual strains and the mutations in the strain paralleled the prior results: 1) the level was decreased in the mitochondria from cells that had a heterozygous mutation in ATP3 or ATP6, 2) the decrease was a result of the decrease in the gene number from 2 to 1, and 3) the atp2δ heterozygous mutation was epistatic to the atp3δ and atp3δ mutations.
The synthase complex that was devoid of the growth phenotypes were because of the assembly of an ATP to explain the epistatic effect and the growth defects of the

These enzyme complexes were proposed to be defective and provides biochemical and additional genetic evidence that sup-

In the diploid strain, the deletion of a single copy of either ATP3 or ATP6 (going from 2 to 1) would be expected to decrease their expression thereby reducing the level of these subunits in the cell (Fig. 2B). In turn, this decrease in expression of these subunits would be expected to result in an increase in the level of the subunit-deficient enzyme complex, which is thought to be responsible for the partial uncoupling of the mitochondrial membrane. This model would explain the semi-dominant negative effect of the heterozygous mutation in either ATP3 or ATP6.

The epistatic effect of the heterozygous mutation in ATP2 is also explained by this model. The heterozygous atp2Δ or atp6Δ mutant cells were phenotypically like the heterozygous atp2Δ mutant cells. This implies that the cells with a single mutation in ATP3 or ATP6 resulted in a gain of activity, because when paired with the atp2Δ mutation, the negative effects of the mutation are lost. This gain of activity is thought to be the increased proton permeability
across the mitochondrial membrane because of the γ- and δ-less enzyme complexes (Fig. 2A). The question remains, however, how does a heterozygous mutation in ATP2 prevent the formation of the subunit-deficient enzymes? One possible explanation is that the level of the β-subunit is decreased in the mutant cells with a heterozygous atp2Δ mutation. Because the γ- and δ-deficient enzymes require the β-subunit for their assembly, the decrease in the level of the β-subunit as a result of the atp2Δ mutation may be sufficient to decrease the level of the γ- and δ-deficient enzyme complexes to a level that is not seen as a negative phenotype.

The model also explains the epistatic effect of the heterozygous atp2Δ mutation on the level of the mitochondrial ATPase (Table III) in strains with mutations in ATP3 and ATPδ. Specifically, the model suggests the subunit-deficient enzyme is formed in a diploid strain with a heterozygous mutation in ATPδ, which is then rapidly degraded so as to leave just a fraction of the level at steady state. This would have the effect of reducing the level of the holo-enzyme because of the formation and subsequent degradation of the subunit-deficient enzyme. However, when paired with a heterozygous mutation in ATP2, the rate of formation of the subunit-deficient enzyme complexes is also decreased, allowing for the synthesis of the more stable holo-enzyme.

The results in this and the prior study (9) have profound implications for the mammalian cell. If the γ- and δ-less subunit complexes also form in the mammalian cell, possibly in the normal or disease state, then the level of the γ- or δ-less ATP synthase may be high enough to partially uncouple the mitochondria. This effect could have a large impact on the metabolism of the cell and the physiology of the animal.

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