The ATP synthase is under a number of mechanisms of regulation. The chloroplast ATPase has a unique mode of regulation in which activity is controlled by the redox state in the organelle. This mode of regulation is determined by a small unique region within the γ-subunit and this region contains two cysteine residues. Introduction of this region within the yeast γ-subunit causes a defect in oxidative phosphorylation. Oxidative phosphorylation is restored if the cysteine residues are replaced with serine. Biochemical analysis of the chimeric mitochondrial ATPase indicates that the ATP synthase is not largely altered with the cysteine residues in either the oxidized or reduced states. However, the level and activity of cytochrome c oxidase are decreased by about 90%, whereas that of NADH dehydrogenase and cytochrome c reductase are unchanged as compared with the wild-type enzymes. The level and activity of cytochrome c oxidase are restored with replacement of the cysteine residues with serine in the regulatory region. These results indicate that the chimeric ATP synthase containing cysteine, but not serine, decreases the expression or assembly of cytochrome c oxidase with little effect on the activity of the ATP synthase.

The mitochondrial ATP synthase (560,000 Da) is composed of the F₁-ATPase, which contains the catalytic sites and F₀, the proton pore. The F₁-ATPase is comprised of α₁β₁γδε (360,000 Da) with the three catalytic sites formed by the αβ pairs (1). The γδε-subunits form the central stalk and are located within the core formed by the three pairs of αβ-subunits (2, 3). F₀, is minimally composed of abc₁₀ and acts as a proton turbine whose rotation is coupled to the central stalk. A peripheral stalk formed by subunits b, d, h, and subunit 5, links the α-subunit to the periphery of the α/β core and acts as a stator, preventing the core from following rotation of the central stalk (4–6).

The ATP synthase is subject to regulation by a number of different means. In eukaryotes, the mitochondrial inhibitor protein inhibits hydrolysis of ATP to presumably prevent the wasteful loss of ATP under conditions where ATP is not being made (7). The inhibitor protein is not present in bacteria, but analogous regulation is thought to be effected by the C-terminal of the ε-subunit (8–10). The bacterial ε-subunit is homologous to the mitochondrial δ-subunit. The γ-subunit of the chloroplast ATPase contains a unique disulfide bond that inhibits ATP hydrolysis by CFI, but in the reduced form, is non-inhibitory (11). This regulatory region thus acts as a redox sensor to inhibit the chloroplast enzyme under conditions where ATP is not being made and may act in concert with the inhibitory action of the C-terminal domain of the ε-subunit (12).

Two cysteine residues, Cys¹⁹⁹ and Cys²⁰⁵, in the chloroplast γ-subunit are responsible for the redox regulation of the chloroplast ATP synthase (11). The region containing Cys¹⁹⁹ and Cys²⁰⁵ is unique to the chloroplast γ-subunit. However, introduction of this region into the bacterial enzyme has been shown to convey the redox regulation to the bacterial enzyme (13).

There are five enzyme complexes that participate in oxidative phosphorylation: complexes I, II, III, IV, and V corresponding to NADH dehydrogenase, succinate dehydrogenase, cytochrome c reductase, cytochrome c oxidase, and the ATP synthase, respectively. In yeast, supercomplexes have been identified that are composed of Complexes III and IV (14, 15). In mammalian cells, almost all of Complex I is in a supercomplex with Complexes III and IV. Furthermore, the ATP synthase has been reported to be in a supercomplex with the phosphate carrier and ATP/ADP translocase (14–18). These supercomplexes are thought to add an additional level of efficiency to the reaction pathway. In addition, in yeast and mammalian cells, the ATP synthase forms a dimer (14), and in yeast, it is at least partly responsible for formation of cristae (19). The ATP synthase dimer is mediated by subunit g of the ATP synthase. Deletion of subunit g eliminates the dimer form of the ATP synthase and also eliminates the cristae in the yeast mitochondrion suggesting a role of the ATP synthase in the formation of cristae (19). Deletion of subunit g also has an effect on the activity of cytochrome c oxidase suggesting the importance of the dimer form of the ATP synthase in expression or stability of cytochrome c oxidase (20).

We have investigated the structure function relationship of the redox regulatory region of the chloroplast ATP synthase by making chimeric constructs of the yeast and chloroplast γ-subunit. By use of homologous recombination in yeast, five chimeric constructs have been made to test the capacity of regions within the chloroplast γ-subunit to confer redox regulation to the yeast ATPase. The chimeric construct containing the entire
Chimeric Yeast/Chloroplast ATP Synthase

putative regulatory region of the chloroplast γ-subunit, when inserted in the yeast γ-subunit, inactivated oxidative phosphorylation in the yeast, as judged by biochemical assays and the ability of the yeast to grow on a non-fermentable carbon source. However, the defect was due to the nearly complete loss of cytochrome c oxidase activity, with only mild alterations in the ATP synthase or in the other complexes of the electron transport chain. The results of this study provide new structure/function information on the chloroplast regulatory region and suggest that the ATP synthase is either directly or indirectly related to the function of cytochrome c oxidase.

MATERIALS AND METHODS

Yeast Strains and Media—Yeast Saccharomyces cerevisiae yeast strain W303-1B (MAT α, ade2, his3, leu2–3, trp1, ura3) (A gift from Dr. Alex Tzagoloff, Columbia University, NY) is the parent strain of those generated in this study. Supplemental Table S1 shows a list of the yeast strains used in this study. Yeast was grown at 30 °C in YPAD (1% yeast extract, 2% peptone, 2% glucose, 30 mg/liter adenine), YPGal (1% yeast extract, 2% peptone, 2% galactose), YPG (1% yeast extract, 2% peptone, 3% glucose, 30 mg/liter adenine), and YPGal (1% yeast extract, 2% peptone, 3% glucose supplemented with amino acids at 20 mg/liter). Yeast transformation was performed by the LiAc method as described (21).

Construction of Yeast/Chloroplast Chimeric γ-Subunits—Yeast/chloroplast chimeric γ-subunits were made using gap repair (22). The region of the gene coding for the regulatory region of the chloroplast γ-subunit was amplified by polymerase chain reaction (PCR). The 5’ ends of the PCR primers contained 30 bases that corresponded to the target point for homologous recombination. The PCR products were co-transformed with a linear plasmid DNA (pYATP3NcoI) containing yeast ATP3 into W303-1A (23). The plasmid DNA was made linear by making a single restriction cut within ATP3. Upon transformation into yeast, homologous recombination occurs between the PCR product and the corresponding linear plasmid. This recombination event replaced the corresponding domain in ATP3 with the regulatory region from chloroplast enzyme and circularized the plasmid DNA. The recombinants were selected by growth of cells on minimum media devoid of tryptophan. The plasmids were extracted from the transformants and transformed into Escherichia coli XL1-Blue by electroporation as detailed in the instrument manual (Bio-Rad). The plasmid DNA was purified from E. coli and sequenced (University of Chicago Cancer Research DNA Sequencing Facility). Supplemental Table S2 shows a list of plasmids used in this study.

Expression and Purification of Chimeric F1-ATPase and F1F0-ATPase—The plasmids encoding wild-type and different chimeric γ subunits (pYATP3, pYCATP3(II), pYCATP3(I+II), pYCATP3(II+III), pYCATP3(I+II+III), and pYCATP3(I+II+III, C25) (supplemental Table S2) were co-transformed with pYATP2 into HSY201 to generate strains expressing chimeric ATP synthase. F1-ATPase was purified using nickel-affinity chromatography and size exclusion chromatography as described (24). The enzyme was stored at 4 °C as a precipitate in 70% saturated ammonium sulfate. F1F0-ATP synthase was purified using nickel-affinity chromatography and size exclusion chromatography. Mitochondria were purified and converted to submitochondrial particles by washing with SB buffer (0.25 M sucrose, 0.05 M phosphate, 5 mM ε-amino caproic acid, 5 mM benzenamide, and 1 mM EDTA, pH 7.5). The submitochondrial particle (8.5 mg/ml) were extracted with 1% n-dodecyl-β-D-maltoside and the solution was centrifuged at 158,000 g at 4 °C. The extract was loaded at 2 ml/min onto a nickel-Sepharose column (GE Healthcare) equilibrated with Buffer A10 (10% glycerol, 0.3 M NaCl, 0.05 M phosphate, 5 mM ε-amino-acaproyc acid, 5 mM benzamidine, 1 mM phenylmethyl-sulfonyl fluoride, 30 mM imidazole, and 0.01% n-dodecyl-β-D-maltoside, pH 7.5). The column was washed with 20 ml of Buffer A10 and eluted with Buffer B10 (same buffer composition as A10 with 300 mM imidazole). The peak fractions were collected and precipitated with 8% polyethylene glycol 6000 and stored at 4 °C. The precipitated protein was resuspended with S300 buffer (20 mM Tris, 50 mM sucrose, 2 mM MgSO4, 1 mM EDTA, 10% glycerol, 0.1% n-dodecyl-β-D-maltoside, 0.1 M NaCl, 5 mM ε-amino-acaproyc acid, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, and 1 mM ATP, pH 8.0) and loaded onto a Superose-12 column (GE Healthcare) at 0.5 ml/min. The ATP synthase eluted at 20 min. Peak fractions were analyzed by SDS-polyacrylamide gel electrophoresis (25) and the fractions containing non-proteolyzed F1F0-ATPase were pooled and stored at 4 °C.

The concentration of the soluble F1-ATPase was determined by the Bradford (26) method. The concentration of F1F0-ATP synthase was determined by densitometry of the Coomassie Blue staining intensity of the α- and β-subunits after separation by SDS-gel electrophoresis using purified F1-ATPase as a standard.

Biochemical Studies—Mitochondria were isolated from yeast strains as described (27). The concentration of mitochondrial protein was determined using the BCA protein assay (26). Western blot analysis (28) was performed using rabbit antibodies directed against yeast Cox2p, Cox3p, cytochrome b, Rieske FeS protein (Gifts from Dr. Rosemary Stuart, Marquette University, Milwaukee, WI), and chicken antibodies against α- and β-subunits of yeast F1.

Mitochondrial respiratory enzyme activities (NADH dehydrogenase, cytochrome c reductase, and cytochrome c oxidase) were measured as described (29–31). ATPase activity was determined by the coupled enzyme reaction (27) at 30 °C. As indicated, the samples were incubated with 10 mM dithiothreitol (DTT)3 or 100 μM CuCl2 for 30 min before the assay.

ATPase activity of F1F0-ATP synthase was measured after reconstituting the enzyme into liposomes as described (32) with some modifications. α-Phosphatidylcholine (60 mg) was suspended in 2 ml of REV buffer (50 mM KCl, 0.2 mM EDTA, 20 mM TrisSO4, pH 8.0). The lipid dispersion was placed in a 10-ml glass tube and sonicated for 3 min at 10 °C (model GI12SP1G, Laboratory Supplies Co., Hixson, NY). Reconstitution of the ATP synthase in liposomes was performed by mixing 0.3 ml of the liposomes (30 mg/ml), 0.15 ml of REV buffer, and F1F0 (90

3 The abbreviation used is: DTT, dithiothreitol.
Chimeric Yeast/Chloroplast ATP Synthase

The primary structure comparison between yeast and chloroplast γ-subunit is shown in Fig. 1A. Overall, all the subunits share 34% identical amino acids and 46% homology. This is similar to the percent identity and homology between γ-subunits from yeast and bovine (42 and 54%, respectively). The central region, residues 174–232 in the chloroplast γ-subunit, contains regions that are distinct from those in the yeast γ-subunit and within that region reside the cysteine residues required for redox regulation, Cys199 and Cys203. This is the only region identified by analysis of the primary sequence that obviously differed between the yeast and chloroplast γ-subunit, suggesting that no other regions are required for redox regulation. The putative redox regulatory region was divided into three regions, I, II, and III, colored in blue and red, respectively. These regions are highlighted in the model of the structure of the yeast F1–ATPase with the corresponding colors for regions I and III (blue and red, Fig. 1, B and C). Region II (green) does not have any corresponding residues in the yeast γ-subunit and is thus not present in the model. To test the importance of each of these regions in the redox regulation, four chimeric constructs were made in which the regions of the chloroplast γ-subunit were inserted at the corresponding regions of the yeast γ-subunit. These four chimeric constructs correspond to insertion or replacement of regions: (II), (I, II), (II, III), and (I, II, III).

The four constructs were made in yeast using homologous recombination, which allowed the precise insertion of these regions within the gene. The chimeric gene constructs were transformed into a yeast strain devoid of the genes encoding the γ-subunit (ATP3) and that encoding the β-subunit (ATP2). The resulting strains were transformed with the gene encoding a His6 tagged derivative of the ATP2 gene. The atp2Δ mutation was necessary because yeast with an atp3Δ mutation rapidly become petite (loss or deletion of the mitochondrial DNA), which prevents biochemical analysis of the ATP synthase.
Chimeric Yeast/Chloroplast ATP Synthase

However, the percentage of petites formed is dramatically reduced in the \( atp^{2-}, atp^{3-} \) double mutant strain and low in the \( atp^{2-} \) mutant strain (33). The His\(_6\) derivative of ATP2 also allows rapid purification of the F\(_1\)-ATPase or the F\(_1\)F\(_0\)-ATPase for biochemical studies. Supplemental Table S1 shows a list of all of the strains used in this study.

Fig. 2 shows the growth of the yeast cells on complete medium containing glycerol as the carbon source (YPG) or minimal medium with glucose as the carbon source (synthetic media). Cells defective in oxidative phosphorylation are unable to grow on YPG medium, but can grow on medium with glycerol as a carbon source. The results indicate that insertion of region (II) alone did not alter the cells’ ability for growth on YPG medium, whereas insertion of regions (I, II) had a modest negative affect, insertion of regions (II, III) had a moderate affect, and insertion of regions (I, II, III) eliminated the ability of the cell to grow on YPG medium. To determine whether the Cys residues in region II were responsible for the defect in chimera (I, II, III), these residues were mutated to Ser and tested in the same manner. Replacement of Cys with Ser in the largest chimeric construct (I, II, III) (C225S,C231S) restored the ability of the cell to grow on YPG. This result indicates that the insertion of regions (I, II, III), but not any of the smaller inserts, largely altered by the insertion of the chloroplast regulatory region (not shown).

The ATPase activity was measured for the wild-type and mutant mitochondrial F\(_1\)-ATPase in the isolated mitochondria, the chloroform released enzyme, purified F\(_1\), and in some cases, the F\(_1\)F\(_0\)-ATP synthase (Table 1). The results indicate that the total activity was reduced by about 60% for the yeast strain containing (I, II, III), with a corresponding decrease in oligomycin sensitivity. The F\(_1\)-ATPase is selectively released with chloroform extraction and releases only assembled enzyme (34). The total activity of the chloroform-released enzyme from chimera (I, II, III) mirrored the activity of the isolated mitochondrial ATP synthase. It was about twice that for the (I, II, III) mutant strain. This was consistent with the results of the growth studies supporting the conclusion that the ATPase activity is from the F\(_1\)-ATPase. In contrast, the level of the ATPase activity from the mitochondria isolated from the remaining mutant strains, and the corresponding chloroform-released enzyme, were not decreased by more than 25% compared with the wild-type strain. The mitochondrial enzyme showed a corresponding small decrease in oligomycin sensitivity. Surprisingly, in comparison to the wild-type enzyme, oxidation with CuCl\(_2\) did not largely inhibit the ATPase activity relative to reduction with DTT. Separate experiments using purified F\(_1\)-ATPase from the HSY205 indicated that disulfide bond formation occurred readily between the cysteines in the regulatory region (not shown).

The F\(_1\) and F\(_1\)F\(_0\)-ATPase were purified from the strains to determine whether the specific activities of the enzymes were altered with the insertion of the chloroplast regions (Table 1). The ATPase activity from the F\(_1\) purified from the chimera strain (I, II, III) did not decrease by more than 20% as compared with the wild-type enzyme. The specific ATPase activity of F\(_1\)F\(_0\) isolated from chimera (I, II, III) was not lower than that of either the wild-type or the C2S chimera strain (HSY204C2S). The largest alteration was in the \( K_m \) for ATP of the chimeric F\(_1\)F\(_0\)-ATP synthase. It was about twice that for the (I, II, III) mutant enzyme (105 \( \mu \)M) as compared with that of the wild-type strain (59 \( \mu \)M) (when measured under oxidizing conditions) and this difference was eliminated after reduction with DTT. Thus, based on these studies, the activities of F\(_1\) and F\(_1\)F\(_0\) were not largely altered by the insertion of the chloroplast regulatory region (I, II, III).

Selective Decrease in Cytochrome c Oxidase Activity—Prior studies have indicated that the activity of the ATP synthase must be decreased by more than 85% before there is a negative growth phenotype on YPG medium (35). The biochemical studies of the chimeric yeast/chloroplast enzyme (I, II, III) indicated that the activity of this enzyme was not nearly sufficiently inhibited to be responsible for the negative growth phenotype. Thus, studies were performed to determine whether there were defects in any of the enzymes of the electron transport chain, which may be responsible for the inability of the yeast to grow on medium containing a nonfermentable carbon source. Specific activities were determined for NADH dehydrogenase, cytochrome c reductase, and cytochrome c oxidase (Table 2).

### TABLE 1

ATPase activities of the chimeric enzymes

| Strain | Region | Mito % Oligo | CHCl3 | F1 | F1F0 | K_m |
|---------|--------|--------------|-------|----|------|------|
| WT | NA | 1.1 (25%) | 95 | 10.7 (18%) | 115 | 8.0 | 59 |
| HSY205 | I, II | 0.78 (11%) | 88 | 12.1 (25%) | 83 | ND | ND |
| HSY203 | I, II | 0.72 (3%) | 90 | 12.8 (7%) | 87 | ND | ND |
| HSY204 | I, II, III | 0.39 (35%) | 56 | 4.5 (21%) | 92 | 13.8 | 67 (105) |
| HSY206 | I, II, III | 0.72 (31%) | 67 | 6.5 (17%) | 63 | ND | ND |
| HSY204C2S | I, II, III | 0.77 | 87 | 10.6 | 78 | 12.9 | ND |

FIGURE 2. Growth phenotype of the yeast strains with the chimeric constructs. The growth of the cells is shown on solid plates containing minimal medium (synthetic media, SD) and complete medium containing glycerol as the carbon source (YPG). The cells were added to water at three dilutions, as indicated, and grown at 30 °C. The negative control is HSY201 transformed with vectors pRS304 and pRS306.
TABLE 2
Activity measurements of respiratory enzyme complexes

| Strain     | NADH dehydrogenase (μmol/min/mg of protein) | Cytochrome c reductase (μmol of Fe(CN)$_6$3-/min/mg of protein) | Cytochrome c oxidase (nmol of O$_2$/min/mg of protein) |
|------------|---------------------------------------------|---------------------------------------------------------------|------------------------------------------------------|
| Wild-type  | 0.40                                        | 1.8                                                           | 2903                                                 |
| HSY204     | 0.41                                        | 1.2                                                           | 169                                                  |
| HSY204C2S  | 0.58                                        | 2.4                                                           | 1873                                                 |

Figure 3. Selective loss of cytochrome c oxidase. A, difference absorbance spectra (reduced minus oxidized) of detergent-dissolved mitochondrial proteins isolated from the strains and antibodies, as indicated, as described under “Materials and Methods.”

The results indicate that cytochrome c oxidase activity is decreased by nearly 95% in the HSY204 strain (I, II, III), but activity is decreased by just 35% when the Cys$_{225}$ and Cys$_{231}$ are replaced with Ser (HSY204C2S).

Studies were performed to determine whether the decrease in cytochrome c oxidase activity was due to an inhibition of the activity leaving an assembled and stable complex or if the cytochrome c oxidase is absent from the mitochondria. Fig. 3A shows difference spectra of mitochondria isolated from the corresponding wild-type and mutant strains. The spectra indicate that heme aa$_3$ is largely reduced in the HSY204 strain containing the regulatory region (I, II, III), at near normal levels in the HSY204C2S strain. In contrast, cytochromes b, c, and c$_1$ were at normal levels in the HSY204 strain (I, II, III). Fig. 3B shows the Western blot analysis used to determine whether the subunits of cytochrome c oxidase are present in the HSY204 strain (I, II, III). The results indicate that Cox2p and Cox3p were both decreased but the percentage decrease was not consistent with the observed decrease in cytochrome c oxidase activity. The Western blot also demonstrates that the level of the α- and β-subunits of the ATPase are decreased in the HSY204 strain (I, II, III), but not by more than about 50%. Analysis of the level of heme and activity of cytochrome c oxidase were measured from mitochondria isolated from strains HSY203, HSY205, and HSY206, and the levels were consistent with their growth phenotype on YPG medium (supplemental Fig. 2S).

Association of Cytochrome c Oxidase with the ATP Synthase—

The results suggest that the ATP synthase is either directly or indirectly associated with cytochrome c oxidase. To determine whether there is a direct association of cytochrome c oxidase with the ATP synthase, the ATP synthase was partially purified by nickel-affinity chromatography (using the His$_6$ tag on the β-subunit) and analyzed for copurification of cytochrome c oxidase (Fig. 4). The results indicate that Cox2p and Cox3p of cytochrome c oxidase co-purified with the ATP synthase suggesting a direct interaction of cytochrome c oxidase with the ATP synthase. The control indicated that cytochrome c oxidase did not bind to the nickel-Sepharose column in the absence of the His$_6$ tag on the ATP synthase. However, the association of ATP synthase and cytochrome c oxidase is not strong, as it is not observed when the chloroplast membrane is treated with 1% dodecyl-β-D-maltoside (data not shown).

Model of the Chloroplast Regulatory Loop—

The conformation of region (I, II, III) within the context of the yeast γ-subunit and F$_{1}$-ATPase was homology modeled using Molecular Operating Environment (MOE 2007.09, Chemical Computing Group, Inc., Montreal, Canada). In addition, a loop in the γ-subunit (residues 60–70) was modeled with MOE because this region is missing in the yeast model and the corresponding residues are also missing in the bovine model. The resulting model shown in Fig. 5 provides a framework within which to interpret the results. The regulatory region is on the face opposite the δ- and ε-subunits. The model shows that the regulatory region is capable of interacting with β$_{γ}$, β$_{γ}$p, and α$_{γ}$p. The model indicates that potentially, the regulatory region can interact with the catalytic and non-catalytic subunits of the ATPase and has the potential to insert into the interface between β$_{γ}$ and α$_{γ}$p. The model places the two regulatory Cys residues at the end of a loop. This loop structure is similar to the redox loop in the periplasmic domain of the transmembrane electron transporter, DsbD (Protein Data Bank 1JPE) (36). Like the chloroplast regulatory region, this redox loop undergoes oxidation/reduction of two cysteine residues, which are spaced...
similarly (residues 103 and 109 in DsbD). This model thus provides an explanation as to how this region might interact with the \( \gamma \)-subunit of the bovine ATPase (39). The model and mechanism proposed here differ considerably from an earlier model (12, 40). The prior model of the chloroplast regulatory region places it at the bottom of F1, far from the catalytic interface formed by \( \alpha_T \) and \( \beta_T \). Thus, a second possible mechanism is that the regulatory loop moves into the interface and prevents the proper conformational changes during the catalytic cycle. This mechanism is similar to that proposed for the inhibitor protein that has been shown to bind to the catalytic \( \beta_L \) interface of the bovine ATPase (39).

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interaction with the $F_{0}$ components and rotation of the central stalk making this location unlikely. Finally, there are no easy explanations as to how redox regulation could occur if this regulatory element was so distant from either the central stalk or the active site of the enzyme. For these reasons, we favor the model proposed here over the prior model.

The results of this study also provided the surprising finding that when introduced into yeast mitochondria, whereas the chloroplast regulatory region impairs oxidative phosphorylation, it impairs cytochrome $c$ oxidase but not the ATP synthase or Complex I or III of the electron transport chain (Tables 1 and 2). This suggests that the ATP synthase either directly or indirectly interacts with cytochrome $c$ oxidase. The interaction of the ATP synthase appears to be guided by interaction directly with the cysteine residues in the regulatory region, because serines at these positions have no effect. Alternatively, the oxidation of these sites might cause a conformational change that is required for the yet unidentified interaction. Although the conformational specific interaction seems unlikely, it is supported by the fact that the smaller chimeric constructs did not have that same effect on cytochrome $c$ oxidase despite the presence of the cysteine residues.

There have been other reports on the possible interaction of the ATP synthase with cytochrome $c$ oxidase. Deletion of subunit g of the yeast ATP synthase has been shown to both decrease the formation of the dimer form of the ATP synthase and reduce the level of cytochrome $c$ oxidase (20, 41, 42). In addition, electron paramagnetic resonance and differential scanning calorimetric studies have indicated a direct interaction between cytochrome $c$ oxidase and the ATP synthase (43). Thus, in conjunction with the results of this study, there is growing evidence that the ATP synthase interacts with cytochrome $c$ oxidase, possibly forming a supercomplex. However, the results in this study still do not exclude the possibility that the reduction in cytochrome $c$ oxidase is due to an interaction with a component involved in the assembly or synthesis of cytochrome $c$ oxidase. Current studies are underway to clarify this further.

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