The Modulation in Subunits e and g Amounts of Yeast ATP Synthase Modifies Mitochondrial Cristae Morphology*

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Subunits e and g of Saccharomyces cerevisiae ATP synthase are required to maintain ATP synthase dimorphic forms. Mutants devoid of these subunits display abnormal mitochondrial morphologies. An expression system regulated by doxycycline was used to modulate the expression of the genes encoding the subunits e and g. A decrease in the amount of subunit e induces a decrease in the amount of subunit g, but a decrease in the amount of subunit g does not affect subunit e. The loss of subunit e or g leads to the loss of supramolecular structures of ATP synthase, which is fully reversible upon removal of doxycycline. In the absence of doxycycline, mitochondria present poorly defined cristae. In the presence of doxycycline, onion-like structures are formed after five generations. When doxycycline is removed after five generations, cristae are mainly observed. The data demonstrate that the inner structure of mitochondria depends upon the ability of ATP synthase to make supramolecular structures.

FₐFₒ-ATP synthase is a molecular rotary motor that is responsible for aerobic synthesis of ATP. It exhibits a head piece (catalytic sector), a base piece (membrane sector), and two connecting stalks. The sector F₁, containing the head piece is a water-soluble unit that retains the ability to hydrolyze ATP when in soluble form. Fₒ is embedded in the membrane and is mainly composed of hydrophobic subunits forming a specific proton-conducting pathway. When the F₁ and Fₒ sectors are coupled, the enzyme functions as a reversible H⁺-transporting ATPase or ATP synthase (1–4). The two connecting stalks are made of components from F₁ and Fₒ. The central stalk is a part of the rotor of the enzyme. The second stalk, which is part of the stator, connects F₁ and hydrophobic membranous components of the enzyme probably via a flexible region (5). High resolution x-ray crystallographic data have led to solving the structure of F₁ (6–9) from different sources. Stock et al. (10) reported the 3.9-Å resolution x-ray diffraction structure of the Saccharomyces cerevisiae F₁ associated with a c₆₀-ring oligomer.

In Escherichia coli, Fₒ is composed of only 3 subunits, whereas the mitochondrial Fₒ of mammals is composed of 10 different subunits (11). The same 10 components have been identified in the S. cerevisiae enzyme (12–14). Among these additional subunits not present in bacterial and chloroplast ATP synthases, subunits e and g are not involved in ATP synthesis function but are involved in the dimerization/oligomerization of the mitochondrial ATP synthase (13, 15) because the absence of subunits e and g in the respective null mutants abolishes the ability of ATP synthase to make supramolecular structures. Subunits e and g are small hydrophobic proteins with an Nₐ–Cₐ orientation in the inner mitochondrial membrane (12, 16) with a unique transmembrane span probably located at the interface between two ATP synthase monomers. Subunit e can form homodimers upon oxidation via its unique cysteine residue (17), and it has been reported that the transmembrane span contains a conserved GXXXG membranous dimerization motif that is essential for the stability of supramolecular structures of the yeast ATP synthase (18).

Surprisingly, mutant mitochondria devoid of either subunit e or g were found to have numerous digitations and onion-like structures, thus suggesting a link between dimerization/oligomerization of the ATP synthase and cristae morphology (15, 19, 20). Because these alterations in the inner structure of mitochondria were observed on mutant cells grown for at least 30 generations after inactivation of the genes, it was important to check mitochondrial morphology during modulation of the expression of the genes encoding subunits e and g. In the present work the expression of the TIM11 (ATP21) and ATP20 genes encoding subunits e and g, respectively, was modulated by using a tetracycline-regulatable promoter system (21). The addition of the tetracycline derivative, doxycycline, in the growth medium decreased the amount of subunits e and g efficiently and led to the loss of supramolecular structures of ATP synthase and an alteration of the cristae morphology, two features that were reversible upon removal of doxycycline.

EXPERIMENTAL PROCEDURES

Materials—Doxycycline was purchased from ICN Biochemicals Inc. All other reagents were of reagent grade quality.

Yeast Strains and Plasmids—The S. cerevisiae strain D273-10B/ HU (MATα, met6, ura3, his3) (22) was the wild type strain. The null mutants ΔTIM11 and ΔATP20 were constructed by a PCR-based mutagenesis, and the kan+ gene was removed (23).

The TIM11 gene was amplified by PCR and cloned in BamHI and PstI sites of the pCM189 vector (21). The ATP20 gene was amplified by PCR and cloned in NotI and PstI sites of the pCM189 vector. The PvuII fragments containing TIM11 and ATP20 genes of the resulting vectors were purified and ligated in the integrative vector pRS316 cleaved by EcoRV. The integrative vectors were cleaved by NheI and inserted in the his 5 locus of the respective null mutants ΔTIM11 and ΔATP20,
were analyzed by Western blot (Fig. 1). Subunit i, a membrane containing or not doxycycline (20 μg/ml). The rhö cell production in cultures was measured on glycerol plates supplemented with 0.1% glucose. Mitochondria were isolated from cells, grown with or without doxycycline, and harvested at an A_{600 nm} of 2–3 corresponding to the logarithmic growth phase. They were prepared from protoplasts as described previously (25). Protein amounts were determined according to Lowry et al. (26) in the presence of 5% SDS using bovine serum albumin as standard. The ATPase activity with and without oligomycin was measured at pH 8.4 in the presence of 0.375% Triton X-100 to remove the endogenous inhibitor of F_{1} (27).

Electrophoretic and Western Blot Analyses—SDS-gel electrophoreses were conducted as described in Ref. 28 in the absence of a reducing agent. Whole yeast cell extracts were performed according to Egner et al. (29). Western blot analyses were described previously (30). Polyclonal antibodies against subunits e and g were raised against amino acid residues 69–82 and 31–45, respectively. Antibodies against subunits e, g, and i were used with dilutions of 1:10,000. Membranes were incubated with peroxidase-labeled antibodies and visualized with the ECL reagent of Amersham Biosciences. Molecular mass markers (Benchmark Prestained Protein Ladder) were from Invitrogen. BN-PAGE experiments were conducted as described previously (31, 32). Mitochondria (1 mg of protein) were incubated for 30 min at 4 °C with 0.1 ml of digitonin solution with the indicated digitonin to protein ratios. The extracts were centrifuged at 4 °C for 15 min at 40,000 × g and aliquots (40 μl) were loaded on the top of a 3–13% polyacrylamide slab gel. After electrophoresis the gel was incubated in a solution of 5 mM ATP, 5 mM MgCl2, 0.05% lead acetate, 50 mM glycine-NaOH, pH 8.4, to reveal the ATPase activity (33, 34), or the gel was stained with Coomassie Brilliant Blue.

Ultrastructural Studies—Freezing and freeze substitution of yeast cell pellets were performed as described previously (15). Preparation of mitochondria for electron microscopy was as follows. The mitochondrial pellets were suspended at 4 °C in the isolation buffer containing 1% glutaraldehyde for 1 h and then 2.5% glutaraldehyde for 2 h. Mitochondria were washed at 4 °C with the isolation buffer and then with 25 mM phosphate buffer, pH 7.2, in 0.6 M mannitol. The samples were incubated overnight at 0 °C with 1% osmic tetroxide in 0.1 M phosphate buffer, pH 7.2. The samples were washed with cold water, stained for 1 h in 2% uranyl acetate at 4 °C, embedded in 1% agarose, dehydrated progressively with acetone, and infiltrated with Araldite. Grids were examined at 120 kV using a Philips Tecnai 12 Biotwin microscope.

RESULTS

The Decrease in the Amount of Subunits e and g Destabilizes the Supramolecular Structures of the Yeast ATP Synthase—To modulate the expression of the TIM11 and ATP20 genes that encode subunits e and g, respectively, we used an expression system in which transcription was driven by a hybrid tetO-CYC1 promoter through the action of a tetR-VP16 activator (21). With this system, expression from the promoter is regulated by tetracycline or derivatives such as doxycycline that are added to the medium currently used to grow yeast cells.

The growth of the wild type and respective mutant cells was followed during a 10-h period after doxycycline addition. The doubling time was not affected by the presence of doxycycline in the growth medium, which contained lactate as a non-fermentable carbon source (Table I). We have previously reported that null mutants in either TIM11 or ATP20 genes converted into rho− cells with high frequency (15). Indeed, the rho− conversion occurred only after a long incubation period (20 h) with doxycycline (Table I).

The repression of the TIM11 gene was followed as a function of time in the presence of doxycycline in the growth medium. tetO-TIM11 cells were harvested, the cell pellets were treated as described in Egner et al. (29), and aliquots of the samples were analyzed by Western blot (Fig. 1). Subunit i, a membraneous component of the yeast ATP synthase, was used as a control. A fast decrease in the amount of subunit e was observed in the presence of doxycycline, whereas the subunit i amount was not modified (Fig. 1A). Although the ATP20 gene encoding subunit g was not dependent on doxycycline, the amount of subunit g also decreased in the same way as subunit e (Fig. 1B), which is in agreement with previous observations showing the loss of subunit g in mutants either devoid of subunit e (13) or altered in the putative membranous dimerization domain of subunit e (18).

Mitochondria were prepared from tetO-TIM11 cells grown with or without doxycycline. Western blot analyses showed also a simultaneous decrease in the amounts of subunits e and g at the mitochondrial level and no decrease in the amount of the control protein, subunit i (data not shown). In a control experiment, mitochondria were isolated from the wild type cells grown for 20 h in the presence or in the absence of doxycycline. From Western blot analyses no decrease in the amount of nuclear encoded subunits e, i, and g and mitochondrially encoded protein subunit 6 (another component of F_{0}) was observed upon incubation with the antibiotic (Fig. 2, A−C), indicating that doxycycline at the concentration used in the culture medium did not alter the mitochondrial protein synthesis in a wild type context. Consequently, the decrease in the amounts of subunits e and g reported in Fig. 1 was the result of the decrease in the expression of the TIM11 gene.

The ATP20 gene that encodes subunit g was also made dependent on doxycycline. Mitochondria were prepared from tetO-ATP20 cells grown for 4, 10, and 20 h in the presence of the antibiotic. By Western blot analyses (Fig. 2, D and E) only a faint band corresponding to subunit g was still present in
mitochondria after 10 and 20 h of growth with doxycycline. In contrast to the observed decrease in the amounts of subunits e and g induced by the attenuated expression of subunit e, the progressive decrease in the subunit g amount had no effect on the presence of subunit e. This result was expected because as reported previously, the loss of subunit g in ∆ATP20 cells did not alter the presence of subunit e (13). A 21.4-kDa band was present in Fig. 2E. This band corresponds to a homodimer of subunit e resulting from the formation of a disulfide bond between two subunits e and involving the Cys-28, which is located in the intermembrane space (17). We have previously reported that such a band pre-exists in the absence of oxidizing agent in mitochondrial membranes of mutants devoid of subunit g, thus showing an alteration of the environment of subunit e by the loss of subunit g (18).

The involvement of subunits e and g in the formation of supramolecular structures of ATP synthase has been reported previously (13, 15). BN-PAGE analyses of mitochondrial digitonin extracts prepared from tetO-TIM11 cells grown for 2, 6, and 12 h in the presence of doxycycline are shown in Fig. 3. The positions of F₁ and ATP synthase in the gel are indicated by the white bands resulting from the precipitation of lead phosphate upon incubation of the slab gel with ATP and lead acetate. In this experiment, digitonin to protein ratios of 0.75 and 1.5 g/g were chosen to extract the membranous proteins. With such ratios, F₁F₀ oligomer, F₁F₀ dimers, F₁F₀ monomer, and F₁ are clearly observed. The two bands migrating at an acrylamide concentration of 6% correspond to the dimeric form of the enzyme. Why the dimeric form is a doublet is not clear. It could correspond to different associations of ATP synthases or different compositions in subunits and associated lipids. The rationale in the experiment of Fig. 3 was to detect the presence of oligomeric forms of the ATP synthase by using a digitonin to protein ratio of 0.75 g/g and the presence of the dimeric and the monomeric forms of the enzyme by using a digitonin to protein ratio of 1.5 g/g. As shown previously, the high detergent to protein ratio removes subunits e and g, leading to the dissociation of the supramolecular structures of the wild type ATP synthase, which converts to the monomeric form (13). Thus, with a digitonin to protein ratio of 1.5 g/g to extract the ATP synthase, the densitometric analyses of the Coomassie Blue-stained gel indicated that 16% of the ATP synthase was in the monomeric form in the control experiment (without doxycycline). After 2 h of growth in the presence of doxycycline, oligomeric forms of ATP synthase were still present at a digitonin/protein ratio of 0.75 g/g, and 70% of the ATP synthases were in the dimeric forms at a digitonin/protein ratio of 1.5 g/g. 30–35% and only 5% of ATP synthases were in the dimeric forms after 6 and 12 h of growth with doxycycline, respectively (Fig. 3). The arrow indicates the position of the IIIIV₂ supercomplex that is present in digitonin extracts at a digitonin to protein ratio of 1.5 g/g (48).

BN-PAGE analyses of tetO-ATP20 mitochondrial digitonin extracts indicated that growth with doxycycline also greatly altered the stability of supramolecular structures of ATP synthase (Fig. 4). The densitometric analysis of the Coomassie Brilliant Blue-stained gel revealed that with a digitonin to protein ratio of 1.5 g/g, 40% of ATP synthases were in the dimeric forms in the mitochondrial digitonin extract obtained from tetO-ATP20 cells grown for 4 h with doxycycline. With the same digitonin to protein ratio the dimeric forms represented 10 and 6% of the sum of monomeric and dimeric forms present in the mitochondrial digitonin extracts of tetO-ATP20 cells grown for 10 and 20 h with doxycycline, respectively. In addition a high ATPase activity corresponding to free F₁ was detected after 20 h of growth with doxycycline, which is in agreement with the spontaneous rho− cell conversion shown in Table I.

The Loss of Supramolecular Structures of ATP Synthase Is Fully Reversible—The subsequent inability of ATP synthase to make high molecular structures was reversed by incubation of the cells in the absence of doxycycline. The tetO-TIM11 cells were incubated first for 12 h with the antibiotic in conditions that eliminate subunits e and g. The cells were harvested and grown in the same medium but without doxycycline for 6, 12, and 20 h. Mitochondria were prepared for each incubation time and analyzed by Western blot (Fig. 5) and by BN-PAGE (Fig. 6). Clearly, the increase in the amount of subunit e correlates with
an increase in the amount of subunit g and in the amount of dimeric and oligomeric forms of ATP synthase, whereas a concomitant decrease in the monomeric form is observed.

Next, the evolution of the ATPase activity was followed during the decrease in the expression of the TIM11 gene and during its expression upon removal of doxycycline. The ATPase activity was measured with mitochondria prepared from cells grown in the presence of doxycycline for 6, 12, and 20 h (Fig. 7A). In another experiment, cells grown for 12 h in the presence of doxycycline were harvested and incubated in the culture medium without the antibiotic for 6, 12, and 20 h. Mitochondria were prepared, and aliquots (25 μg of protein) were submitted to Western blot analysis. The blots were probed with antibodies against subunits e and i (A) and against subunits g and i (B).

Fig. 4. The decrease in the expression level of subunit g leads to the loss of supramolecular structures of the ATP synthase. Mitochondria were prepared from tetO-ATP20 cells grown with (+) or without (−) doxycycline (dox.) for 4, 10, and 20 h. Mitochondria (500 μg of protein) were incubated with 50 μl of a digitonin solution at the indicated digitonin to protein ratio. After centrifugation, 40 μl of the supernatant was loaded on the top of a 3–13% polyacrylamide slab gel. After BN-PAGE analysis, the bands were revealed by the ATPase activity (top panel) and by Coomassie Blue staining (lower panel). %T, acrylamide concentration.

Fig. 5. The removal of doxycycline from the culture medium of tetO-TIM11 cells induces the synthesis of subunit e and allows the presence of subunit g in mitochondrial membranes. Cells were grown with (+) or without (−) doxycycline (dox.) for 12 h and then harvested and incubated in the culture medium without the antibiotic for 6, 12, and 20 h. Mitochondria were prepared, and aliquots (25 μg of protein) were submitted to Western blot analysis. The blots were probed with antibodies against subunits e and i (A) and against subunits g and i (B).

Fig. 6. The removal of doxycycline from the culture medium of tetO-TIM11 cells allows the presence of supramolecular structures of ATP synthase in mitochondrial digitonin extracts. Cells were grown with (+) or without (−) doxycycline (dox.) for 12 h and then harvested and incubated in the culture medium without the antibiotic for 6, 12, and 20 h. Mitochondria were prepared, and mitochondrial digitonin extracts were submitted to BN-PAGE analysis as described in Fig. 3. After BN-PAGE analysis, the bands were revealed by the ATPase activity (top panel) and by Coomassie Blue staining (lower panel). %T, acrylamide concentration. The arrow indicates the position of the III1IV2 supercomplex that is present in digitonin extracts at a digitonin to protein ratio of 1.5 g/g.

Fig. 7. The decrease in the expression level of subunit e decreases the ATPase activity. A, mitochondria were prepared from tetO-TIM11 cells grown without and with doxycycline (dox.) for 6, 12, and 20 h. B, mitochondria were prepared from tetO-TIM11 cells grown for 12 h in the presence of doxycycline and then harvested and grown again for 6, 12, and 20 h in the absence of doxycycline. The ATPase activity in the absence (O) or presence (□) of oligomycin was measured as described under “Experimental Procedures.” Units are in micromoles of Pi/min/mg of protein.

The Modulation of the Expression of TIM11 and ATP20 Genes Correlates with an Altered Mitochondrial Morphology—We have reported previously that the mitochondrial morphology of yeast mutants devoid of either subunit e or g was altered, showing onion-like structures inside the yeast cells. The purpose of the following experiments was to follow the gradual change of cristae during the repression and the derepression of TIM11 and ATP20 genes. tetO-TIM11 cells grown with and without doxycycline
The alteration of the inner structure of mitochondria is reversible. TetO-TIM11 cells were grown without doxycycline (A), with doxycycline for 4 h (B), with doxycycline for 8 h (C), with doxycycline for 12 h (D), and with doxycycline for 12 h and then harvested and grown again for 12 h in the absence of doxycycline (E). The samples were prepared as described in under “Experimental Procedures.” They were observed by transmission electron microscopy. The black triangles indicate mitochondrial cristae of cells grown without doxycycline. The black arrows indicate inner membrane structures, which stem from the same place on the organelle. The bars indicate 100 nm. m, mitochondria.

In the absence of doxycycline, mitochondria displayed poorly defined cristae (Fig. 8A). After 4 h of growth in the presence of doxycycline, mitochondria had inner structures different from those found in the control mitochondria. These structures are clearly defined sheets separated by a constant width and which stem from the same place on the organelle (Fig. 8B). After 6 h of growth with doxycycline they are parallel structures that traverse the organelle and induce elongating mitochondria (Fig. 8C). Finally, after 12 h of growth with doxycycline, elongated and large mitochondria containing the typical onion-like structures were observed in null mutants ΔTIM11 and ΔATP20 cells were observed (Fig. 8D). In another experiment, tetO-TIM11 cells were grown first for 12 h with doxycycline, washed, and grown again for 12 h without doxycycline. The resulting cells had mitochondria without the onion structures and poorly defined cristae but still with inner membrane structures traversing the organelle (Fig. 8E), thus indicating a partial recovery of cristae morphology. Mitochondria were also prepared from tetO-TIM11 cells grown without doxycycline (Fig. 8F) and with doxycycline for 2, 6, and 12 h (Fig. 9, A, B, C, and D, respectively). Electron microscopy of isolated mitochondria revealed more clearly the inner structures of organelles. As a function of time of growth with doxycycline, the inner structure of isolated mitochondria changed progressively, showing large vesicles and circular membranes inside the organelles (Fig. 9D). Mitochondria were also prepared from tetO-TIM11 cells grown for 12 h with doxycycline and then washed and grown again for 12 h without doxycycline. In agreement with data shown in Fig. 8E, the removal of doxycycline from the growth medium led to a partial recovery of the inner structure of organelles (Fig. 9E). In another set of experiments tetO-ATP20 cells were grown first with doxycycline and second without doxycycline. Cells displayed the same pattern of structures for mitochondria as those observed with tetO-TIM11 cells grown with and without doxycycline (data not shown). As reported in Fig. 9F mitochondria isolated from tetO-ATP20 cells grown for 10 h with doxycycline displayed membrane sheets of inner membrane traversing the organelles.

**DISCUSSION**

The importance of the subunits e and g in the edification of supramolecular structures of ATP synthase and in the mitochondrial morphology has been already reported. Immunogold electron microscopy has shown that the null mutants cells in either TIM11 or ATP20 genes encoding subunits e and g, respectively, displayed elongated mitochondria and onion structures composed of two to three concentric layers of double leaflets of inner mitochondrial membrane contained inside a continuous envelope of outer membrane, a feature that is different from the mitochondrial nebkenkern occurring during Drosophila melanogaster spermatogenesis (35). On the other hand it has been demonstrated in S. cerevisiae that subunits e and g of the mitochondrial ATP synthase are involved in the dimerization/oligomerization of the enzyme (13, 15). Moreover abnormal associations of ATP synthase resulting from cross-linking in vivo eliminates mitochondrial cristae (20). As a consequence there is a link between ATP synthase and mitochondrial cristae morphology. However, the link between the ability of the mitochondrial ATP synthase to make supramolecular structures and the cristae morphology is still non-elucidated. The hypothesis that the association of ATP synthase dimers generates the tubular cristae by protrusion of the inner peripheral membrane has been proposed by Allen (36) on the basis of observations of the tightly ordered pattern of ATP synthases on the tubular cristae in Paramecium multimicronucleatum mitochondria (37). The absence of tubular cristae in null mutants devoid of either subunit e or g and therefore devoid of supramolecular structures of ATP synthases favors this hypothesis. Because these observations were made from null mutants grown for at least 30 generations after inactivation of the TIM11 or ATP20 genes encoding subunits e and g this hypothesis is likely to be correct.
genes, it was important to search for the correlation between the progressive loss of supramolecular structures of ATP synthase and the alteration of cristae morphology but also between the recovery of supramolecular structures of ATP synthases and the recovery of cristae morphology. In the absence of conditional mutants in subunit e or g we chose the modulation of...
the expression of subunits e and g by using a tetracycline-regulatable promoter system. A correlation between the loss of subunit e or g and the loss of supramolecular structures of ATP synthases was found from Western blot and BN-PAGE experiments. In addition, electron microscopy experiments showed that the progressive loss of subunit e or g during a period corresponding to five generations led to mitochondria whose inner structure was altered more and more. Moreover, the alteration of the inner mitochondrial structure was reversible upon removal of doxycycline, which corresponded to a full recovery of supramolecular structures of ATP synthase and of subunits e and g. However, whole recovery of the inner structures of mitochondria was not fully obtained after 12 h of growth without antibiotic (corresponding to five generations), whereas amounts of subunits e and g were recovered, and all ATP synthases were under supramolecular forms in digitonin extracts. We make the hypothesis that the sheets of inner membranes in onion-like structures are not immediately replaced by new cristae containing newly synthesized ATP synthases bearing the e and g subunits. Additional experiments will be done to test this proposal.

Interesting points raised by the transmission electron microscopy observations are the differences in the type of mitochondria observed in situ and isolated mitochondria. Isolated wild type mitochondria are circular and appear more relaxed than those present in their cellular context. Mitochondria isolated from cells grown in the presence of doxycycline are also circular but display concentric membranes in the matrix space. This aspect probably resulted from an alteration of the structure of organelles during isolation. Another point raised is the type of inner structures of mitochondria inside the yeast cells. Wild type cells display mitochondria with thin, short, and poorly defined cristae, whereas mitochondria containing low amounts of subunit e or g have well defined sheets of inner membrane that traverse the organelle. We hypothesize that this difference in the structure reflects two aspects of the inner structure of mitochondria, most probably tubular shaped cristae without doxycycline and lamellar cristae in the presence of doxycycline and that the presence or absence of supramolecular structures of mitochondrial ATP synthase controls both aspects. We interpret the formation of onion-like structures as an uncontrolled biogenesis of sheets of inner membrane, which promotes elongation of mitochondria and leads to round organelles inside the cells. Finally these organelles fuse to produce the onion-like structures observed in ΔTIM11 and ΔATP20 null mutant strains.

We report in Fig. 7 that the full loss of subunits e and g decreased the mitochondrial ATPase activity by 50%. Indeed, such a decrease is not sufficient to affect growth by oxidative phosphorylation (38). This decrease corresponds either to a decrease in the amount of ATP synthase per mg of mitochondrial protein or to a reduced specific ATPase activity. Western blot analyses performed with isolated tet-O-TIM11 and tetO-ATP20 mitochondria revealed that the amount of subunit i, another F0 component, was not modified in the presence or in the absence of doxycycline (Figs. 2 and 5), which means a decrease in the turnover of the enzyme is likely. Such an ATPase activity decrease has not been reported with TIM11 and ATP20 genes displayed mitochondrial ATPase activities that are only 44–45% inhibited by oligomycin. The result was corroborated by an increase in the spontaneous conversion of null mutant cells in rho cells (15), a conversion that is always observed upon modulations of genes encoding ATP synthase subunits of S. cerevisiae. As a result, it is concluded that the anomalous mitochondrial morphologies found either in null mutants in TIM11 and ATP20 genes or upon the increase in the expression of subunits e and g are the consequence of the inability of the ATP synthase to make supramolecular structures and not the consequence of the spontaneous conversion of yeast cells in rho cells.

Other components such as Mmm1p (39, 40) and Mgm1p are also involved in the internal morphology of mitochondria. The dynamin-related GTPase Mgm1p is an inner membrane protein that is peripherically associated with the inner membrane. It participates in mitochondrial fusion (39–44), and it has been shown to have a major function in structuring the cristae membrane (45, 46). A relationship between Mgm1p and subunit e has been reported recently (47). The mature form of Mgm1p has been shown to be required for subunit e stability, and it has been proposed that Mgm1p might be functionally linked to a protease that degrades subunit e. As a consequence Mgm1p should be considered as an upstream regulator of subunit e stability and therefore of ATP synthase associations and as a result a regulator of cristae morphology.

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