In the Absence of the First Membrane-spanning Segment of Subunit 4(b), the Yeast ATP Synthase Is Functional but Does Not Dimerize or Oligomerize*

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The N-terminal portion of the mitochondrial b-subunit is anchored in the inner mitochondrial membrane by two hydrophobic segments. We investigated the role of the first membrane-spanning segment, which is absent in prokaryotic and chloroplastic enzymes. In the absence of the first membrane-spanning segment of the yeast subunit (subunit 4), a strong decrease in the amount of subunit g was found. The mutant ATP synthase did not dimerize or oligomerize, and mutant cells displayed anomalous mitochondrial morphologies with onion-like structures. This phenotype is similar to that of the null mutant in the ATP20 gene that encodes subunit g, a component involved in the dimerization/oligomerization of ATP synthase. Our data indicate that the first membrane-spanning segment of the mitochondrial b-subunit is not essential for the function of the enzyme since its removal did not directly alter the oxidative phosphorylation. It is proposed that the unique membrane-spanning segment of subunit g and the first membrane-spanning segment of subunit 4 interact, as shown by cross-linking experiments. We hypothesize that in eukaryotic cells the b-subunit has evolved to accommodate the interaction with the g-subunit, an associated ATP synthase component only present in the mitochondrial enzyme.

The F$_0$F$_1$-ATP synthase is a molecular rotary motor that is responsible for the aerobic synthesis of ATP. It exhibits a tripartite structure consisting of a headpiece (catalytic sector), basepiece (membrane sector), and two connecting stalks. The sector F$_1$ containing the headpiece is a water-soluble unit retaining the ability to hydrolyze ATP when in a soluble form. F$_0$ is embedded in the membrane and is mainly composed of hydrophobic subunits forming a specific proton conducting pathway. When the F$_1$ and F$_0$ sectors are coupled, the enzyme functions as a reversible H$^+$-transporting ATPase or ATP synthase (1–3). The enzyme displays two connecting stalks that are constituted of components from both F$_1$ and F$_0$. The first stalk is the rotor part of the enzyme. The second stalk is part of the stator which relays the catalytic domain F$_1$ and hydrophobic membraneous components of the enzyme. High resolution x-ray crystallographic data have been used to solve the structure of the F$_1$ (4–7) from different sources. Recently, Stock et al. (8) reported the 3.9-Å resolution x-ray diffraction structure of yeast F$_1$ associated with the c-ring oligomer, but a large part of the structure of F$_0$ remains unknown. In *Escherichia coli*, F$_0$ is composed of subunits a, b, and c. The mitochondrial F$_0$ of mammalian is composed of 10 different subunits (9). The same 10 components have been identified in the *Saccharomyces cerevisiae* enzyme (10). However, two of them (subunits e and g) have been classified as associated proteins since they are not essential in the structure or for the activity of the yeast complex (11–13). In addition, two new components named subunits i, j, and k have recently been identified in the yeast F$_0$ as associated proteins (14, 15). It has been reported that subunits e and g are involved in the dimerization of the yeast enzyme (12). We have recently reported that null mutants in either TIM11 or ATP20 genes encoding subunits e and g, respectively, led to anomalous mitochondrial morphology with proliferation of the inner mitochondrial membrane, in turn leading to onion-like structures enclosed inside a continuous envelope of outer mitochondrial membrane (16). It was hypothesized that ATP synthase itself participates in the mitochondrial morphogenesis through a oligomerization process mediated at least by subunits e, g, and 4.

Subunit 4, which is homologous to the mammalian b-subunit, is a major component of the stator. It shows interactions with other components of F$_0$ such as subunits d, F$_{00}$, OSCP, and g (17, 18). There is only one copy of subunit b per mitochondrial ATP synthase molecule (17, 19–21) whereas there are two in the prokaryotic and chloroplastic enzymes. Subunit 4 is also able to dimerize with another subunit 4 belonging to another complex (20). Unlike the prokaryotic and chloroplastic b-subunits which have only one membrane-spanning segment in their N-terminal part, the mitochondrial b-subunit has two membrane-spanning segments. In a previous work, we have shown that the N-terminal part of subunit 4 is close to subunit g (18). The purpose of the present work was to investigate the role of the first additional 43 amino acid residues bearing the first membrane-spanning segment of the mitochondrial b-subunit, and the involvement of the latter in the interaction with subunit g. We now show that the first membrane-spanning segment of the b-subunit is essential for the ATP synthase dimerization/oligomerization but is not essential for the structure and function of the yeast ATP synthase.

* This work was supported in part by the Center National de la Recherche Scientifique, the Ministère de la Recherche et de l’Enseignement Supérieur, the Université Victor Segalen, Bordeaux 2, and the Etablissement Public Régional d’Aquitaine. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a research grant from the Ministère de la Recherche et de la Technologie.

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**EXPERIMENTAL PROCEDURES**

**Materials**—N,N’-(1,2-Phenylene)dimaleimide and digitonin were from Sigma. Oligonucleotides were purchased from MWG-BIOTECH. All other reagents were of reagent grade quality.

**Yeast Strains and Nucleic Acid Techniques**—The *S. cerevisiae* strain D273-10B/A/HU (MATa, met6, ura3, his3) (22) was the wild type strain. The strains containing modified versions of subunit 4 were obtained by integration of the mutated versions of *ATP4* gene at the chromosomal locus in the deleted-disrupted yeast strain PYV01 (MATa, met6, ura3, his3, ATP4::URA3) (23). The yeast mutants with a deletion 4N- and 4TM1 were constructed according to the following strategy: Nol sites were introduced into the ATP4 gene (24) by directed mutagenesis on single-stranded DNA (18). We used mutagenesis with T4MPSA oligonucleotide (5’-TATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’). We used mutagenesis with T4MP5A oligonucleotide (5’-ATGCTTCTCAGCCCGAAACAGAC-3’).

**Biochemical Procedures**—Cells were grown aerobically at 28 °C in a complete liquid medium containing 2% lactate as carbon source (26) and harvested in logarithmic growth phase. The *rba-1* cell production in cultures was measured on glycerol plates supplemented with 0.1% glucose. Mitochondria were prepared from protoplasts as previously described (27). Protein amounts were determined according to Lowry et al. (28) in the presence of 5% SDS, 0.5 M urea, and 0.05% sodium dodecyl sulfate. Oxygen consumption rates were measured with NADH as substrate (29). The ATPase activity was measured at pH 8.4 (30). Variations in transmembrane potential (∆Ψ) induced by the phosphorylation and the ATP-dependent proton-pumping activity were evaluated by measurement of fluorescence quenching of rhodamine 123 (15, 31) with a SPM25 Kontron fluorescence spectrophotometer.

**BN-PAGE** experiments were done as described in Refs. 32 and 33. Mitochondria (1 mg of protein) were incubated for 30 min at 4 °C with 0.1 ml of digitonin with the indicated digitonin/denitrogen ratio. The extracts were centrifuged at 4 °C for 15 min at 40,000 × g and aliquots (50 μl) were immediately loaded on the top of a 3-13% polyacrylamide slab gel. After electrophoresis the gel was either stained with Coomassie Brilliant Blue or incubated in a solution of 5 mM ATP, 5 mM MgCl2, 0.05% lead acetate, 50 mM glycine-NaOH, pH 8.6, to reveal the ATPase activity (34, 35). Protein standards were thiryoglobin (669 kDa), apo-ferritin (443 kDa), β-amylose (200 kDa), and serum albumin (132 and 66 kDa). Nitrocellulose membranes (Membrane Protein BA83, 0.2 μm from Schleicher and Schuell) were used for Western blot analyses. Polyclonal antibodies against subunits e, g, and g were raised against amino acid residues 69–82 and 31–45, respectively. Antibodies against subunits 4, e, g, and i were used with dilutions of 1:1,000. Mitochondria were incubated with peroxidase-labeled antibodies and visualized with the ECL reagent of Amersham Biosciences, Inc. Molecular mass markers (BENCHMARK Prestained Protein Ladder) were from Invitrogen.

**Cross-linking Experiments**—Mitochondria isolated from wild-type and mutant cells were suspended in 0.6 M mannitol, 2 mM EGTA, 50 mM HEPES, pH 7.5, at concentrations of 10 mg/ml. To this suspension was added an equal volume of 0.75% (w/v) Triton X-100 and solubilization was performed for 20 min at 4 °C. The mixture was centrifuged at 100,000 × g for 15 min at 4 °C and the supernatant was incubated with 300 μM N,N’-(1,2-phenylene)dimaleimide for 1 h at room temperature. The reaction was quenched by addition of 2-mercaptoethanol and aliquots (50 μg of protein) were analyzed by Western blot.

**RESULTS**

**Proximity of Subunits 4 and g**—The proximity of subunits 4 and g has been reported in a previous work (18). The cross-linking experiments had been performed with the heterobifunctional reagent azido phenacyl bromide. This reagent, with a maximum cross-linking distance of 9 Å, linked the matricial domain of subunit g and the N-terminal part of subunit 4 upon incubation of either mitochondrial membranes or mitochondrial Triton X-100 extracts of mutants 4K7C and 4K14C, but the identification by mass spectrometric analyses of targets in subunit g was unsuccessful. Subunit g has a unique endogenous cysteine residue at position 75, a position which is predicted to be close to the membrane. The Cys75 of subunit g and cysteine residues inserted by site-directed mutagenesis at positions 7 and 14 of subunit 4 (two positions which are also predicted to be close to the membrane) were used as targets for the bifunctional reagent N,N’-(1,2-phenylene)dimaleimide (average S-S distance of 9.39 ± 0.47 Å) (36). In this way a 36-kDa band, which reacted with polyclonal antibodies raised against subunits 4 and g, was detected by Western blot analysis of 4K7C and 4K14C mutant mitochondria (Fig. 1). This band is a heterodimer resulting from the cross-linking between subunit 4 (23 kDa) and subunit g (13 kDa), thus indicating that the engineered Cys75 and Cys51 of subunits 4 and the endogenous Cys75 of subunit g are close. Such a proximity between subunits 4 and g suggests that the unique membrane-spanning segment of subunit g could be in contact with the first membrane-spanning segment of subunit 4. To test this hypothesis, two shortened versions in the N-terminal part of subunit 4 were constructed. The modified subunits 4 were obtained by insertion of NcoI sites into the ATP4 gene sequence followed by cleavage and ligation. The leader sequence which targets the subunit to the mitochondria was kept intact through these constructions. Subunit 4 of the 4N-term mutant was built to eliminate the first 18 amino acid residues that are located in the matrix space and subunit 4 of the 4TM1 mutant was built to remove the first 43 amino acid residues of subunit 4. As a result, the N-terminal matricial part and the first membrane-spanning segment of the wild type subunit were absent (Fig. 2).

**Mitochondria of 4N-term and 4TM1 Mutant Strains Have Efficient Oxidative Phosphorylations**—Mutant strains grew on the two non-fermentable carbon sources glycerol and lactate. The observed increase in the generation time of the 4TM1 mutant strain could have been a consequence of a defect in the oxidative phosphorylations (Table I). However, the ATP/O ratios of 4N-term and 4TM1 mitochondria were similar to that of wild type mitochondria, thus indicating that the oxidative phosphorylation efficiency was not affected. The major difference between either wild type or 4N-term and 4TM1 mitochondria was a 40% decrease in the basal, coupled, and uncoupled respiration rates of 4TM1 mitochondria. The same results were obtained with the ΔATP20 mutant mitochondria of 4N-term and 4TM1 mitochondria.
which were devoid of the ATP synthase associated subunit g, a component involved in the dimerization of the enzyme. The low respiration rates reflected the spontaneous rho− cell conversion, a phenotype which is quite common in yeast when genes encoding the yeast ATP synthase components are modified (37, 38). Our interpretation is that although growing on a non-fermentable carbon source, a part of the cellular population while converting to rho− cells no longer grew, and then accumulated. As a result, the mitochondrial preparations of ΔTM1 and ΔATP20 mutant strains contained a mixed population of mitochondria isolated from rho− and rho+ cells. This affected the mitochondrial respiration rates (expressed as per milligram of protein) and the generation time, but did not alter the respiratory control or the ATP/O ratio.

ATPase activity measurements were performed at pH 8.4 in the presence of 0.375% Triton X-100, experimental conditions which reveal the full ATPase activity by removing the natural inhibitor IF1. The specific ATPase activities of mutants and wild type mitochondria were identical (Table II). However, the sensitivity to the F0 inhibitors oligomycin and DCCD was around 70–74 and 57–60% for 4ΔN-term and 4ΔTM1 mitochondria, respectively, instead of 87% for wild type mitochondria. The decrease in the sensitivity to F0 inhibitors is quite understandable for 4ΔTM1 mitochondria as it reflected the presence of a mixed population of mitochondria isolated from rho− and rho+ cells, of which the latter are devoid of subunits that are the targets of F0 inhibitors. The same was true with mitochondria of the null mutant ΔATP20, while it was not the case with 4ΔN-term mitochondria that were isolated from rho− cells. We interpret the slight decrease in oligomycin sensitivity of 4ΔN-term mitochondria under these experimental conditions (basic pH and Triton X-100) as destabilization of the second stalk due to the lack of the first 18 amino acid residues of subunit 4.

Under the respiratory conditions of fluorescent quenching experiments, 4ΔN-term mitochondria display the same behavior as those of wild type mitochondria (15). In the presence of ethanol, 4ΔN-term mitochondria displayed a fast transient decrease in the fluorescent quenching of rhodamine 123 upon addition of 50 μM ADP, which corresponds to a decrease in the transmembranous ΔΨ attributed to a proton influx through F0 during ADP phosphorylation (Fig. 3). 4ΔTM1 mitochondria displayed a lower decrease in the fluorescent quenching of the dye upon ADP addition. In addition, the time during which ADP was consumed was significantly increased. Although ATP/O ratios were similar for both types of mitochondria, these data reflected the presence in the assay of a mixed population of mitochondria from rho+ and rho− cells, the latter being devoid of intact F1,F0-ATP synthase. The variations in the transmembrane ΔΨ mediated by the ATPase proton-pumping activity were also analyzed after transiently energizing mitochondria by ethanol, an activation step which is necessary to remove the natural inhibitory peptide IF1 of the mitochondrial ATPase which would otherwise inhibit the ATPase activity (39). Subsequent 1 mM ATP addition induced similar fluorescent quenching of the dye with ΔΨ/F values = 0.43 and 0.42 for 4ΔN-term and 4ΔTM1 mitochondria, respectively, thus reflecting a normal proton pumping activity mediated by the mutant ATP synthases (wild type mitochondria displayed a mean ΔΨ/F value of 0.425 ± 0.015). Under these conditions, the ATP-dependent proton pumping activity only concerns F1,F0-ATP synthases. The ATP-dependent fluorescent quenching was DCCD sensitive for both types of mitochondria. The same fluorescent quenching upon ATP addition and its stability with time suggested that the F1,F0-ATP synthases of the two mutants were fully functional.

**The Lack of the First Membrane-Spanning Segment of Subunit 4 Alters ATP Synthase Dimerization and Oligomerization**—The repression of subunit 4 alterations in mutants 4ΔN-term and 4ΔTM1 was investigated by Western blot analyses. Mitochondrial membranes of 4ΔN-term and 4ΔTM1 strains showed a decrease in the relative molecular mass of subunit 4 (theoretical molecular masses of 21,250 and 18,848 Da, respectively), which is in agreement with the respective relative molecular masses of 20,000 and 18,000 that were calculated from comparison with the migration of standard proteins (Fig. 4A). 4ΔTM1 mitochondria displayed an additional faint band of Mr 21,000 (Fig. 4A, lane 3). When the 4ΔTM1 mutant enzyme was solubilized by Triton X-100 and pelleted, the additional band disappeared, thus showing that it was probably poorly associated with the enzyme (Fig. 4A, lane 5). The N-terminal part of the subunit 4ΔTM1 is likely to be located in the intermembrane space with an orientation opposite to that of native subunit 4. Taking this into account, we suggest that the 21,000 Mr band corresponds to the subunit 4ΔTM1 with its leader sequence (theoretical mass 22,608 Da). This point was not further investigated.

The similarity of the phenotypes of 4ΔTM1 and ΔATP20 strains prompted us to examine the presence or the absence of ATP synthase-associated subunits e and g, which are known to be involved in the dimerization/oligomerization of the enzyme (Fig. 4, B and C). Subunit i, another associated membranous component of the yeast ATP synthase (14, 15), was used as a standard. Western blot analyses of mitochondrial membranes of 4ΔATP1 strain indicated that the lack of the first 43 amino acid residues of subunit 4 led to a strong decrease in the amount of subunit g (Fig. 4C, lane 3), whereas the amount of subunit e was not modified. Mitochondria isolated from 4ΔN-term strain contained subunits e and g. As a result, the consequences of the lack of the first membrane-spanning segment of subunit 4 led to a similar phenotype to that of the null mutant in ATP20 gene that encodes subunit g. Since subunit g is involved in the dimerization of the yeast enzyme (12), dimerization was examined by BN-PAGE analyses of mitochondrial digitonin extracts. These extracts were loaded on a 3–13% acrylamide slab gel and mitochondrial complexes were separated under native conditions. The gel was stained either with Coomassie Brilliant Blue (Fig. 5A) or incubated with ATP-Mg2+ and Pb2+ to reveal the ATPase activity (Fig. 5B). The wild type digitonin extracts mainly contained ATP synthase dimers but also higher molecular mass bands corresponding to ATP synthase oligomers (16) that were destabilized when the digitonin/protein ratio was increased. Why the dimeric form was a doublet is unclear. Either this doublet reflects different associations of ATP synthases or different compositions in subunits and associated lipids. Indeed, SDS-PAGE analysis of the
TABLE I
Phenotype of yeast cells and oxidative phosphorylation of isolated mitochondria

The wild type strain was D273–10B/A/H/U. The null mutant in the ATP20 gene was constructed by a PCR-based mutagenesis. Yeast cells were grown on complete medium containing 2% lactate as carbon source. The rho− cell production in cultures was measured on glycerol plates supplemented with 0.1% glucose. Mitochondria were prepared from protoplasts. Data of time generation are from typical experiments. Measurements of oxidative phosphorylations were performed four times. ATP/O ratios were determined with NADH as substrate. CCCP concentration was 3 µM.

| Strains         | Doubling time (min) | % of rho− cells in cultures | Respiration rates |
|-----------------|---------------------|-----------------------------|------------------|
|                 |                     |                             | CCCP State 4     | State 3     | ATPO |
|                 |                     |                             | nmol of O2/min/mg of protein | % | nmol of O2/min/mg of protein | % |
| Wild type       | 164                 | 0.9 ± 0.2                   | 1199 ± 42        | 302 ± 39   | 759 ± 58 | 1.09 ± 0.13 |
| 4ΔN-term        | 175                 | 1.3 ± 0.2                   | 1470 ± 30        | 305 ± 20   | 900 ± 30 | 1.16 ± 0.06 |
| 4ΔTM1           | 208                 | 31.2 ± 2.9                  | 810 ± 20         | 175 ± 15   | 475 ± 10 | 1.13 ± 0.05 |
| ΔATP20          | 220                 | 40.0 ± 2.4                  | 795 ± 50         | 175 ± 7    | 480 ± 20 | 1.22 ± 0.04 |

TABLE II
ATPase activities of isolated mitochondria

D273–10B/A/H/U (wild type) and mutant mitochondria were prepared from yeast cells grown with 2% lactate as carbon source. Three different preparations were made from each strain, and measurements were performed in triplicate with 50 µg of mitochondrial protein. Data presented are from typical experiments. ATPase activities and the sensitivity to the Fo inhibitors oligomycin (6 µg/ml) and DCCD (29 µM) were measured at pH 8.4 in the presence of 0.375% Triton X-100 to remove the Fo′ inhibitor.

| Strains          | Control | + Oligomycin | Inhibition | + DCCD | Inhibition |
|------------------|---------|--------------|------------|--------|------------|
|                  | µmol of Pi/min/mg of protein | % | µmol of Pi/min/mg of protein | % |
| Wild type        | 4.98 ± 0.10 | 0.67 ± 0.02 | 87 | 0.67 ± 0.03 | 87 |
| 4ΔN-term         | 4.27 ± 0.11 | 1.10 ± 0.05 | 74 | 1.29 ± 0.01 | 70 |
| 4ΔTM1            | 5.00 ± 0.10 | 2.18 ± 0.05 | 56 | 2.01 ± 0.11 | 60 |
| ΔATP20           | 6.61 ± 0.04 | 3.68 ± 0.09 | 44 | 3.84 ± 0.14 | 42 |

4ΔTM1 preparations since rho− mitochondria contain a catalytic domain F1, which is not firmly bound to the inner mitochondrial membrane.

The 4ΔTM1 Mutant Is Defective in the Mitochondrial Morphology—We have already reported that the null mutants in either TIM11 or ATP20 genes have anomalous mitochondrial morphologies (16). Thus, transmission electron microscopy of yeast cell sections was performed to examine the effect of subunit g shortening on the ultrastructure of mitochondrial membranes. Mitochondria from wild type (16) and 4ΔN-term mutant cells appeared normal with numerous cristae (Fig. 6A). In contrast to this, electron micrographs of 4ΔTM1 cells showed mitochondria with very different morphologies such as onion-like structures surrounding other subcellular organelles (the vacuole) (Fig. 6B). The same abnormal mitochondrial struc-
The gel was either stained with Coomassie Brilliant Blue (with the indicated digitonin/protein ratio and analyzed by BN-PAGE. The figure shown in B is a negative picture of the original gels. As a result, bands appear in black on a white background. wt, wild type extract. S, standard proteins.

| 4AN-term | 4ΔTM1 | ΔATP20 | wt |
|----------|-------|--------|----|
| digitonin / protein (g/g) | 0.75 | 0.75 | 0.75 | 0.75 | 1 | 0.75 | 1 | 0.75 | 1 |
| kDa | 669 | 669 | 669 | 443 | 443 | 443 | 132 | 132 | 132 |

**DISCUSSION**

The Lack of the First Membrane-spanning Segment of Subunit 4 Leads to the Same Phenotype as That of Null Mutants Devoid of Components Involved in the Dimerization of the Yeast ATP Synthase—The yeast F₀ is composed of eight essential components for its structure. In addition, proteins e, g, i, and k are associated to F₁ and inactivation of their structural gene does not significantly alter the enzyme (11–15, 40). Two of them (e and g) that are present only in the mitochondrial ATP synthase are involved in its dimerization. Inactivation of their respective genes, TIM11 and ATP20, does not alter the efficiency of oxidative phosphorylations but prevents dimerization of the ATP synthase. However, many rho− cells (40%) appear during growth, thus increasing the generation time of the whole population. Additionally, it has been reported that in the absence of either e- or g-subunits, the mitochondrial morphology is highly altered with a lack of the tubular cristae and proliferation of the inner mitochondrial membrane (16). The present findings suggest an interaction between the unique membrane-spanning segment of subunit 4 and the first membrane-spanning segment of subunit 4. The proximity between these two subunits has already been proposed (18). Subunit g is composed of 115 amino acid residues and displays a unique membrane-spanning segment. The major part of subunit g is located in the matrix space with its N-terminal part inside (12, 41). Subunit 4 (b) is also mainly located in the matrix space. The two membrane-spanning segments of subunit 4 are linked by a short hydrophilic loop (amino acid residues 46–55) whose residue 54 is accessible to non-permeant reagents from the intermembrane space (42). The 4 + g cross-linking products involving subunit g and targets in the N-terminal part of subunit 4 (18) are in agreement with the orientation of both subunits. The 36-kDa cross-linking product obtained with the N,N′-(1,2-phenylene)dimaleimide involving the unique Cys376 of subunit g and positions 7 and 14 of subunit 4 is also in favor of a very close relationship between the two partners. Since the targets involved in this cross-linking are close to the membrane, we suggest that the membranous domains of both subunits are also close and probably interact. Fig. 7 shows a schematic representation of both subunits 4 and g interacting at mem-

**FIG. 6.** The 4ΔTM1 strain displays anomalous mitochondrial morphologies. Transmission electron microscopy of yeast cell sections of 4AN-term (A), 4ΔTM1 (B), and ΔATP20 strains (C). The abbreviations are: m, mitochondria; n, nucleus; v, vacuole. The bars indicate 0.5 μm.
ATP Synthase Dimerization and Subunit b

**Fig. 7.** Schematic representation showing the relationships between subunits g and 4. Numbering of wild type subunit 4 begins at the methionine residue 1 of the mature sequence (24). The two membrane-spanning segments and a part of the matrix domain of subunit 4 are shown. The arrows indicate the cross-links involving the endogenous Cys17 of subunit g and either K7C or K14C positions of subunit 4. The black circles represent the lysine residues replaced by cysteine residues at positions 7 and 14 of subunit 4.

The membrane-spanning segment levels.

*The Additional N-terminal Part of the Mitochondrial b-subunit (Subunit 4) and the ATP Synthase Dimerization and Oligomerization—The b-subunit is an essential component of the second stalk connecting the F1 and F0 sectors of the enzyme. It has a structural role even though some authors consider that this component could also serve as an elastic element which transiently stores energy during catalysis (43, 44). Like that of other prokaryotes, the E. coli F0F1-ATP synthase has two identical b-subunits to build the second stalk. The chloroplast enzyme has subunits I and II that are homologous to the b-subunit, thus leading in association with subunit e to the dimerization/oligomerization of the enzyme and to a normal biogenesis of the mitochondrial cristae.

**Acknowledgments**—We are grateful to Drs. D. Brethes, X. Grandier-Vazeille, and C. Napias for stimulating discussions.

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ATP Synthase Dimerization and Subunit b

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