Identification of a Nuclear Gene (FMC1) Required for the Assembly/Stability of Yeast Mitochondrial F₁-ATPase in Heat Stress Conditions*

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We have identified a yeast nuclear gene (FMC1) that is required at elevated temperatures (37 °C) for the formation/stability of the F₁ sector of the mitochondrial ATP synthase. Western blot analysis showed that Fmc1p is a soluble protein located in the mitochondrial matrix. At elevated temperatures in yeast cells lacking Fmc1p, the α-F₁ and β-F₁ proteins are synthesized, transported, and processed to their mature size. However, instead of being incorporated into a functional F₁ oligomer, they form large aggregates in the mitochondrial matrix. Identical perturbations were reported previously for yeast cells lacking either Atp12p or Atp11p, two specific assembly factors of the F₁ sector (Ackerman, S. H., and Tzagoloff, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4986–4990), and we show that the absence of Fmc1p can be efficiently compensated for by increasing the expression of Atp12p. However, unlike Atp12p and Atp11p, Fmc1p is not required in normal growth conditions (28–30 °C). We propose that Fmc1p is required for the proper folding/stability or functioning of Atp12p in heat stress conditions.

F₁F₃-ATP synthases play a major role in cellular energy production. They are found in the plasma membranes of bacteria, thylakoid membranes of chloroplasts, and in the inner membrane of mitochondria. They use a proton gradient across their host membrane to produce ATP from ADP and inorganic phosphate (1, 2). This enzyme contains two distinct parts, called F₃ and F₁. The F₃ mediates the transmembrane transport of protons, and the synthesis of ATP takes place on the F₁.

The F₁ contains five different types of subunits in the stoichiometric ratio α₃β₃γδε (3, 4). The three-dimensional structures of F₁ from bovine heart (5), rat liver (6) and yeast (7) show that the α- and β-subunits alternate in a hexagonal array with a central cavity occupied by the amino and carboxyl termini of the γ-subunit. The interfaces between the α- and β-subunits form three catalytic and three noncatalytic nucleotide binding sites.

In the yeast Saccharomyces cerevisiae, the F₁ subunits are encoded in the nucleus (8–12), synthesized in the cytoplasm, imported into mitochondria as unfolded polypeptide chains (13), and then folded in the mitochondrial matrix with the help of Hsp60p and Hsp10p (14). The oligomerization of the F₁ monomers is assisted by two proteins called Atp12p and Atp11p. These interact directly with the α-F₁ and β-F₁ proteins, respectively (15, 16). In yeast strains lacking either Atp11p or Atp12p, the α-F₁ and β-F₁ proteins aggregate in the mitochondrial matrix (17). Thus it is believed that Atp12p and Atp11p facilitate the formation of αβ heterodimers by protecting these two F₁ subunits from non-productive interactions (16).

We report in this study the identification of Fmc1p, a novel protein required for the formation or stability of the F₁ oligomer. Like Atp11p and Atp12p, its absence also results in the aggregation of the α-F₁ and β-F₁ proteins. However, this is seen only at elevated temperatures (37 °C), whereas Atp11p and Atp12p are required both in normal (28–30 °C) and heat stress conditions. Interestingly, the formation/stability of the F₁ oligomer was restored in cells lacking Fmc1p by increasing the expression of Atp12p. We propose that Fmc1p assists the folding/stability or functioning of Atp12p and that this role becomes essential at elevated temperatures.

MATERIALS AND METHODS

Strains and Media

The S. cerevisiae strains used are listed in Table I. Escherichia coli XL1-Blue (Stratagene) was used for the cloning and propagation of plasmids. Yeast strains were grown in either YPGA (1% yeast extract, 1% bactopeptone, 2% glucose, 30 mg/liter adenine), YPGAL (1% yeast extract, 1% bactopeptone, 2% galactose, 30 mg/liter adenine), or synthetic complete media SC (2% glucose, 0.67% yeast nitrogen base, 20 mg/liter adenine containing either 1% casamino acids or appropriate drop-out mix (CSM series from BIO 101) at the concentration recommended by the manufacturer. The SC media with casamino acids were always supplemented with 50 mg/liter tryptophan and 20 mg/liter methionine. The growth of yeast strains on nonfermentable carbon sources was tested on N1 (1% yeast extract, 1% bactopeptone, 2% ethanol, 0.05 M sodium-potassium phosphate, pH 6.2) and N3 (1% yeast extract, 1% bactopeptone, 2% glycerol, 0.05 M sodium-potassium phosphate, pH 6.2). All media were solidified by adding 2% Bacto-Agar Difco.

Construction of Yeast Strains Carrying a Null Mutation in FMC1

FMC1 was deleted according to the procedure described by Wach (18). The 297- and 470-base pair DNA sequences located, respectively, upstream of the ATG initiator codon and downstream of the stop codon...
of FMC1 were PCR-amplified from yeast genomic DNA (W303–1B) with the primers P1 (5'-GTATGGTCTGATACCCTGTCAGATCACC and 5'-CTCTTCAGTAACTTAGTTGGACATG) and P2 (5'-CTATCTCGGTAGAAGGAGAGCGGCTAGATGTAATATATTAGTGGCTCTTCTCCTCCTTC, P3 (5'-GAGGTTGTTAGGAGGCCAAGGCCGACATGATCATCATTATATATC), P4 (5'-ATTCTGGGAGAATGAGGCGAAGGCTATTTG). DNA fragments carrying HIS3 were generated by PCR from plasmid pUC18-HIS3 (19) with the primers Ps (5'-TTGATTGACAGGATGAATTGATTGATTGATCTCTTCTCCTCCTCCTTC, P5 (5'-CTCTGGGAGAATGAGGCGAAGGCTATTTG). The three PCR products (P1-P2, P3-P4, and Pa-Pb) were mixed, and PCR with the primers P1 and P4 gave a DNA fragment in which HIS3 was flanked by sequences adjacent to the FMC1-coding region. The strain W303–1B was transformed with the deletion cassette, and resulting His+ clones were analyzed by Southern blot. Out of 12 His+ transformants analyzed, 11 gave the expected hybridization signals (not shown).

**Plasmid Constructions**

Cloning of FMC1—A DNA fragment carrying FMC1 was amplified by PCR from the genomic DNA of W303–1B with the primers 5'-ATTCCTCGGTAGAAGGAGAGCGGCTAGATGTAATATATTAGTGGCTCTTCTCCTCCTCCTTC and 5'-GGGCCAAGGCCGACATGATCATCATTATATATC. The PCR product was cut by BsaI to produce a blunt end fragment containing the FMC1 coding sequence with 75 base pairs upstream of the initiator ATG and 498 base pairs downstream from the Stop codon. This fragment was cloned at the HindII site of pUC19 to give pJR22. From this plasmid, the HindIII-BamHI fragment containing FMC1 was cloned into the centromeric plasmid pRS316 (20) cut with the same pair of enzymes to give pLL1.

Cloning of ATP12 in a Low Copy Number Vector—The ATP12-containing HindIII-HindIII fragment of plAE10 was inserted at the HindIII site of pRS316 to give pLL5.

**Isolation of ATP12 as a Multicopy Suppressor of the Null Allele fmc1**

The fmc1 strain MC6 was transformed with a partial HindIII digest of yeast chromosomal DNA cloned into the URA3-containing multicopy vector pEMBLYe23 (a gift of Dominique Thomas, Gif-sur-Yvette). About 100,000 Ura+ transformants were selected on SC medium lacking uracil and were then replica-plated onto glycerol medium (N3) prewarmed for at least 2 h at 37 °C. After an incubation of 5 days at 37 °C, clones that showed good growth were tested for their growth on glycerol at 37 °C after curing the plasmids they contained on SC medium supplemented with 0.1% of 5-fluoroorotic acid. When growth on glycerol at 37 °C was plasmid-dependent, the suppressor plasmids were recovered in E. coli and tested again by retransformation of fmc1 cells. Several rescue plasmids contained the same insert, a HindIII-HindIII 1599-base pair fragment corresponding to a segment of chromosome X between the HindIII sites 7249 and 8882. The HindIII plasmid was labeled by nick translation using the nick translation kit from Roche Molecular Biochemicals.

**Production of anti-Fmc1p Antibodies**

Anti-Fmc1p antibodies were prepared by Eurogentec (Seraing, Belgium) with the synthetic peptide NH2-YNPGNKLTQDEK as an immunogen.

**RESULTS**

**Genetic and Molecular Biology Methods**

Genetic experiments were carried out as described by Rose et al. (21). Standard recombinant DNA techniques were used as described by Sambrook et al. (22). Yeast transformations were performed using procedures (23). Yeast genomic DNA was isolated as described previously (24). Yeast mitochondrial RNAs were isolated as described procedures (23). Yeast genomic DNA was isolated as described by di Rago et al. (25). Yeast mitochondrial RNAs were isolated as described by Rouillard et al. (36). Antibodies raised against yeast ATP synthase subunits, Asn2p, Atp12p, and Fmclp were used after dilution 1:10,000; 1:10,000, 1:2,500, and 1:5,000, respectively. ProBlott membranes were incubated with peroxidase-labeled antibodies at a dilution of 1:10,000 and revealed with the ECL reagent of Amersham Pharmacia Biotech. The chloromethyl/methanol extraction of subunits 6, 8, and 9 of ATP synthase was as described by Michon et al. (39). Mitochondrial proteins were labeled in cycloheximide-blocked cells according to Claisse et al. (40). Mitoplasts were prepared according to Daum et al. (41).
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Fig. 1. Yeast cells carrying a null mutation in the FMC1 gene fail to grow at elevated temperatures on media containing a non-fermentable carbon source. Wild type FMC1^{+} and mutant Δfmc1, containing either an intronless mtDNA or a mtDNA with 13 introns, were grown overnight at 28 °C in glucose (YPGA). The cultures were diluted and 5 μl of each dilution spotted on glucose (YPGA) and ethanol (N1) media. The plates were photographed after 3 days of incubation at 37 °C.

Mitochondria from the mutant and wild type strains cultured at 28 °C had approximately the same DCCD-sensitive ATP hydrolytic activity (Table III). By contrast, Δfmc1 cells grown at 37 °C had a very low mitochondrial ATPase activity (less than 10% of the control). This residual activity was essentially insensitive to DCCD and also about two times lower than that of mitochondria isolated from wild type rho° cells cultivated at 37 °C. Thus, the F_{1} sector of the ATP synthase is severely affected in yeast cells lacking FMC1 grown at 37 °C, whereas it is fully active when these cells are grown at 28 °C.

It should be noted that the ATPase activity measured for the wild type grown at 37 °C was much less sensitive to DCCD than that of wild type cells grown at 28 °C (see Table III). This suggests that a substantial part of the F_{1} sector is not physically or functionally coupled with the F_{0} sector when wild type yeast is cultivated at elevated temperatures. This observation has been reported previously (42).

The proton-pumping activities of Δfmc1 mitochondria were probed by fluorescence quenching of rhodamine 123. The Fig. 4 shows the results obtained with mitochondria isolated from mutant and wild type cells grown at 37 °C. For the wild type, the addition of ethanol produced a fluorescence quenching of the dye, which was transiently decreased by adding 50 μM ADP, thus reflecting an electrogenic exchange of internal ATP against external ADP and a proton influx through the F_{0} sector during phosphorylation of the added ADP. By contrast, with mutant mitochondria, although ethanol was still able to energize the membrane, a subsequent addition of ADP could not substantially decrease the membrane potential (ΔΨ). Changes in ΔΨ mediated by the ATPase proton-pumping activity were analyzed after energizing mitochondria by ethanol, an activation step necessary to remove the natural inhibitory peptide (IF1) of the mitochondrial ATPase, which would otherwise in-
hibit ATPase activity (43). As expected, with wild type mitochondria, inhibition by KCN of the proton pumping by the respiratory chain resulted in a collapse of ΔΨ, and subsequent addition of ATP promoted a fluorescence quenching of the dye that was DCCD-sensitive. With mutant mitochondria, an addition of ATP after collapsing ΔΨ by KCN promoted a lower significance portion of the F1 sector of the ATP synthase, the F1-

Taken together, the results of these different analyses show that oxidative phosphorylation is severely affected at elevated temperatures when the FMC1 gene is absent, with the most dramatic consequences seen at the level of the ATP synthase.

**FMC1 Is Required for the Assembly or Stability of the ATP Synthase at Elevated Temperatures**—As described above, the respiratory enzyme defect of the Δfmc1 strain is probably due to a failure in mitochondrial ATP synthesis. To determine whether this was caused by a block in the assembly of the ATP synthase, we examined the steady state levels of several subunits of this enzyme in the mutant grown at elevated temperatures.

**TABLE I**

Yeast strains used in this study

| Name         | Genotype                                                                 | Origin     |
|--------------|--------------------------------------------------------------------------|------------|
| W303–1B      | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [rho+]                  | R. Rothstein |
| W303–1B/A    | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [rho+]                  | R. Rothstein |
| W303–1B/60   | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [rho+]                  |            |
| CKYL2        | MATa leu1 kor1–1 can4 [ΔE6 Op]                                           |            |
| CR01         | MATa leu1 kor1–1 can4 [mtDNA 777–3A, 13 introns]                        |            |
| FA213/5      | MATa/MATa ade2–1 ade2–1 his3–11,15 his3–11,15 trp1–1/trp1–1             | This study  |
|              | leu2–3,112 [mtDNA 777–3A, 13 introns]                                    |            |
| FA213/5–2A   | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 fmc1–1 HIS3 [rho+]     | This study  |
| FA213/5–2A/50| MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 fmc1–1 HIS3 [rho+]     | This study  |
| MC6          | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [Δfmc1–1 HIS3]           | This study  |
|              | (ΔE6 Op), Cytoductant of CKYL2 in FA213/5–2A/50                         |            |
| MC8          | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [Δfmc1–1 HIS3]           | This study  |
|              | (mtDNA 777–3A, 13 introns). Cytoductant of CK01 in FA213/5–2A/50        |            |
| MC1          | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [Δfmc1–1 HIS3]           | This study  |
|              | Cytoductant of CKYL2 in W303–1B/60                                       |            |
| MC3          | MATa ade2–1 his3–11,15 trp1–1 leu2–3,112 ura3–1 [mtDNA 777–3A, 13      | This study  |
|              | introns]. Cytoductant of CK01 in W303–1B/60                             |            |

**TABLE II**

Production of petite (rho−/rho−) cells by the Δfmc1 mutant

The strains were cultivated for five to six generations in glucose (YPGA) at the indicated temperature (28 or 37 °C). The cultures were then diluted and spread for single colonies on a glycerol medium containing 0.1% glucose. The petite (rho−/rho−) and grande (rho−/rho+) colonies were scored after 4 days of incubation at 28 °C.

| Strain | 28 °C | 37 °C |
|--------|-------|-------|
|        | Petites | % Petites | Petites | % Petites |
| Wild type [13 introns] | 18 | 5 | 5 | 13 |
| (MC3)  |      |     |    |     |
| Δfmc1 [13 introns] | 7 | 4 | 2 | 3 |
| (MC8)  |      |     |    |     |
| Wild type [w/o intron] | 2 | 1 | 1 | 1 |
| (MC1)  |      |     |    |     |
| Δfmc1 [w/o intron] | 1 | 0 | 0 | 0 |
| (MC6)  |      |     |    |     |

mitochondrial ATP synthase (ATP9 and ATP8), although these two proteins may be synthesized at a slower rate (see above). We do not know whether the missing subunits of nuclear origin are synthesized in the Δfmc1 mutant, but it is reasonable to assume that their absence also results from proteolytic degradation after a block in the assembly of the enzyme. It has been shown previously that in the absence of either Atp12p or Atp11p, two chaperones specifically involved in the assembly of the F1 sector of the ATP synthase, the F1-α and F1-β proteins accumulate normally, but instead of being incorporated into a functional F1 oligomer, they form large aggregates in the mitochondrial matrix (17). Interestingly, a significant portion of the F1-α and F1-β proteins also behaved as large protein aggregates in the Δfmc1 mutant, as shown by sucrose gradient analysis (Fig. 5C).

Immunoprecipitation with antibodies against the F1-α protein were made to see whether this protein was associated with the remaining ATP synthase subunits in the Δfmc1 mutant. The immunoprecipitates obtained from mitochondria isolated from the mutant grown at 37 °C contained only the F1-α and F1-β subunits (not shown). This indicates that these two proteins are part of an entity that is not, or is poorly, associated with the remaining subunits of the enzyme.

**Fmc1p Is a Soluble Mitochondrial Protein**—An analysis of the 155-amino acid (18,352 Da) sequence deduced from FMC1 using the P-sort program of Nakai and Kanehisa (44) indicates that Fmc1p is a mitochondrial protein. This prediction was confirmed with the use of antibodies raised against a 14-amino acid peptide corresponding to the nucleotide sequence of FMC1 between positions 390 and 432. A Western blot analysis of
mitochondrial proteins isolated from wild type cells using these antibodies produced a specific 14-kDa signal that could not be detected in the \( \Delta m f c 1 \) mutant (Fig. 6A). The apparent size of the protein indicates that Fmc1p is synthesized as a precursor containing an amino-terminal presequence of \(-4\) kDa. After an osmotic disruption of both the outer and inner mitochondrial membranes in the presence of carbonate, the protein was recovered in a water-soluble form (Fig. 6B). After disruption of just the outer membrane by a mild osmotic treatment of mitochondria, a portion of the immunological signal was recovered in the mitoplast fraction and preserved after treatment of the mitoplasts with proteinase K (the recovery of a part of the signal in the supernatant fraction is probably due to partial damage of the mitoplasts) (Fig. 6C). These data indicate that Fmc1p is a protein of the mitochondrial matrix, either free or loosely bound to the inner face of the inner mitochondrial membrane.

The \( \Delta m f c 1 \) Mutant Can Be Rescued by Increasing the Copy Number of ATP12—The results described above show that Fmc1p is a mitochondrial protein needed at elevated temperatures for the assembly or stability of the \( F_1 \) sector of the ATP synthase. To gain more insight into its function, we decided to determine whether the loss of Fmc1p can be compensated for by overexpressing another yeast protein(s), a current approach to identifying proteins with related cellular functions. To this end, the \( \Delta m f c 1 \) mutant was transformed with a yeast wild type genomic library in a high copy number vector, and the resulting transformants were tested for their growth on glycerol at 37 °C. Thus, a small increase in the ATP12 expression of Atp12p may be apparently sufficient to overcome the very specific action of Atp12p in the assembly of the \( F_1 \) oligomer (16, 17), they strongly suggest that Fmc1p has a function also confined to this process. Second, because the \( \Delta m f c 1 \) mutant exhibits at elevated temperatures all the characteristics of the \( \Delta a t p12 \) mutant and because the absence of Fmc1p can be overcome by increasing the production of Atp12p, a logical view is that the function of Atp12p is impaired at elevated temperatures when Fmc1p is missing. Consistent with this, we found that the abundance of Atp12p was significantly reduced in

These results are particularly interesting. First, given the very specific action of Atp12p in the assembly of the \( F_1 \) oligomer (16, 17), they strongly suggest that Fmc1p has a function also confined to this process.
mitochondria isolated from Δfmc1 cells cultivated at 37 °C (Fig. 8). We have also shown that the multi-copy suppressor relationship between FMC1 and ATP12 is not reciprocal, as the Δatp12 mutant remained unable to grow on glycerol after transformation with FMC1 cloned in a high copy number vector. Altogether these data suggest that Fmc1p may be required for the formation/stability or functioning of Atp12p at elevated temperatures.

Since Atp11p cooperates with Atp12p in the assembly of the F₁ oligomer (15–17), we decided to determine directly whether Atp11p could, like Atp12p, rescue the Δfmc1 mutant. ATP11 may have been poorly represented in the library we used and, hence, not isolated in the search of multicopy suppressors. We therefore constructed a high copy number vector containing ATP11. After transformation with this plasmid, the Δfmc1 mutant remained unable to grow on glycerol at 37 °C (not shown). Also, we found that the Δatp11 mutant was not rescued by overexpression of Fmc1p. Thus, contrary to Atp12p, it seems that the proper folding/stability or functioning of Atp11p at elevated temperatures does not require the presence of Fmc1p.

**DISCUSSION**

We have identified a novel nuclear-encoded yeast mitochondrial protein and showed that its presence is required for the assembly/stability of the F₁ sector of the ATP synthase in heat stress conditions. In its absence and at elevated temperatures, the α-F₁ and β-F₁ proteins are synthesized, transported, and processed to their mature size, but instead of being incorporated into a functional F₁ oligomer, they form large aggregates in the mitochondrial matrix. Identical defects in the assembly of the F₁ oligomer were observed previously for yeast cells lacking either Atp12p or Atp11p (17). However, unlike Fmc1p, these two latter proteins are required for the formation of the F₁ oligomer not only at elevated temperatures but also in normal growth conditions.

The oxygen consumption rate of mitochondria isolated from the Δfmc1 mutant grown at elevated temperatures was strongly reduced (by 70%) in comparison to the wild type. This is probably a secondary consequence of the defect in the assembly of the F₁ oligomer. Indeed a decreased respiratory activity was also seen for strains carrying null mutations in the genes of α-F₁, β-F₁ (8, 9, 46), Atp11p, and Atp12p (17).

Interestingly, the Δfmc1 mutant recovered the ability to assemble the F₁ oligomer at elevated temperatures by increasing the expression of Atp12p, suggesting that the function of Atp12p may be compromised at elevated temperatures when Fmc1p is lacking. Consistent with this, the steady state level of Atp12p was found to be substantially reduced in mitochondria isolated from Δfmc1 cells grown at elevated temperatures.

### Table III

| Strain         | Temperature | Respiration rates | ATP hydrolytic rates |
|----------------|-------------|-------------------|----------------------|
|                |             | NADH + CCCP       | Ascorbate + TMPD + CCCP | −DCCD | +DCCD |
| Wild type rho⁻ (MC1) | 37 °C       | 592               | 2031                  | 7502 ± 390 | 3613 ± 103 |
| Δfmc1 rho⁻ (MC6)  | 37 °C       | 191               | 401                   | 1051 ± 112 | 947 ± 102  |
| Wild type rho⁺ | 37 °C       | 0                 | 0                     | 2133 ± 185 | 2170 ± 234 |
| Δfmc1 rho⁻ (MC1)  | 28 °C       | 877               | 2050                  | 6547 ± 124 | 2274 ± 105 |
| Δfmc1 rho⁻ (MC6)  | 28 °C       | 1006              | 2744                  | 6582 ± 802 | 1426 ± 50  |

![Fig. 4. Proton-pumping activities of mitochondria.](http://www.jbc.org/) Proton-pumping activities were monitored by fluorescence-quenching of rhodamine 123 with intact mitochondria isolated from wild type FMC1⁺ (strain MC1) and mutant Δfmc1 (strain MC6) grown in galactose (YPGALA) at 37 °C for 6–8 generations. The additions were 0.3 mg of mitochondrial proteins (mito), 10 µl of ethanol (EtOH), 50 µM ADP, 3 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP), 6 µg of DCCD, and 200 µM KCN; 1 mM ATP was added 1 min after KCN.

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Based on these observations, a reasonable hypothesis is that Fmc1p helps the folding/stability or functioning of Atp12p at elevated temperatures.

The molecular mass of native Atp12p, estimated from its sedimentation properties in sucrose gradients, is at least twice as great as that of the monomer (47). Chemical modifications and two-hybrid genetic studies argue against the formation of oligomers of Atp12p (48). Furthermore, in a strain unable to express Atp11p, the native size of Atp12p was not found to be modified, indicating that Atp11p and Atp12p are not part of the same complex (49). Based on the results reported here, it will be particularly interesting to see whether Fmc1p belongs to or is required for the formation of the Atp12 oligomer. Experiments to resolve this question are in progress.

Atp12p and Atp11p interact directly with the F1α and F1β proteins, respectively, and this is presumed to facilitate the formation of αβ heterodimers by protecting these two subunits of the ATP synthase from non-productive interactions (15, 16).

If Fmc1p actually mediates the αβ dimerization by assisting Atp12p at elevated temperatures, how can we explain that...
antibodies against the F₁-α protein coimmunoprecipitated the F₁-β protein in mitochondrial extracts of the fmc1 mutant. As will be presented elsewhere, the F₁-α and F₁-β proteins were found by immunocytochemistry to be part of the same inclusion bodies in mitochondria of the Δfmc1 mutant.⁢ Thus, even when they fail to interact properly, the F₁-α and F₁-β proteins still remained associated physically, which could explain their co-immunoprecipitation from the Δfmc1 mutant.

Severe perturbations at the level of the F₁ sector of the ATP synthase were seen in the Δfmc1 mutant. It is not known whether or not the F₁ sector is assembled in the Δatp11 and Δatp12 mutants. We believe that Fmc1p is not directly re-

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Acknowledgments—We are very grateful to A. Tzagoloff for the gift of Δatp12 and Δatp11 strains and antibodies against Atp12p and to G. Lauquin for antibodies against Aac2p. We thank A. Tzagoloff and our colleagues B. Guérin, M. Rigoulet, G. Dujardin, C. Herbert, O. Groudinski, M.-F. Giraud, and D. Brethis for their interest and helpful discussions. We are very grateful to C. Herbert for a critical reading of the manuscript and English revisions. We are grateful to X. Grandier-Vazeille for help in preparing anti-Fmc1p antibodies.

³ B. Coulary, L. Lefebvre-Legendre, D. Savouré, J. Schaeffer, and J.-P. di Rago, manuscript in preparation.
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J. Biol. Chem. 2001, 276:6789-6796.
doi: 10.1074/jbc.M009557200 originally published online November 28, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M009557200

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