F_1-dependent translation of mitochondrially encoded Atp6p and Atp8p subunits of yeast ATP synthase

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The ATP synthase of yeast mitochondria is composed of 17 different subunit polypeptides. We have screened a panel of ATP synthase mutants for impaired expression of Atp6p, Atp8p, and Atp9p, the only mitochondrially encoded subunits of ATP synthase. Our results show that translation of Atp6p and Atp8p is activated by F_1 ATPase (or assembly intermediates thereof). Mutants lacking the α or β subunits of F_1, or the Atp11p and Atp12p chaperones that promote F_1 assembly, have normal levels of the bicistronic *ATP8*/*ATP6* mRNAs but fail to synthesize Atp6p and Atp8p. F_1 mutants are also unable to express $ARG8^m$ when this normally nuclear gene is substituted for *ATP6* or *ATP8* in mitochondrial DNA. Translational activation by F_1 is also supported by the ability of *ATP22*, an Atp6p-specific translation factor, to restore Atp6p and to a lesser degree Atp8p synthesis in the absence of F_1. These results establish a mechanism by which expression of *ATP6* and *ATP8* is translationally regulated by F_1 to achieve a balanced output of two compartmentally separated sets of ATP synthase genes.

F1-ATPase | mitochondria | *Saccharomyces cerevisiae* | translational regulation

The preservation of functional mitochondria and chloroplasts during cell growth and division depends on a large pool of genetic information resident in the nucleus and of a more limited set of genes present in the genomes of the organelles themselves. The proteins encoded by the chloroplast and mitochondrial genomes interact with partner proteins derived from nuclear genes to form hetero-oligomeric complexes that function, respectively, in photosynthesis and oxidative phosphorylation. This circumstance has necessitated the evolution of mechanisms for insuring a balanced output of the two spatially separate sets of genes. Since mitochondrial and chloroplast proteins are not exported to the cytoplasm, it stands to reason that each organelle must bear the burden of adjusting expression of its genes to the demands imposed by the nucleo-cytoplasmic system.

Regulation of organellar gene expression was first demonstrated by studies of the b_6f complex of *Chlamydomonas rheinhardtii* chloroplasts (1). Translation of the cytochrome *f* component on chloroplast ribosomes was shown to depend on its association with subunit IV and cytochrome b_6, two other subunits of the complex, which unlike cytochrome *f*, are synthesized on cytoplasmic ribosomes. This regulatory mechanism, termed "control by epistasis of synthesis" (CES), although differing in detail, was shown to operate in the biogenesis of other chloroplast proteins including photosystem II (2). The generality of CES as a means for adjusting the rate of translation of specific organellar gene products to the availability of their imported partners is underscored by the discovery that in *Saccharomyces cerevisiae* expression of the mitochondrial gene for the Cox1p subunit of cytochrome oxidase is coupled to a downstream assembly event through Mss51p, a translational activator of *COX1* mRNA (3).

Currently, little is known about the mechanism by which biogenesis of the mitochondrial ATP synthase (F_1-F_0 ATPase) is regulated. Assembly of a catalytically active F_1 oligomer occurs independently of F_0 (4) and entails an initial chaperone-dependent association of the α and β subunits into a hexamer constituting most of the protein mass of F_1 (5). Chaperones also play important roles in assembly of F_0, having been shown to promote oligomerization of the Atp9p ring (6) and association of the ring with Atp6p (7). Interaction of Atp6p with the Atp9p ring is probably a late assembly event as the resultant complex can cause an unregulated proton leak leading to dissipation of the mitochondrial membrane potential. The incorporation of Atp6p into the complex has, therefore, been inferred to occur at a stage when the structural elements necessary for coupling proton transfer to ATP synthesis or hydrolysis are already in place.

In the present study we present evidence that translation of both Atp6p and Atp8p in yeast mitochondria is contingent on the presence of assembled but not necessarily catalytically active F_1. Our evidence, however, indicates that unlike the CES assembly-dependent mechanism for regulation of Cox1p expression, Atp6p and Atp8p translation is subject to direct activation by F_1, thus defining an alternate mechanism for self regulation of a mitochondrial enzyme with a dual genetic identity.

Results

Impaired F_1 Assembly Affects Expression of *ATP6 and ATP8*. In the absence of the Atp11p and Atp12p chaperone or of their β and α subunit substrates, assembly of the F_1 $\alpha_3\beta_3$ hexamer is blocked and the two subunits are deposited as large inactive aggregates in the mitochondrial matrix (5). To assess if F_1 affects expression of the mitochondrially encoded subunits of F_0, we assayed their translation in vivo in Δ*atp11*, Δ*atp12*, Δ*atp1*, and Δ*atp2* mutants by pulse-labeling of cells in the presence of cycloheximide to inhibit cytoplasmic protein synthesis. Under these conditions incorporation of radiolabel into Atp6p and Atp8p was 10–18% of that measured in the wild-type strain (Fig. 1).

To exclude the possibility that the reduced labeling of Atp6p and Atp8p is an artifact resulting from the α and/or β subunit aggregates present in the F_1 mutants, we also examined a double Δ*atp1* Δ*atp2* null mutant, devoid of such aggregates. The results of the in vivo translation assay indicated that the double mutant, like the single α and β subunit mutants, failed to show any significant incorporation of the radioactive precursors into Atp6p and Atp8p (Fig. 1).

The in vivo assays also revealed a significant inhibition of Cox1p synthesis (Fig. 1). This is probably a secondary effect of the ATP synthase deficiency as mutations in Atp6p and other subunits of F_0 have been shown to suppress translation of Cox1p (8).

***ATP6*/*ATP8* mRNA Is Normally Transcribed and Processed in F_1 Mutants.** The marked reduction of Atp6p and Atp8p labeling in the F_1 mutants could be the product of decreased transcription, mRNA maturation, translation, or enhanced turnover of the newly translated but unassembled subunits. *ATP6* and *ATP8* are cotranscribed with *COX1*, the gene for subunit 1 of cytochrome oxidase (Fig. 2*A*) (9). The primary transcript is processed in several steps to produce two distinct bicistronic *ATP8*/*ATP6* mRNAs of 5.2 and 4.6 kb (10) and a *COX1* precursor RNA that undergoes a series of splicing

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The authors declare no conflict of interest.

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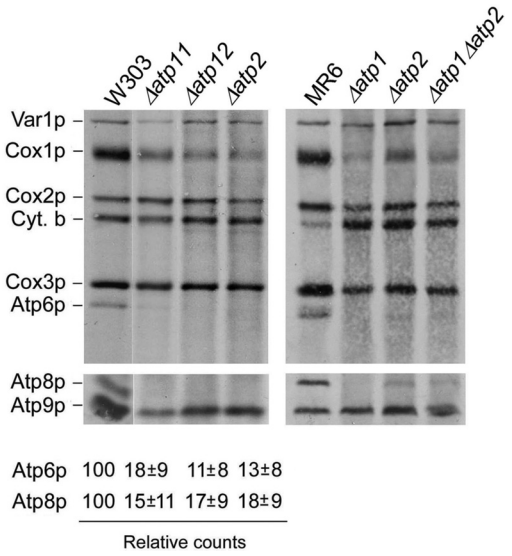

Fig. 1. In vivo labeling of mitochondrial gene products in wild-type and F_1 mutants. The respiratory competent strains W303 and MR6, and F_1 mutants with the indicated genotypes were grown in rich galactose medium and labeled for 20 min with [^{35}S]methionine + [^{35}S]cysteine in the presence of cycloheximide. Total cellular extracts were separated by SDS/PAGE in two different polyacrylamide gels prepared with a 30:0.8 ratio of acrylamide and bisacrylamide. Upper gel: 12% polyacrylamide gel containing 4 M urea and 25 glycerol. Lower gel: 17.5% polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane and exposed to X-ray film. The bands corresponding to Atp6p and Atp8p were quantified with a phoshorimager and normalized to Cox3p. The averages of 4–5 independent experiments are expressed as percentage of wild-type.

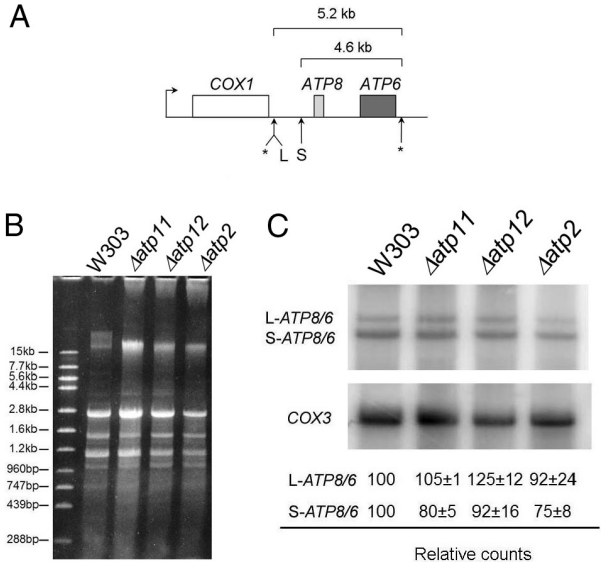

Fig. 2. *ATP8/6* mRNAs are normally transcribed and processed in F_1 mutants. (*A*) Diagram showing transcription of the primary polycistronic *COX1/ATP8/ATP6* RNA from a site upstream of *COX1*. Cleavage sites that produce the mature messengers are shown by asterisks. The 5.2- and 4.6-kb mRNAs are the result of cleavages at L and S, respectively. *COX1*, which contains multiple introns in the W303 strain, is not drawn to scale. (*B*) Mitochondrial RNAs isolated from W303 and Δ*atp11*, Δ*atp12*, and Δ*atp2* mutants were separated on a 1% agarose gel, stained with ethidium bromide (left panel) and transferred to a Nytran membrane that was hybridized with ^{32}P labeled *ATP6*- and *COX3*-specific probes. The *ATP6/ATP8* mRNAs was quantified with a phosphorimager and normalized to the *COX3* mRNA.

reactions to form the mature mRNA. Some strains of yeast contain the *ENS2* gene downstream of *ATP6* (11). *ENS2* codes for a DNA endonuclease, and when present is part of the *ATP8/6* mRNAs. The W303 strain used in the present study lacks this gene.

A requirement of F_1 for transcription or processing of the *COX1/ATP8/ATP6* region was excluded by Northern analysis of total mitochondrial RNAs in the parental respiratory competent strain and in Δ*atp2*, Δ*atp11*, and Δ*atp12* mutants. The *ATP8/ATP6* mRNAs were quantified and normalized to the *COX3* mRNA to correct for any differences ascribable to secondary ρ^- or ρ^0 mutants in the cultures. The Northern hybridizations indicated that the two *ATP8/ATP6* mRNAs in the F_1 mutants were present at levels comparable to those of the wild-type strain (Fig. 2*B*).

F_1 Upregulates Translation of Atp6p and Atp8p. The Northern hybridization results pointed to either reduced translation or enhanced turnover of newly synthesized Atp6p and Atp8p in F_1 mutants. To distinguish between these two possibilities, the mitochondrial *ATP6* and *ATP8* genes were replaced with *ARG8^m*, a version of nuclear *ARG8* recoded for expression in mitochondria (12). *ARG8* codes for acetylornithine aminotransferase, a mitochondrial protein that functions in arginine biosynthesis (13). The Δ*atp6*::*ARG8^m* (8) or Δ*atp8*::*ARG8^m* (this study) alleles were substituted for wild-type mitochondrial DNA (mtDNA) in Δ*atp12* Δ*arg8*, Δ*atp2* Δ*arg8* double mutants, and in an Δ*atp1* Δ*atp2* Δ*arg8* triple mutant. Expression of mitochondrial *ARG8^m*, detected as arginine-independent growth, should not be affected by mutations in *ATP12* or *ATP2* if F_1 is required for stability of Atp6p and Atp8p. Alternatively, if F_1 upregulates translation of *ATP6* and *ATP8*, expression of the *ARG8^m* reporter gene should be compromised in an Δ*atp2* or Δ*atp12* mutant.

The introduction into MR6, a respiratory competent Δ*arg8* strain, of mtDNA with the Δ*atp6*::*ARG8^m* or Δ*atp8*::*ARG8^m* allele abolished its arginine requirement, confirming expression of *ARG8^m* from the *ATP6* and *ATP8* loci (Fig. 3). Neither allele, however, was able to confer arginine-independent growth when combined with Δ*atp2*, Δ*atp12*, or both Δ*atp1* and Δ*atp2* mutations (Fig. 3). Since ATP synthase mutants tend to convert at high frequency to ρ^- and ρ^0 mutants that are deficient in mitochondrial protein synthesis (6, 14), the arginine auxotrophy could also have resulted from a loss of mtDNA. This was excluded by the presence of 40–60% of ρ^+ cells in the cultures (Fig. 3). These results confirm that the compromised expression in F_1 mutants of *Atp6p* and *Atp8p* (or of *ARG8^m* when present at either the *ATP6* or *ATP8* locus) is a consequence of translational downregulation.

To determine if overexpression of any single F_1 subunit can

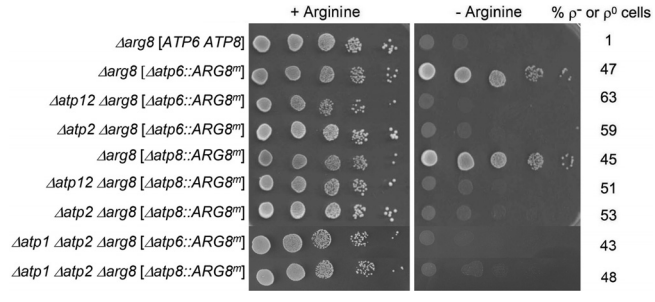

Fig. 3. F_1 mutants do not express *ARG8^m* from the *ATP6* or *ATP8* locus of mitochondrial DNA. The parental strain MR6 (Δ*arg8* [*ATP6 ATP8*]) and the different respiratory deficient mutants with either the mitochondrial Δ*atp6*::*ARG8^m* or Δ*atp8*::*ARG8^m* allele were grown overnight in minimal glucose medium supplemented with auxotrophic requirements including arginine. Serial dilutions were spotted on minimal glucose with or without arginine and incubated at 30 °C for 2 days. The percentage of ρ^- or ρ^0 mutants in the cultures is indicated next to each strain in the right hand margin. The mitochondrial genotypes are enclosed by the straight brackets.

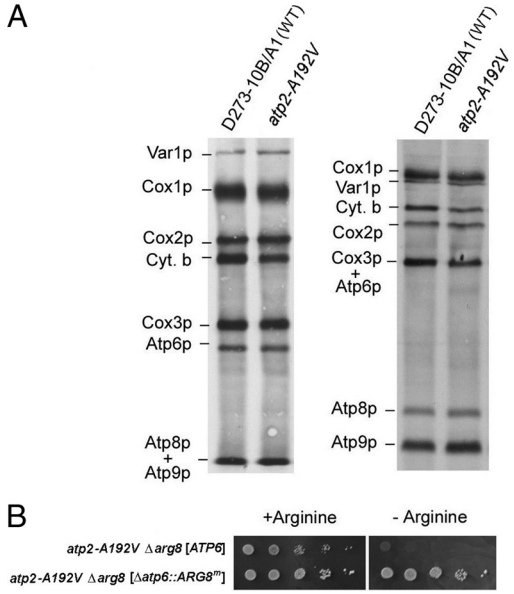

Fig. 4. *ATP6* and *ATP8* are expressed in a catalytic inactive F_1 mutant. (*A*) The parental strain D273–10B/A1 and the point mutants P159 (*atp2-A192V*) were labeled in vivo as in Fig. 1 and separated by SDS/PAGE on a 12% gel containing 6 M urea and 25% glycerol (left panel) and a 17.5% polyacrylamide gel (right panel). (*B*) The point mutant (*atp2-A192V* Δ*arg8*) with wild-type mtDNA and with the Δ*atp6*::*ARG8*^{m} allele were grown and spotted on minimal glucose with or without arginine as in Fig. 3.

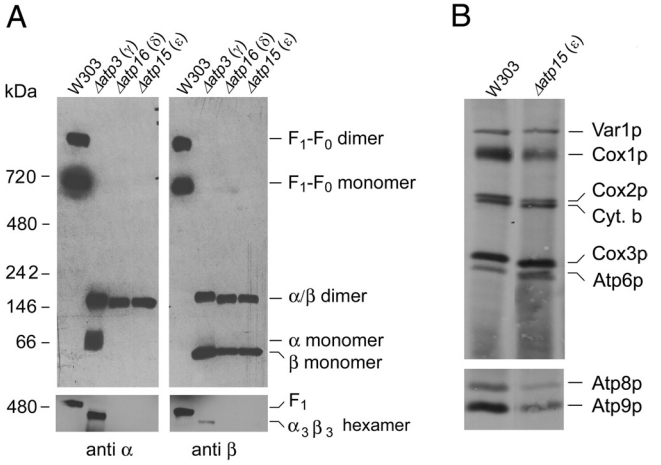

Fig. 5. Characterization of F_1 and expression of Atp6p and Atp8p in γ, ε, and δ subunit null mutants. (*A*) Mitochondria were extracted with 2% digitonin and samples representing 250 μg of starting mitochondrial protein were analyzed by BN-PAGE as described in *Materials and Methods*. (*B*) The respiratory competent strain W303 and the ε null mutant (Δ*atp15*) were labeled in vivo and separated on two different SDS/PAGE gel systems as in Fig. 1.

restore expression of the *ATP6* locus in an F_1 mutant, the Δ*atp12* Δ*arg8* double mutant with the Δ*atp6*::*ARG8*^{m} allele was transformed with high-copy plasmids containing the α, β, γ, δ, or ε subunit genes. None of the genes were able to suppress the growth requirement for arginine indicating that overexpression of the single F_1 subunits is not a sufficient condition for expression of Atp6p.

Translation of Atp6p and Atp8p Does Not Depend on Catalytically Active F_1. We next asked whether mutants with assembled but catalytically inactive F_1 are able to translate Atp6p? The respiratory defective mutant P159 was previously shown to have a loss of function mutation (A192V) in the β subunit that does not interfere with assembly of the F_1 oligomer (15). Centrifugation of the solubilized enzyme in a sucrose gradient confirmed that the β subunit of F_1 in the mutant sedimented nearly identically to the β-galactosidase size standard, indicative of an assembled F_1-F_0 complex. P159 was, therefore, likely to have a complete F_0 unit with Atp6p and Atp8p. This was confirmed by in vivo labeling of mitochondrial gene products in the mutant. The pattern of labeled proteins in the mutant was identical to that of wild-type with no evidence of diminished translation of the three F_0 subunits or of Cox1p of cytochrome oxidase (Fig. 4*A*).

The ability of a catalytically damaged F_1 to support Atp6p translation was confirmed by testing expression of *ARG8*^{m} at the *ATP6* locus in the *atp2-A192V* Δ*arg8* double mutant. As expected the double mutant with the Δ*atp6*::*ARG8*^{m} allele was able to grow in the absence of arginine (Fig. 4*B*) indicating that *ATP6*/*ATP8* expression is not dependent on the hydrolase activity of F_1.

Are Soluble But Incompletely Assembled Forms of F_1 Sufficient to Activate Translation of Atp6p and Atp8p? Mitochondrial F_1 ATPase consists of five different subunits with an $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry. In contrast to the Δ*atp11*, Δ*atp12*, Δ*atp1*, and Δ*atp2* mutants, which accumulate the α and/or β subunits of F_1 as bulk aggregates, ε, δ, or γ mutants maintain both α and β subunits as soluble proteins (16–18).

The oligomeric status of the α and β subunits in ε, δ, and γ mutants was examined by BN-PAGE separation of digitonin solubilized mitochondria. The α and β subunit specific antibodies detected the same bands, which differed from F_1 and the F_1-F_0 complex (Fig. 5*A*). Based on their migration, the two most prominent bands, present in all three mutants, but absent in wild-type, were estimated to have masses of 50 kDa and 150 kDa probably corresponding to the β monomers and the αβ dimer, respectively. The mass of the αβ dimer should be approximately 110 kDa. The tendency of the BN-PAGE system to overestimate the masses of the native ATP synthase complex and of F_1 could account for discrepancy in the mass of the αβ dimer. However, it is not excluded that what we identify to be the dimer could have an extra F_1 subunit. The γ mutant, and in some experiments the δ and ε mutants as well, had a band detected by the α subunit-specific antibody that migrated slightly slower than the monomeric β subunit. The third band, recognized by both antibodies in the γ subunit mutant, migrated slightly ahead of F_1. This band required longer exposure time to be visible. Based on its size and reactivity this band is probably the $\alpha_3\beta_3$ hexamer. At present, it is not possible to say if the αβ dimer and the monomeric subunits are breakdown products of an unstable hexamer or if they are *bona fide* assembly intermediates of F_1.

It was of interest to ascertain if the partially assembled, soluble forms of the α and β subunits are capable of activating Atp6p and Atp8p translation. Direct measurements of Atp6p and Atp8p translation in the δ and γ subunit mutants were not possible because of the extensive loss of wild-type mtDNA, a hallmark of these strains. For the same reason we were unable to construct δ and γ subunit mutants with the *ARG8*^{m} substitutions at the *ATP6* and *ATP8* loci of mtDNA. Although the ε subunit mutant is also unstable, the percentage of ρ^+ cells in a freshly grown culture was adequate for detection of radiolabeled mitochondrial gene products by in vivo translation. These assays indicated that the ε subunit is not essential for translation of Atp6p and Atp8p (Fig. 5*B*), which is consistent with a recent report showing that an ε mutant can assemble a complete ATP synthase complex, albeit at very reduced levels (19).

***ATP6*/*ATP8* Translation in F_0 Assembly Mutants.** Mutants blocked in expression of Atp9p retain the ability to translate Atp6p and Atp8p

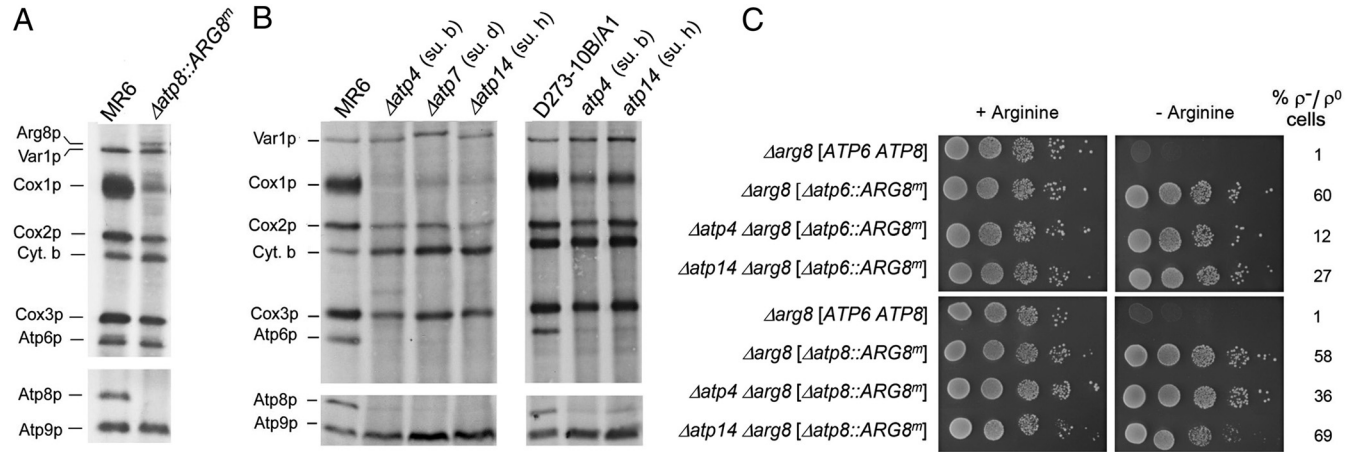

Fig. 6. Expression of Atp6p, Atp8p in F_0 and peripheral stalk mutants. (*A*) Mitochondrial gene products of the parental strain MR6 and of the Δ*atp8*::*ARG8*^{m} mutant were labeled in vivo and analyzed by SDS/PAGE as in Fig. 1. (*B*) The respiratory competent parental strains MR6 and D273–10B/A1, the subunit b, d, and h null mutants (Δ*atp4*, Δ*atp7*, and Δ*atp14*, respectively) and the subunit b and h point mutants (*atp4* and *atp14*) (20) were labeled and analyzed as in Fig. 1. (*C*) Strains with the indicated genotypes were serially diluted, spotted on minimal glucose with or without arginine and grown as in Fig. 3.

(6). Similarly, the Δ*atp8* mutation does not affect translation of Atp6p and Atp9p as evidenced by the comparable labeling of Atp6p and Atp9p (relative to Cox3p) in the wild-type and the Δ*atp8* mutant (Fig. 6*A*). Earlier studies indicated that Atp8p and Atp9p translation is also not affected in the Δ*atp6* mutant (8). These results demonstrate that expression of the three mitochondrially encoded subunits of the ATP synthase is not interdependent.

Information bearing on the effect of mutations in peripheral stalk subunits on expression of mitochondrial gene products is scant. We, therefore, studied the synthesis of Atp6p and Atp8p in cells expressing fully assembled and functional F_1 but missing subunits of the peripheral stalk. Mitochondrial gene products were labeled in vivo in Δ*atp4* (subunit b), Δ*atp7* (subunit d), and Δ*atp14* (subunit h) null mutants. All three peripheral stalk mutants displayed a deficit of Atp6p and Atp8p (Fig. 6*B*). The *atp4* and *atp14* point mutants also showed large reductions in labeling of Atp6p and to a lesser extent Atp8p (Fig. 6*B*). The inability of Δ*atp5* mutants to maintain a full length mitochondrial genome precluded assessing the effect of absence of it product, OSCP, the fourth peripheral stalk subunit, on translation of Atp6p and Atp8p.

Decreased in vivo labeling of a mitochondrial gene product is not necessarily indicative of a translation defect as rapid turnover of a newly synthesized but unassembled protein would be expected to elicit the same phenotype. The *ARG8*^{m} expression assay was used to discriminate between an effect of the peripheral stalk mutations on translation and turnover of Atp6p and Atp8p. The mitochondrial Δ*atp6*::*ARG8*^{m} and Δ*atp8*::*ARG8*^{m} alleles were introduced into Δ*atp4* Δ*arg8* and Δ*atp14* Δ*arg8* double mutants. The ability of these mutants to grow in the absence of arginine indicated that absence of the peripheral stalk subunits did not affect expression of *ARG8*^{m} from the *ATP6* or *ATP8* loci of mtDNA (Fig. 6*C*). The decreased labeling of Atp6p and Atp8p is, therefore, most likely the result of enhanced turnover of the two subunits.

***ATP22* Is a High Copy Suppressor of the Atp6p/Atp8p Translation Defect.** The yeast nuclear *ATP22* gene codes for an Atp6p-specific translational activator (21). In the present study this gene was isolated based on its ability to suppress the arginine requirement of the Δ*atp12* Δ*arg8* double mutant with the Δ*atp6*::*ARG8*^{m} mitochondrial allele. Transformation of the double mutant with a high-copy yeast genomic plasmid library produced arginine-independent clones, some of which contained plasmids with *ATP22*. Subcloning confirmed that *ATP22* was responsible for restoring arginine-independent growth of the double mutant (Fig. 7*A*).

In earlier studies, the function of Atp22p was inferred from the phenotype of *atp22* mutants, which are blocked in translation of Atp6p but not Atp8p (21). In view of this, the ability of *ATP22* to partially suppress the arginine auxotrophy of Δ*atp12*Δ*arg8* double mutants with the mitochondrial Δ*atp8*::*ARG8*^{m} allele was unexpected (Fig. 7*A*). To further validate this result we constructed an Δ*atp22* Δ*arg8* double mutants with either the Δ*atp6*::*ARG8*^{m} or Δ*atp8*::*ARG8*^{m} alleles. Growth of the two strains on minimal medium lacking arginine, when compared to the wild-type, indicated that whereas the Δ*atp22* mutant with the Δ*atp6*::*ARG8*^{m} allele was auxotrophic for arginine, the Δ*atp8*::*ARG8*^{m} allele was sufficiently well expressed in the Δ*atp22* mutant to allow arginine-independent growth, albeit not as well as the wild-type (Fig. 7*B*). These results are consistent with the only partial rescue of the arginine auxotrophy of the Δ*atp8*::*ARG8*^{m} allele in the background of the Δ*atp2* or Δ*atp12* mutation.

To ascertain if *ATP22* also rescues translation of Atp6p and Atp8p in F_1 mutants, Δ*atp12* null mutants containing wild-type mtDNA with and without *ATP22* on a high copy plasmid were radiolabeled in the presence of cycloheximide and mitochondrial gene products analyzed in two different gel systems optimized for the resolution of Atp6p and Atp8p (Fig. 7*C*). Translation of Atp6p in the transformants, when quantified and normalized to Cox3p, was very similar to that of the wild-type. The results of the in vivo translation assay are consistent with the growth phenotype of the Δ*atp2* and Δ*atp12* mutants harboring the mitochondrial Δ*atp6*::*ARG8*^{m} allele (Fig. 7*A*). Surprisingly, overexpression of *ATP22* also rescued translation of Atp8p.

Discussion

Biogenesis of the mitochondrial ATP synthase is a complex process because of its numerous constituent polypeptides that need to be coordinately expressed from two separate genomes. Those derived from mtDNA are more likely to be targets for regulation by their nuclear counterparts, rather than the converse, simply based on the fact that they do not cross the mitochondrial barrier and, therefore, are physically barred from exerting an influence on the transcriptional and translational machineries of the nucleus and cytoplasm.

In this study we show that translation of Atp6p and Atp8p, two subunits vital for the function of the F_0 sector, is controlled by F_1. In the absence of the α or β subunits of F_1, or of their chaperones Atp11p and Atp12p, expression of Atp6p and Atp8p but not of the other gene products of mtDNA is sharply reduced. Although Cox1p synthesis is also lower in F_1 mutants, this is a hallmark of ATP

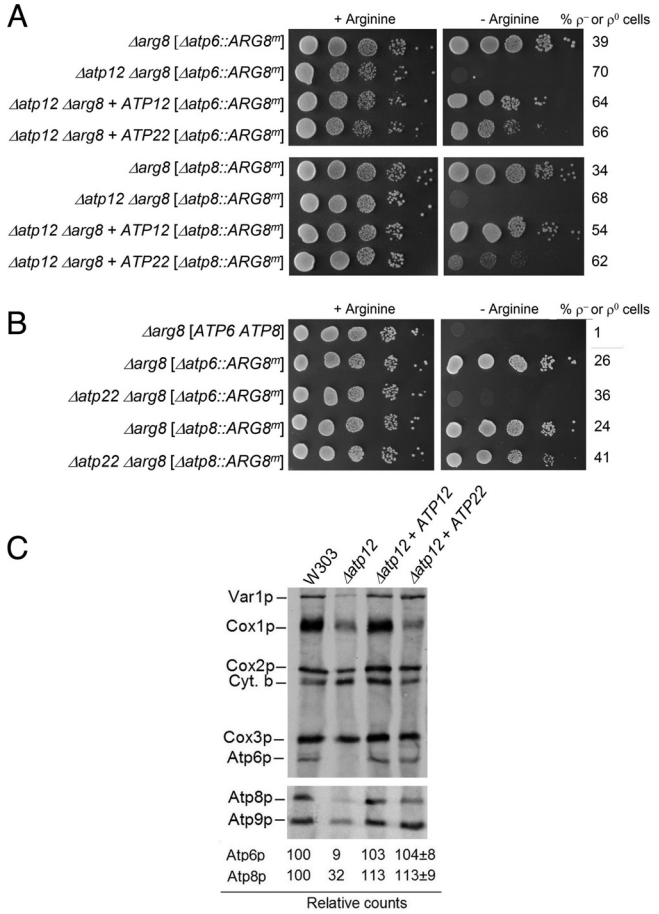

Fig. 7. Suppression of the Atp6p and Atp8p translation defect in F_1 mutants by *ATP22*. (*A*) Mutants with the indicated genotypes were transformed with *ATP12* and *ATP22* cloned in high-copy plasmids and were grown overnight in minimal glucose supplemented with all of the amino acids requirements including arginine. Serial dilutions were spotted on minimal glucose with or without arginine and grown as in Fig. 3. (*B*) $ARG8^m$ is expressed from the *ATP8* but not *ATP6* locus. The parental strain MR6 (Δ*arg8* [*ATP6 ATP8*]) and the different mutants were grown in minimal glucose containing arginine and serial dilutions were spotted on minimal medium with and without arginine as in Fig. 3. (*C*) *ATP22* restores translation of Atp6p and Atp8p in an F_1 mutant. The respiratory competent strain W303, the Δ*atp12* mutant and transformants harboring *ATP12* and *ATP22* on high copy plasmids were labeled in vivo as in Fig. 1 and separated by SDS/PAGE on a 12% gel containing 6 M urea (upper panel) and a 17.5% polyacrylamide gel (lower panel).

synthase strains in general (8, 14). The presence of normal levels of *ATP8/ATP6* mRNA in the F_1 assembly mutants excludes a defect in either transcription or processing of the *COX1/ATP8/ATP6* multicistronic transcript. A high turnover rate of the two subunits is also unlikely. This follows from the finding that expression of $ARG8^m$ at the *ATP6* or *ATP8* locus of mtDNA is also arrested in F_1 mutants. The *ARG8* product is not normally translated in mitochondria and has no functional or structural relationship to the ATP synthase. It is, therefore, difficult to rationalize how the absence of F_1 would affect its stability. Moreover, respiratory deficient mutants, including ATP synthase mutants, are prototrophic for arginine when acetylornithine aminotransferase is expressed from nuclear *ARG8* and imported into mitochondria. These results constitute compelling evidence that F_1, or components thereof, upregulate translation of Atp6p and Atp8p.

Activation of Atp6p and Atp8p translation by F_1 does not depend on its hydrolytic activity. This is evident from the ability of mutants with assembled but catalytically incompetent F_1 to synthesize Atp6p and Atp8p and to express $ARG8^m$ from either the *ATP6* and *ATP8* loci. We also tried to answer the question of whether the presence of native α and β subunits of F_1 is a sufficient condition for translational activation. Native gels of the γ, δ, and ε mutants revealed the presence of soluble monomeric α and β (only in the γ mutant), dimeric $\alpha\beta$ and to a much lesser extent of hexameric $\alpha_3\beta_3$ oligomers. The ability of the ε subunit mutant to synthesize Atp6p and Atp8p indicates that assembly of a complete F_1 unit is not a precondition for translational regulation. The soluble monomers or $\alpha\beta$ dimers present in mutants lacking the minor subunits of F_1 could be assembly intermediates or breakdown products of $\alpha_3\beta_3$ hexamers. Since our data do not distinguish between the two, it is uncertain which of these F_1 subassemblies functions in translational activation. The fact that translation is reduced in Δ*atp11* and Δ*atp12* mutants, which contain aggregated forms the α and β subunits (5), indicates that a minimal requirement for translation activation is that the α and β subunits of F_1 be present as soluble proteins. A requirement of γ and δ subunit mutants for translation of Atp6p and Atp8p proved to be difficult to assess directly because of the extensive loss of ρ^+ mitochondrial genome in these strains (17, 18). The low level of γ and δ subunits reported in the ε mutant (16), however, favors the notion that neither of these F_1 components is essential for translational activation. It is also worth noting that overexpression of different F_1 subunits was ineffective in restoring $ARG8^m$ expression in a Δ*atp12* mutant. Taken together these results point to soluble α or β subunit alone, the $\alpha\beta$ dimer or the $\alpha_3\beta_3$ hexamer as the minimal structural elements of F_1 required for activation of translation.

Deletions of any single mitochondrially encoded subunit of the ATP synthase had no noticeable effect on translation of the other two. This was shown previously for strains deficient Atp6p and Atp9p (6, 8) and in the present study for the Δ*atp8* null mutant. Interestingly, in vivo translation assays of Δ*atp4* and Δ*atp14* mutants lacking, respectively, subunits b and h of the peripheral stalk, disclosed a severe reduction of both Atp6p and Atp8p. We attribute this to turnover rather than lower translation because null alleles of these peripheral stalk genes did not significantly affect expression of $ARG8^m$ when it replaced either *ATP6* or *ATP8*. These results argue against a contribution of F_0 components in regulating translation of Atp6p and Atp8p.

ATP22, which was previously shown to code for an Atp6p-specific translational activator (21), is a high copy suppressor of the Atp6p translation defect in F_1 mutants. This further substantiates the in vivo translation results and the $ARG8^m$ expression data indicating that regulation by F_1 is exerted at the translational stage. *ATP22* was also able to restore translation of Atp8p, although not as effectively as Atp6p. The earlier conclusion that Atp22p is a specific activator of *ATP6* (21) is supported by the finding that the Δ*atp8*::$ARG8^m$ allele is expressed in an Δ*atp22* mutant. Growth of the Δ*atp22* mutant with the Δ*atp8*:::$ARG8^m$ allele, however, is somewhat slower than wild-type in the absence of arginine. An explanation for partial effect of Atp22p on translation of the *ATP8* locus will require a better understanding of how this translational activator functions.

Activation of Atp6p translation by F_1 does not involve the CES mechanism proposed to regulate some chloroplast genes in *Chlamydomonas* (1, 2) and the mitochondrial *COX1* gene in yeast (3). An essential feature of this mechanism is that a factor needed for translation of a specific mRNA also binds to the newly synthesized but unassembled protein product. For example, Mss51p, a *COX1*-specific translation factor (22) was shown to bind to Cox1p before incorporation of this subunit into cytochrome oxidase (23). The release of the factor from the complex, a necessary step for initiating a new round of translation, occurs after the newly translated subunit interacts with a partner protein imported from the cytoplasm. In this scenario the release of a translational factor (for example Atp22p) from Atp6p and/or Atp8p would be driven by F_1. This mechanism is inconsistent with our data showing that F_1 mutants are severely compromised in expressing $ARG8^m$. Since

Atp6p and Atp8p are not related to acetylornithine aminotransferase, interaction of this arginine biosynthetic enzyme with an *ATP6* or *ATP8* specific translation factor is highly dubious. Accordingly, translation of the mitochondrial *ARG8^m* mRNA would not be expected to be affected in F_1 mutants. This evidence is more consistent with a direct effect of F_1 or of a partially assembled F_1 on mitochondrial translation of the two ATP synthase subunits. Even though control of Atp6p and Atp8p translation by F_1 differs from CES at the mechanistic level, both share the common feature of coupling translation of organellar gene products to assembly of their parent complex. Since the relationship between activation of Atp6p translation by Atp22p and by F_1 is not presently know, it is not excluded that a CES regulation may operate in the Atp22p-dependent step of Atp6p translation.

Finally, it is worth pointing out that in addition to achieving a coordinate output of the two compartmentally segregated sets of ATP synthase-related genes, regulation of Atp6p translation by F_1 also serves the important physiological function of preventing the accumulation of an Atp6p-Atp9p intermediate complex with a capacity to dissipate the membrane potential of mitochondria.

Materials and Methods

Strains and Growth Media. The genotypes of the yeast strains used in this study are indicated in the figures. Unless otherwise indicated all of the translation assays were done with mutants in the W303 (**MAT**a or **MAT**α *ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1*) genetic background (24). The mutants used for growth tests were derived from crosses to MR6 (**MAT**a *ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 arg8::HIS3*), a respiratory competent strains with an *arg8* null mutation (8). The compositions of the solid and liquid YPD, YPGal, YPEG, and minimal glucose media have been described previously (18).

Deletion and Replacement of *ATP8* with *ARG8^m*. The *Δatp8::ARG8^m* deletion cassette was constructed by PCR amplification of 300 bp of the 5′flanking sequence of *ATP8* and sequences with primers 5′- ggctctagatttaataattattaaatatattc and 5′- ggcggatcctatatattattaatttatttattc and 400 bp of the 3′-flanking sequence with primers 5′- ggcggatccttttatatatatttttttaataag and 5′-ggcgagctcttattttatatataaatatagg. The two PCR fragments were digested with a XbaI/BamH1 and BamH1/SacI and ligated to pJM2 (25). The plasmid obtained from this ligation was digested with BamH1 and ligated to *ARG8^m* as a BamH1 fragment obtained by PCR amplification of the gene in pDS24 (25) with primers 5′- ggcggatccatgttcaaaagatatttatcatc and 5′-ggcggatccttaagcatatacagc. The resulting plasmid (pATP8/ST12) was introduced into αDFS160, ρ^0 strain with a *kar1* mutation by biolistic transformation using the Bio-Rad PDS-1000/He particle delivery system as described previously (26). Mitochondrial transformants were identified by their ability to correct a *cox2* mutant. A transformant (αDFS160/ATP8/ST12) was crossed to the respiratory competent *arg8* mutant MR6 (8). Respiratory defective recombinants with the *Δatp8::ARG8^m* were confirmed to be arginine independent indicating mitochondrial expression of *ARG8^m*.

Nucleic Acid Manipulations. Standard techniques were used for DNA cloning and for transformation and purification of plasmid DNA from *Escherichia coli* (27). The LiAc procedure was used for yeast transformation (28). Northern analysis of mitochondrial RNAs was performed as described previously (29).

Miscellaneous Procedures. Mitochondria were isolated from the cells grown in YPGal at 30 °C by the methods of Herrmann et al. (30). Mitochondrial gene products were labeled in vivo with [^{35}S] Methionine + [^{35}S] Cysteine (1,000 Ci/mmol, MP Biochemicals), transferred to a nitocellulose membrane and visualized by autoradiography as previously described (31). For BN-PAGE mitochondrial proteins were extracted with 2% final concentration of digitonin and separated on a 4–13% linear polyacrylamide gel (32). Proteins were transferred to a PVDF membrane and probed with rabbit polyclonal antibodies against yeast α and β subunits of F_1. The antibody complexes were visualized with the Super Signal West Pico Chemiluminescent substrate kit (Pierce Chemical Co.).

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