Formation of the Yeast F₁F₀-ATP Synthase Dimeric Complex Does Not Require the ATPase Inhibitor Protein, Inh1*

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The yeast F₁F₀-ATP synthase forms dimeric complexes in the mitochondrial inner membrane and in a manner that is supported by the F₀-sector subunits, Su e and Su g. Furthermore, it has recently been demonstrated that the binding of the F₁F₀-ATPase natural inhibitor protein to purified bovine F₁-sectors can promote their dimerization in solution (Cabezón, E., Arechaga, I., Jonathan P., Butler, G., and Walker J. E. (2000) J. Biol. Chem. 275, 28353–28355). It was unclear until now whether the binding of the inhibitor protein to the F₁ domains contributes to the process of F₁F₀-ATP synthase dimerization in intact mitochondria. Here we have directly addressed the involvement of the yeast inhibitor protein, Inh1, and its known accessory proteins, Stf1 and Stf2, in the formation of the yeast F₁F₀-ATP synthase dimer. Using mitochondria isolated from su e null mutants deficient in Inh1, Stf1, and Stf2, we demonstrate that formation of the F₁F₀-ATP synthase dimers is not adversely affected by the absence of these proteins. Furthermore, we demonstrate that the F₁F₀-ATPase monomers present in su e null mutant mitochondria can be as effectively inhibited by Inh1, as its dimeric counterpart in wild-type mitochondria. We conclude that dimerization of the F₁F₀-ATP synthase complexes involves a physical interaction of the membrane-embedded F₀ sectors from two monomeric complexes and in a manner that is independent of inhibitory activity of the Inh1 and accessory proteins.

Mitochondria, eukaryotic organelles, produce energy in the form of adenosine 5'-triphosphate (ATP) in a process termed oxidative phosphorylation (for recent reviews, see Refs. 1–3). Under conditions of high proton motive force (ΔميرH<sup>+</sup>), the F₁F₀-ATP synthase catalyzes the formation of ATP from adenosine 5'-diphosphate (ADP) in a manner that is coupled to the transport of protons from the intermembrane space across the inner membrane to the matrix. A decrease in the ΔميرH<sup>+</sup> as a result of oxygen deprivation to the cell, or because of the presence of an uncoupler of oxidative phosphorylation can, however, cause a reversal of the action of the F₁F₀-ATP synthase, resulting in the hydrolysis of ATP to ADP and Pᵢ. This hydrolytic activity of the F₁F₀-ATP synthase is regulated directly by the natural inhibitor protein, termed IF₁ in bovine and Inh1 in yeast. Under conditions of low ΔميرH<sup>+</sup>, the IF₁ protein forms homodimers (its active state) and binds directly to the F₁-sector and by doing so promotes the inhibition of ATP hydrolysis and thereby preserves cellular ATP levels (4–7). Homodimerization of IF₁ is supported by a coiled-coil structural motif in the IF₁ protein and occurs under conditions of matrix acidification (4–7). IF₁ binds to the F₁-sector in a 1:1 stoichiometry (8). Using purified F₁-sectors, the active IF₁ dimer has been shown to be able to bind to two separate F₁ complexes concomitantly, thereby promoting their dimerization (6, 7). Although the IF₁ protein can promote dimerization of F₁ complexes in solution, it was not apparent until now whether the inhibitor protein may play a direct role in the formation of dimers of the membrane-bound F₁F₀-ATP synthase complex in mitochondria.

It has been recently demonstrated that the yeast mitochondrial F₁F₀-ATP synthase forms dimeric complexes in the inner membrane (9, 10). The dimeric ATP synthase complex was identified following mild detergent (digitonin) solubilization of mitochondrial membranes, followed by either size exclusion chromatography or by blue-native polyacrylamide gel electrophoresis (BN-PAGE)1 (9, 10). The proximity between two F₁F₀-ATP synthase complexes was also independently shown by the ability to form a disulfide bridge between two subunits 4 proteins from neighboring ATP synthase complexes (11). Analysis of the subunit composition of the dimeric and monomeric complexes following BN-PAGE, by high-resolution two-dimensional gel electrophoresis resulted in the identification of dimer-specific subunits e and g (Su e and Su g, respectively) (10). Although not essential for the enzyme activity of the complex, Su e and Su g were shown to play an important role in the formation of the dimeric state of the F₁F₀-ATP synthase (10). BN-PAGE analysis indicated that formation of the dimeric form of the F₁F₀-ATP synthase complex was affected in mitochondria isolated from su e and Su g yeast null mutants (Δميرe and Δميرg, respectively) (10). The dimeric state of the ATP synthase is present also in both bovine and human mitochondria, indicating this assembly state of the complex is not unique to yeast mitochondria (12, 13). Consistently, subunits Su e and Su g, required for the formation of the dimeric ATP synthase, are conserved throughout evolution, present in both fungal and mammalian mitochondria.

As integral inner membrane proteins, Su e and Su g are both subunits of the F₀-sector. The dimerization of the F₁F₀-ATP synthase complexes was thus proposed to involve a physical interaction of two membrane-embedded F₀ sectors from two monomeric F₁F₀-ATP synthase complexes (10). The recent ob-

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‡ The abbreviations used are: BN-PAGE, blue-native polyacrylamide gel electrophoresis; STF, stabilizing factor; SFB, stabilizing factor 2-like protein; MCS, multiple cloning site; CCCP, carbonyl cyanide p-chlorophenylhydrazone.
dimerization by centrifugation, washed, and then disrupted with glass beads, and the resulting mitochondrial membrane. In this present study, we have directly addressed the role of the inhibitor protein, Inh1, in the formation of the F₁F₀-ATP synthase dimer in mitochondria isolated from the yeast Saccharomyces cerevisiae.

In yeast the inhibitory action of the Inh1 protein is enhanced by two stabilizing proteins, which are termed Stf1 and Stf2 (STF = stabilizing factors) (14–19). Stf1 shares sequence similarity with Inh1 (51% identity) and like Inh1, displays the potential to form a coiled-coil structure. The amino acid sequence of Stf2 on the other hand is unrelated to the Inh1/Stf1 proteins and does not contain a predicted coiled-coil motif. A data base search, however, indicated the presence of a homolog of Stf2 in yeast, which is encoded by the gene YLR327c. The function of the YLR327c gene product is unknown to date. The predicted protein encoded by this open reading frame is 86 amino acid residues long (Stf2 is 84 residues long) and is referred to here as Stf2, stabilizing factor 2-like protein 2. The amino acid sequence of Stf2 displays 65% identity and 84% similarity with that of the Stf2 protein.

Here we have addressed the role of Inh1 and its accessory proteins Stf1 and Stf2 in the formation of the dimeric F₁F₀-ATP synthase complex. We demonstrate here that the assembly of the mitochondrial F₁F₀-ATP synthase complex is primarily supported through membrane-embedded F₀-sector subunits Su e and Su g, and in a manner that is independent of the Inh1 and accessory proteins. Finally, we demonstrate that both the steady state levels of Inh1 and accessory proteins, and their capacity to inhibit the ATP hydrolysis activity of the F₁F₀-ATP synthase complex under low ΔpH⁺ conditions, are not affected in the absence of the Inh1 protein.

We conclude therefore that dimerization of the mitochondrial F₁F₀-ATP synthase complex is not required for the inhibition of the ATPase activity of the complex by the Inh1 inhibitor and accessory proteins.

**Experimental Procedures**

**Yeast Strains and Growth Conditions**

Yeast strains used in this study were wild-type W303-1A (MATa, leu2, trp1, ura3, his3, ade2) (20) and the su e null mutant, Δsu e (W303-1A, leu2, trp1, ura3, ade2, TIM11::HIS3) (10). The single gene deletion yeast strains, Δinh1::KAN, Δstf1, Δstf2, were constructed in the W303-1A genetic background, as follows. The introduction of the kana-myacin resistance gene (KAN²) into the INH1, STF1, and STF2 loci was confirmed by PCR analysis of the isolated genomic DNA and using oligo-nucleotides, which primed upstream and downstream of the respective disrupted genes (results not shown).

**Construction of the Double Gene Null Strains,** Δinh1/Δstf1 and Δinh1/Δstf2

For the Δinh1/Δstf1 Strain, Δinh1::KAN/Δstf1::HIS3—The STF1 gene was deleted in the Δinh1::KAN yeast strain, as follows. The HIS3 gene was amplified from the pFA6a-HIS3MX6 plasmid (21) using the STF1-specific S1 and S2 primers (see above). The resulting PCR product was transformed into the Δinh1::KAN yeast strain and HIS3-positive transformants were selected. Correct integration of the HIS3 gene into the STF1 gene was verified by PCR analysis of the isolated genomic DNA, as described above.

For the Δstf1/Δstf2 Strain, Δstf1::HIS3/Δstf2::KAN—The STF2 gene was deleted in the Δstf1::KAN yeast strain, as follows. The HIS3 gene was amplified from the pFA6a-HIS3MX6 plasmid using the STF2-specific S1 and S2 primers (see above). The resulting PCR product was transformed into the Δstf1::KAN yeast strain and HIS3-positive transformants were selected. Correct integration of the HIS3 gene into the STF2 gene was verified by PCR analysis of the isolated genomic DNA, as described above. Mitochondria were isolated from the resulting yeast strains, which had been grown in YP-Gal medium (2%) supplemented with 0.5% lactate (22).

**Isolation of Mitochondria for BN-PAGE Analysis and ATPase Measurements**

The individual single null mutant and double null mutant strains were grown on YP-medium containing galactose. Yeast cells were harvested by centrifugation, washed, and then disrupted with glass beads, essentially as described previously (10). For BN-PAGE analysis the cells were disrupted in a sucrose, 6-aminohecanic acid buffer (250 mm sucrose, 5 mm 6-aminohecanic acid, and 10 mm Tris-HCl, pH 7.0). For the ATPase measurements, the cells were grown on YP-glycerol, 0.5% lactate medium and were disrupted in SH buffer (0.6 M sorbitol, 20 mm Hepes, pH 7.2). Following vortexing with the glass beads, the cell debris and glass beads were initially removed by low speed centrifugation. The mitochondrial membranes were then collected by centrifugation at 100,000 × g for 20 min at 4 °C and were stored at −80 °C in a sucrose-containing buffer.

**ATP Hydrolysis Measurements**

ATPase activity measurements at pH 6.0—Isolated mitochondria (100 µg of protein) were resuspended in 525 µl of ice-cold assay buffer.
BN-PAGE

BN-PAGE analysis of digitonin-solubilized mitochondrial membranes (3 g of digitonin/g of mitochondrial protein) was performed essentially as described previously (10, 13). The effect of acidic pH and a low proton motive force (i.e. inhibitor protein binding conditions) on the dimeric state of the ATP synthase, in comparison to that in energized mitochondria at pH 8.4, was assessed by BN-PAGE as follows: mitochondria (wild-type or Δinh1 e null mutant, as indicated) were incubated either with ice-cold, pH 6.0, buffer (0.2 mM NaCl, 3 mM MgCl₂, 20 mM Hepes, pH 6.0, 2 mM ATP) in the presence of antimycin A (11 μM) and CCCP (20 μM) or pH 8.4 buffer (0.2 mM NaCl, 3 mM MgCl₂, 20 mM Tris-HCl, pH 8.4, 2 mM ATP) for 30 s on ice. Mitochondria were then reisolated by centrifugation and were prepared for BN-PAGE following lysis in digitonin, as described above.

Miscellaneous

Protein determinations and SDS-PAGE were performed according to published methods (25, 26). The Western blot analysis and immune precipitation in digitonin, as described above.

RESULTS

Formation of the Dimeric F₁F₀-ATP Synthase Complex Does Not Require the Presence of Inh1 and Its Accessory Proteins Stf1 and Stf2

To investigate the possible involvement of acidic pH and accessory proteins Stf1 and Stf2 in the dimerization of the F₁F₀-ATP synthase, we constructed single gene knock-out mutants for Inh1 and Stf1 antisera (kind gift from Professor Tadao Hashimoto, Muroran Institute of Technology, Japan).

The ATP hydrolysis activity of the F₁F₀-ATP synthase at pH 8.4 in energized mitochondria was determined essentially as described above, with the following exceptions: the assay buffer used was 0.2 M KCl, 3 mM MgCl₂, 20 mM Tris-HCl, pH 8.4, and the mitochondrial membrane potential was not dissipated, as antimycin A and CCCP were omitted.

To exclude the possibility of functional redundancy between Inh1 and accessory proteins, the mutants were then incubated either with ice-cold, pH 6.0, buffer (0.2 mM NaCl, 3 mM MgCl₂, 20 mM Hepes, pH 6.0, 2 mM ATP) in the presence of antimycin A (11 μM) and CCCP (20 μM) or pH 8.4 buffer (0.2 mM NaCl, 3 mM MgCl₂, 20 mM Tris-HCl, pH 8.4, 2 mM ATP) for 30 s on ice. Mitochondria were then reisolated by centrifugation and were prepared for BN-PAGE following lysis in digitonin, as described above.

were also constructed. Using digitonin, the membrane protein complexes were solubilized from the mitochondria isolated from the Δinh1/Δstf1/Δstf2/Δsfl2 single gene deletion yeast strains and also from the Δinh1/Δstf1 and Δstf2/Δsfl2 mutant strains. Solubilization of membrane proteins was performed using a digitonin:protein ratio of 3.0 g/g. Samples were analyzed by BN-PAGE. The dimeric (V,dim) and monomeric (V,mon) forms of the ATP synthase are indicated.

Inh1 and Its Accessory Proteins Do Not Display an Interdependence with Su e for Their Stable Expression

We next addressed whether Su e and Inh1 together with its accessory proteins display an interdependence on each other for their stable expression (Fig. 3). Mitochondria were isolated from the four individual mutant strains, Δinh1, Δstf1, Δstf2, and Δsfl2, and were analyzed by SDS-PAGE and Western blotting, together with mitochondria isolated from the corresponding wild-type strain. The levels of Su e were analyzed in these mutant mitochondria and were compared with a control mitochondrial protein, Tim23. Deletion of the genes encoding Inh1 or its accessory proteins had no appreciable effect on the steady state levels of Su e (Fig. 3A).

To assess the potential functional redundancy between Inh1 and its homolog Stf1, and between Stf2 and its putative homolog Sfl2, the steady state levels of Su e were analyzed in the mitochondria isolated from the double null mutant strains, Δinh1/Δstf1 and Δstf2/Δsfl2, respectively. Western blot analysis using Su e-specific antisera confirmed that no significant alteration in the levels of Su e was observed in both the Δinh1/Δstf1 and Δstf2/Δsfl2 mitochondria, as compared with wild type (Fig. 3B).

We conclude that the presence of the Inh1 and accessory proteins does not appear to influence the steady state levels of...
the dimer-specific subunit, Su e. These results are consistent with the observation that the formation of the dimeric form of the ATP synthase does not appear to be adversely affected in the inh1 or accessory protein mutant mitochondria.

The influence of the dimer-specific subunit Su e on the stability of the Inh1 and Stf1 proteins was next investigated. Mitochondria were isolated from the \( H9004 \) su e null mutant yeast strain and analyzed by SDS-PAGE and Western blotting (Fig. 3C). The levels of Inh1 and Stf1 in the mutant mitochondria were analyzed using subunit-specific antisera. The presence of the Su e was observed not to be required for the stable expression of Inh1 or Stf1, as the levels of these proteins in the \( H9004 \) su e mitochondria were very similar to those in the wild-type control mitochondria. Note, the steady state levels of Stf2 and Sfl2 proteins in the \( H9004 \) su e mitochondria have not been determined, as we do not have specific antisera available for these proteins yet.

As the assembly of the dimeric form of the ATP synthase is defective in the \( \Delta su e \) mitochondria, we conclude that the stable expression of Inh1 and Stf1 does not require the presence of the assembled dimeric \( F_{1}F_{0} \)-ATP synthase. Taken together, the dimer-specific subunit Su e, and the inhibitor protein Inh1 and its accessory proteins do not display an interdependence on each other for their stable expression. These observations are consistent with those presented previously, where the formation of the dimeric ATP synthase, a process required for the stable expression of Su e, does not require the presence of the Inh1 or its accessory proteins, Stf1, Stf2, and its homolog, Sfl2.

Dimerization of the ATP Synthase Mediated by Su e, Is Not Required for the Inhibition of the ATPase Activity by Inh1—We next addressed whether formation of Su e-mediated \( F_{1}F_{0} \) dimers in yeast was necessary for the ability of the Inh1 and accessory proteins to effectively inhibit the ATP hydrolysis activity of the \( F_{1}F_{0} \)-ATPase complex under the adverse conditions of low proton motive force (\( \Delta \mu H^{+} \)). Mitochondria were isolated from the \( \Delta su e \) null mutant and also the \( \Delta inh1/\Delta stf1 \) null mutant strains, and ATP hydrolysis catalyzed by oligomycin-sensitive \( F_{1}F_{0} \)-ATPase was measured at pH 6.0, following dissipation of the mitochondrial membrane potential (Fig. 4). As previously reported (18, 19), conditions of low \( \Delta \mu H^{+} \), i.e. following the addition of an uncoupler such as CCCP, induces the ATP hydrolyzing activity of the \( F_{1}F_{0} \)-ATPase complex in mitochondria isolated from the inhibitor-deficient (\( \Delta inh1 \)) yeast cells (Fig. 4). Furthermore, consistent with previously published results (18, 19), the induction of ATP hydrolysis activity was more pronounced in the \( \Delta inh1/\Delta stf1 \)
mitochondria, where both Inh1 and Stf1 proteins are absent. In contrast, induction of ATP hydrolysis in this manner was not observed in wild-type mitochondria under these low ΔμH⁺ and matrix acidification conditions, because of the presence of the inhibitor protein, Inh1, which binds to and inhibits the F₁F₀-ATPase complex (Fig. 4). As was observed in wild-type mitochondria, the F₁F₀-ATPase hydrolysis activity was not induced in the Δsu e mitochondria, following dissipation of the membrane potential. To control the F₁F₀-ATPase complex was indeed active in our Δsu e and wild-type mitochondrial preparations, oligomycin-sensitive ATP hydrolysis activities were determined in energized mitochondria from each strain at a pH of 8.4. The results obtained indicated that the mitochondria isolated from the wild type, su e null and inh1/Δstf1 null mutant strains all had similar levels of oligomycin-sensitive F₁F₀-ATPase activity, ranging between 890 and 986 nmol of Fu/min/mg of protein.

In summary, under conditions of low ΔμH⁺ and an acidic milieu, it appears that F₁F₀-ATPase monomers in the Δsu e mitochondria can be as effectively inhibited by Inh1 as the dimeric complex in the wild-type mitochondria. As shown earlier, the steady state levels of Inh1 and Stf1 proteins appeared to be very similar between the Δsu e mitochondria and the wild-type mitochondria. Thus, the observed efficient inhibition of the monomeric ATP synthase in the Δsu e mitochondria by Inh1 does not appear to be because of a compensatory effect of overproduction of Inh1 relative to the wild type control.

Association of the Inh1 Protein with the Monomeric F₁F₀-ATP Synthase—The observed inhibition of the F₁F₀-ATPase activity in the Δsu e mitochondria under conditions of acidic pH and low proton motive force, would indicate the ability of the Inh1 protein to effectively bind and inhibit the F₁F₀-ATP synthase monomer. The inhibitory action of the Inh1 protein in the Δsu e mitochondria does not appear to promote the stable dimerization of the monomeric F₁F₀-ATP synthase in the absence of subunit e, however (Fig. 5A). Preincubation of Δsu e mitochondria at pH 6.0 combined with dissipation of the membrane potential with CCCP and antimycin A, i.e. conditions that promote Inh1 binding, did not support dimer formation in the Δsu e mitochondria (Fig. 5A). This result would suggest that the inhibitor protein can effectively inhibit the ATPase activity of the monomeric F₁F₀ complex without promoting its dimerization. Consistently, analysis of the subunit composition of the ATP synthase complexes from wild-type mitochondria indicated the presence of the inhibitor protein Inh1 protein associated with both the dimeric and monomeric forms of the F₁F₀-ATP synthase complex (Fig. 5B). We conclude therefore, that the binding of the Inh1 protein to the monomeric F₁F₀ ATP synthase can occur and that Inh1 binding does not automatically promote ATP synthase dimer formation in intact mitochondria.

**DISCUSSION**

We have reported previously that the yeast F₁F₀-ATP synthase can be isolated as a dimeric complex from the mitochondrial inner membrane (10). Isolation of the dimeric complex was achieved following detergent lysis of the mitochondrial membranes using low detergent to protein ratios (9, 10). The nonessential ATP synthase subunits, Su e and Su g, were shown to play a critical role in formation of a stable F₁F₀-ATP synthase dimer (10). As both of these subunits are integral inner membrane proteins, this led us to propose the model that the formation of the dimeric F₁F₀-ATP synthase required a direct interaction between membrane-embedded F₁ segments (10). Recently, however, the observation that solubilized bovine F₁ domains can dimerize upon binding of the natural inhibitor protein, IF1 (6, 7), has raised the question as to whether the dimerization of the intact F₁F₀ complex in the mitochondrial membrane system may be modulated by the binding and activity of IF₁.

In this present study we have directly addressed the role of Inh1, the yeast homolog of IF₁, in the formation of the dimeric ATP synthase in yeast mitochondria. We have also analyzed the possible roles of the Inh1 accessory proteins, Stf1 and Stf2, together with the putative Stf2 homolog, termed Stf2. On the basis of our observations we argue that in yeast mitochondria,
the presence of the Inh1 or accessory proteins do not play an essential role in the formation of the dimeric ATP synthase complex. First, disruption of the gene encoding Inh1 alone, or in combination with the gene encoding its homolog, Stf1, had no adverse affect on the stability or subunit composition of the ATP synthase dimer. Likewise, the formation and subunit composition of the ATP synthase was also unaffected in the single or double null mutants of the other Inh1 accessory protein, FIG.5.

**Fig. 5. Association of Inh1 with both dimeric and monomeric forms of the F$_1$F$_0$-ATP synthase.** A, mitochondria isolated from wild-type or the Δsu e mutant, were incubated either at pH 8.4 in the presence of an energized membrane (pH 8.4), or at pH 6.0 in the presence of antimycin A and CCCP (pH 6.0), as described under “Experimental Procedures.” Mitochondria were reisolated and were solubilized with digitonin and subjected to BN-PAGE analysis. B, the dimeric and monomeric forms of the F$_1$F$_0$-ATP synthase complex from wild-type mitochondria resolved by BN-PAGE were analyzed in a second dimension by a SDS-urea-PAGE and then Coomassie stained (upper panel). A duplicate gel was subjected to Western blotting and then was immune decorated with antibodies specific for the Inh1 protein (lower panel). The positions of the dimeric (V$_{Dim}$) and monomeric (V$_{Mon}$) forms of the ATP synthase following the BN-PAGE, and the presence of the Inh1 protein in both forms of the ATP synthase complex, are indicated. Note, the majority of the Inh1 protein was not associated with the ATP synthase, and was detected in the running front of the BN-PAGE gel.
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Sif2, and its putative homolog Sif2. Second, if binding of the IF₁/Inh1 protein to the F₁-sector did indeed promote dimerization of the F₁F₀-ATP synthase complexes one may anticipate the dimer form to be a dynamic structure in mitochondrial inner membrane. According to this model, the monomeric ATP synthase would be recruited into a dimeric complex upon the concomitant binding of an active IF₁/Inh1 dimer to two neighboring F₀ domains. We have used the technique of BN-PAGE here to analyze the ratio of dimeric to monomeric ATP synthase in isolated wild-type mitochondria under conditions of low ∆ΔH⁺ or acidic pH, i.e., conditions that should promote IF₁/Inh1 binding, and observed no difference, relative to control mitochondria. Furthermore, dimerization of the monomeric ATP synthase in Δ∆e mitochondrial was not observed following incubation under these conditions optimal for Inh1 binding and inhibition.

Taken together, our current data would support a model that formation of the dimeric ATP synthase is not a dynamic process, which occurs in response to the binding of the Inh1 protein. Although the addition of active IF₁ dimers to purified F₁-sectors could promote their dimerization in solution at the ratio of IF₁:F₁-sector used (6, 7), we have not observed Inh1-mediated dimerization of F₁ complexes in intact mitochondria. We show here that the Inh1 protein can be associated with both dimeric and monomeric forms of the F₁F₀-ATP synthase. On the basis of our observations reported here, we conclude that the binding of IF₁/Inh1 to F₁-sectors in intact mitochondria does not play an essential role in the formation of the F₁F₀-ATP synthase dimers. Rather, as previously proposed (10), dimerization entails the association of the membrane-embedded F₀-ATP synthase subunits, in particular Su e. Furthermore, we propose dimerization of the ATP synthase involves formation of Su e-Su e homodimers, between two neighboring F₀ complexes. Sequence analysis of known Su e proteins show they share a conserved coiled-coil motif, the basis often for homodimerization. Indeed, preliminary evidence for the dimerization of Su e in bovine mitochondria was presented earlier (27). In addition, we have recently been able to directly show that yeast Su e forms homodimers in the mitochondrial inner membrane.²

What is the function of the dimeric form of the F₁F₀-ATP synthase complex? Although required for the formation of the dimeric complex, Su e is not an essential subunit for the enzymatic activity of the ATP synthase complex (10). The amino acid sequence of Su e is strongly conserved throughout eukaryotes, suggesting an important function for this subunit, possibly in the regulation of the ATPase or ATP synthase activities of the enzyme. We considered it is plausible that dimerization of the F₁-sectors, mediated by Su e, may serve to keep two F₁-domains in close proximity of each other. A close spatial arrangement of one F₁-sector with another may support binding of the IF₁/Inh1 inhibitor protein, under conditions when the ATP hydrolysis activity of the enzyme requires regulation. Measurement of the ATPase activity under conditions of low ∆ΔH⁺ and matrix acidification indicated that hydrolysis activity was, however, not induced in the Δ∆e e null mutant mitochondria. Thus, the inhibition of ATP hydrolysis activity by Inh1 of the monomeric ATP synthase in the Δ∆e e mitochondria was as effective as that of the dimeric complex in wild-type mitochondria. Furthermore, the efficient inhibition of the ATP

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