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Biochemistry

Identification of two nuclear genes (*ATP11, ATP12*) required for assembly of the yeast F_1-ATPase

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ABSTRACT **Nuclear respiratory-deficient mutants of *Saccharomyces cerevisiae* (*pet* mutants) have been screened for defects in the mitochondrial ATPase. Mutants in two complementation groups were found to have 10% or less of wild-type ATPase activity. The two wild-type nuclear genes defined by the mutants have been designated *ATP11* and *ATP12*. The proteins encoded by the two genes are not subunits of the ATPase but rather appear to exercise an important function at a late stage in the synthesis of F_1 after transport of the subunits into the internal compartment of mitochondria. Mitochondria of atp11 and atp12 mutants have only marginally reduced levels of the α and β subunits of F_1. Both proteins are processed to their mature size but are not part of a native F_1 structure or associated with the mitochondrial membrane. The most reasonable explanation for the mutant phenotype is a block in the assembly of the F_1 oligomer.**

The F_1-ATPase [ATP phosphohydrolase (H^+-transporting), EC 3.6.1.34] of mitochondria is an oligomeric enzyme composed of five different subunit polypeptides (1). These proteins are synthesized on cytoplasmic ribosomes (2, 3) and transported into the matrix compartment of mitochondria (4, 5) where they engage in an ordered set of physical interactions culminating in the formation of the enzymatically active oligomer. A question central to an understanding of the mechanics of assembly of subunit polypeptides into complex structures such as F_1 is whether the process is guided by other proteins acting in a catalytic capacity, and if so at what stages is their intervention necessary.

The refolding and acquisition of an assembly-competent conformation by some proteins after their translocation into mitochondria has recently been shown to be facilitated by the heat shock protein hsp60 (6–8), a member of the chaperonin family (9). The β subunit of F_1 is one of the proteins whose tertiary structure depended on hsp60 (6, 7). These studies have provided direct evidence for a protein-directed step in F_1 assembly. In the present communication we report evidence indicating that incorporation of the α and β subunits of yeast F_1 into an active oligomer depends on at least two other proteins encoded by the *ATP11* and *ATP12* genes.

MATERIALS AND METHODS

Strains and Growth Media. The genotypes and origins of the yeast strains used in this study are described in Table 1. The respiratory-defective mutants C15 and C264 were derived from *Saccharomyces cerevisiae* D273-10B/A1 by mutagenesis with ethyl methanesulfonate (11). The mutant W303∇ATP11 was constructed by *in situ* disruption of *ATP11* by the one-step gene-replacement procedure (12). The standard media used for cultivation of yeast were YPD [1% (wt/vol) yeast extract/2% (wt/vol) peptone/2% (wt/vol) glucose], YPGal [1% (wt/vol) yeast extract/2% (wt/vol) peptone/2% (wt/vol) galactose], YEPG [1% (wt/vol) yeast extract/2% (wt/vol) peptone/2% (vol/vol) ethanol/3% (vol/vol) glycerol], WO [0.67% nitrogen base without amino acids (Difco)/2% glucose]. Solid medium contained 2% agar. When required, amino acids and other growth supplements were added to a final concentration of 20 μg per ml.

Table 1. Genotypes and sources of *S. cerevisiae* strains

Strain	Genotype	Source
D273-10B/A1	α,ρ^+,*met6*	Ref. 10
D273-10B/A1ρ^0	α,ρ^0,*met6*	Ref. 10
W303-1A	a,ρ^+,*ade2-1,his3-11,15,leu2-3,112, ura3-1,trp1-1*	—*
W303∇ATP11	a,ρ^+,*ade2-1,his3-11,15,leu2-3,112, ura3-1,trp1-1,ATP11::HIS3*	This study
C15	α,ρ^+,*met6,apt11-1*	This study
C264	α,ρ^+,*met6,apt12-1*	This study
C29	α,ρ^+,*met6,atp2-2*	This study
E753	α,ρ^+,*met6,atp1-2*	This study

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Preparation of Yeast Mitochondria, Postribosomal Supernatant Fractions, and Submitochondrial Particles (SMP). Wild-type and mutant yeast were grown aerobically in YPGal medium to early stationary phase, and mitochondria were prepared by one of two methods. The first method was that of Faye *et al.* (13), except that Zymolyase 20,000 (Miles) instead of Glusulase was used to digest cell walls. The postribosomal supernatant fraction was obtained by centrifugation of the postmitochondrial supernatant at 50,000 rpm for 20 min in a 50Ti Beckman rotor. This step removes the bulk of the cytoplasmic ribosomes and small membrane fragments. Mitochondria and postribosomal fractions were also prepared by mechanical disruption of cells with glass beads in a Braun cell homogenizer, as described (14). The same centrifugation schedules were used in both preparative procedures. To remove interfering inorganic phosphate, postribosomal supernatant fractions were dialyzed at room temperature against a buffer containing 10 mM Tris·HCl, pH 7.5/1 mM EDTA/2 mM ATP before the ATPase assays.

SMP were prepared by irradiating a 10-ml suspension of mitochondria (10 mg/ml) for three 15-sec bursts, with cooling in between, by using a Braunsonic 1510, 400 W sonicator. After centrifugation at 50,000 rpm for 20 min, the membranes were resuspended in 0.125 M sucrose/10 mM Tris acetate, pH 7.5.

Immunological and Miscellaneous Assays. The α and β subunits were purified from yeast F_1, and subunit-specific antibodies were obtained as described (15). The preparation of antibodies against core 1, core 2, and cytochrome c_1 fused to the amino-terminal half of *Escherichia coli* component I of anthranilate synthetase has also been reported (16). Proteins were separated on 10% or 12% polyacrylamide gels according to Laemmli (17), except that the separation buffer was adjusted to pH 8 and the composition of the running buffer

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Abbreviation: SMP, submitochondrial particles.

was 0.05 M Tris/0.38 M glycine/0.1% SDS. The proteins were transferred to nitrocellulose and treated with 1:2000 and 1:3000 dilutions of the antibodies against the α and β subunits of F_1, respectively, and with a 1:100 dilution of the other antibodies. The buffers and washing protocols were those of Schmidt *et al.* (18). ATPase activities of mitochondria and postribosomal supernatant fractions were assayed by the colorimetric determination of released inorganic phosphate in a buffer containing 50 mM Tris sulfate, pH 8.0/4 mM $MgCl_2$/10 mM ATP (15). Protein concentrations were measured by the method of Lowry *et al.* (19).

RESULTS

Properties of atp11 and atp12 Mutants. To help understand the genetic information necessary for the maintenance of the oxidative phosphorylation potential of the inner mitochondrial membrane, we have screened a collection of *pet* mutants of *S. cerevisiae* for lesions in the F_1–F_0 ATPase complex. Strains previously assigned to complementation groups G13 and G57 had severely reduced levels of mitochondrial ATPase activity consistent with defects in the F_1 component of the complex. The nuclear genes defined by these mutants are designated *ATP11* (G13) and *ATP12* (G57).

ATPase activities measured in the parental wild type, in a ρ^0 mutant, and in mutants from complementation groups G13 and G57 are summarized in Table 2. Mitochondria of C15, an atp11 mutant and of C267, an atp12 mutant display only 10% of the ATPase activity measured in wild-type mitochondria. In the ρ^0 mutant lacking the mitochondrially synthesized F_0 subunits (20–22) most F_1-ATPase is recovered in the postribosomal supernatant fraction due to leakage of the enzyme from mitochondria as a result of their partial damage during mechanical breakage of cells. No extra ATPase activity, however, was recovered in the postribosomal supernatant fractions of C15 and C264 (Table 2). Mitochondria were also prepared from the mutants by lysis of spheroplasts, an alternative procedure that minimizes leakage of unattached F_1 from mitochondria. The ATPase activities of mitochondria from G13 and G57 mutants were also 10% of the wild-type level under these experimental conditions (data not shown).

In addition to being deficient in ATPase, atp11 and atp12 mutants have lowered concentrations of cytochrome oxidase and coenzyme QH_2-cytochrome *c* reductase. This pleiotropic phenotype is not the result of an effect of the mutations on mitochondrial protein synthesis. Both mutants incorporate radioactive precursor into mitochondrial translation products at normal rates. Nor is the deficiency in the respiratory complexes caused by secondary deletions in mitochondrial DNA because the mutants from both complementation groups accumulate <5% cytoplasmic petite derivatives after long-term subculturing. The pleiotropic phenotype appears to be a general effect of lesions in the mitochondrial ATPase and has also been noted in *pet* strains with mutations in the α or β subunit of F_1 (11, 23, 24).

Table 2. ATPase activities in mitochondrial and postribosomal supernatant fractions

Strain	ATPase activity, units*/mg of protein			
	Mitochondria		Post-ribosomal supernatant	Total units†
	− rutamycin	+ rutamycin		
D273-10B/A1	6.20	1.4	0.21	244
D273-10B/A1ρ^0	1.20	1.30	0.98	260
C15	0.58	0.08	0.02	18
C264	0.83	0.38	0.06	30

Mitochondria and postribosomal supernatants were prepared at room temperature by the Braun homogenization procedure; ATPase activity was measured at 37°C as described. Mitochondria were resuspended in 10 mM Tris HCl, pH 7.5, to a final concentration of 10–20 mg/ml; rutamycin was used at 10 μg/ml.

*A unit of ATPase activity is defined as that amount of enzyme that hydrolyzes 1 μmol of ATP per min under the specified conditions.

†Total unit values are the sums of total ATPase units recovered from the mitochondrial (− rutamycin) and postribosomal supernatant fractions for each sample.

***ATP11* and *ATP12* Do Not Code for Subunits of F_1.** The obvious possibility that the ATPase deficiency of *atp11* and/or *atp12* is caused by mutations in subunits of F_1 can be excluded by the following evidence. In studies to be reported elsewhere we have cloned and characterized *ATP11* and *ATP12*. The amino acid sequences of the encoded proteins bear no homology to sequences reported for the subunits of bovine (1) or *E. coli* (25) F_1.

The alternative explanation—namely, that *ATP11* and/or *ATP12* might code for some unidentified subunit of yeast F_1—was also excluded by the observation that antibodies obtained against hybrid proteins expressed from *ATP11* and *ATP12* fused to the *E. coli trpE* gene do not recognize any component of the purified ATPase.

Mutations in *ATP11* and *ATP12* Affect F_1 Synthesis at a Stage After Import of Subunits into Mitochondria. The exclusion of *ATP11* and *ATP12* as structural genes for subunits of F_1 implies that the absence of functional ATPase in the mutants must result from a block in synthesis, transport, or assembly of the subunits into the active oligomer. Northern (RNA blot) analysis indicated comparable levels of the mRNAs for the α and β subunits in the wild type and in an atp11 mutant W303∇ATP11, with a partially deleted *ATP11* gene (Fig. 1). The concentrations of the mRNAs were not determined in the atp12 mutant. More significantly, Western (immunoblot) analyses indicate that the concentrations of α and β subunits in mitochondria of atp11 and atp12 mutants are only 20–30%

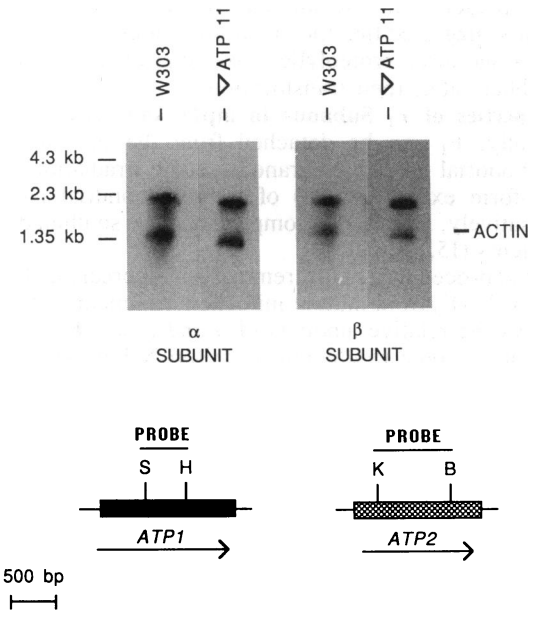

FIG. 1. Northern analysis of ATP1 and ATP2 transcripts. Poly(A)$^+$-enriched RNA was prepared from the parental respiratory-competent strain W303-1A and from W303∇ATP11, a mutant with a deletion in the *ATP11* gene. After electrophoresis on a 1% agarose gel, the RNA was transferred to diazobenzyloxymethyl paper and hybridized to a 550-base-pair (bp) *Sac* I (S)–*Hin*dIII (H) fragment from the *ATP1* gene coding for the α subunit of F_1 (23) and an 850-bp *Kpn* I (K)–*Bam*HI (B) fragment from the *ATP2* gene coding for the β subunit of F_1 (24). The Northern blot was also hybridized to a 600-bp *Cla* I fragment internal to the yeast actin gene (26). Preparation of yeast RNA and hybridization conditions have been described (27). Migration of DNA size standards is indicated at left. Actin mRNA is the lower band in all gels.

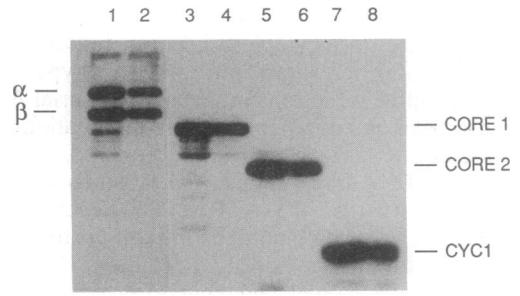

FIG. 2. Immunologic analysis of F_1 and coenzyme QH_2-cytochrome *c* reductase in an atp11 mutant. Mitochondria were prepared from the parental W303-1A and from W303∇ATP11 mutant with a deletion in *ATP11*. Equivalent amounts (10 μg in lanes 1 and 2; 50 μg in lanes 3–8) of total mitochondrial proteins were applied to a 12% polyacrylamide gel. The separated proteins were electrophoretically transferred to nitrocellulose and treated with subunit specific antibodies againt the α and β subunits of F_1 (a mixture of the two antibodies was used in lanes 1 and 2) and against the core 1 (lanes 3 and 4), core 2 (lanes 5 and 6), and cytochrome c_1 (lanes 7 and 8) of coenzyme QH_2-cytochrome *c* reductase. Odd- and even-numbered lanes are mitochondrial samples from the wild type and mutant, respectively.

lower than and, on occasion, are nearly the same as in mitochondria of wild-type yeast. In the experiment of Fig. 2 mitochondria of the parental wild-type strain W303-1A and of the atp11 mutant W303∇ATP11 were probed with antibodies to the α and β subunits of F_1 and with antibodies against several subunits of coenzyme QH_2-cytochrome *c* reductase. The concentration of each protein analyzed was ≈30% lower in the mutant than in the wild type. Because of the lack of suitable antibodies, these analyses have not been extended to the γ, δ, and ε subunits of F_1.

The presence in the mutants of mature size α and β subunits suggested that the mutational block is interposed at some stage after proteolytic processing of the precursors, most likely after their translocation.

Properties of F_1 Subunits in atp11 and atp12 Mutants. Normally, F_1 can be detached from the F_0 unit of the mitochondrial inner membrane by sonic irradiation (15) or chloroform extraction (28) of submitochondrial particles. Alternatively, the F_1–F_0 complex can be solubilized with detergents (15, 28, 29).

These procedures fail to remove any appreciable fraction of F_1 or F_1–F_0 from mutant mitochondrial membranes. Fig. 3 shows the relative amounts of α and β subunits released after either sonic irradiation or Triton X-100 extraction of submitochondrial membranes. Sonic irradiation shears a sizeable fraction of F_1 from the F_0 in wild-type mitochondria.

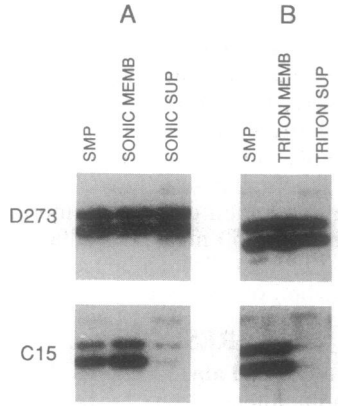

FIG. 3. Extraction of F_1 subunits by sonic irradiation and solubilization with Triton X-100. (*A*) SMP prepared from the wild-type parental strain D273-10B/A1 and the atp11 mutant C15 were suspended at 10 mg/ml in 0.125 M sucrose/10 mM Tris acetate, pH 7.5/2 mM ATP/1 mM EDTA. The pH was adjusted to 8.9 with 2 M Tris, and supensions were sonically irradiated at room temperature with a Branson model 185 sonic oscillator for 2.5 min and centrifuged at 50,000 rpm for 20 min in a 50Ti rotor. (*B*) SMP were suspended at 6.6 mg/ml in 5 mM Tris acetate (pH 7.5), incubated for 10 min on ice with 0.25% Triton X-100, and centrifuged as described above. In both sets of extractions, the membrane (MEMB) pellets were resuspended in the initial volume of buffer. The starting SMP membranes (4 μg) and twice the volume of the extracted pellet and supernatant (SUP) fractions were run on a 10% polyacrylamide gel and probed with a mixture of antibodies against the α and β subunits of F_1.

In contrast, nearly all α and β subunits cofractionate with the membrane fraction of the atp11 mutant. Chloroform extraction of the *atp11* mutant membranes also failed to release F_1 to the aqueous phase (data not shown). Similarly, conditions that extract the F_1–F_0 complex from wild-type membranes are completely ineffective with membranes of the atp11 mutant. Identical results were obtained when these extraction procedures were tried with the atp12 mutant (30).

Extractability of the two major F_1 subunits from mutant mitochondria was also examined at higher concentrations of Triton X-100 and with other detergents and chaotropic reagents. The results of such extractions indicate that only protein-denaturing reagents such as guanidine hydrochloride and urea (data not shown) were effective in solubilizing the F_1 subunits in the two mutants (Fig. 4). When these same samples were analyzed for subunits of the coenzyme QH_2-cytochrome *c* reductase complex, the detergent solubility properties of the core 1, core 2, and cytochrome c_1 proteins were normal (data not shown).

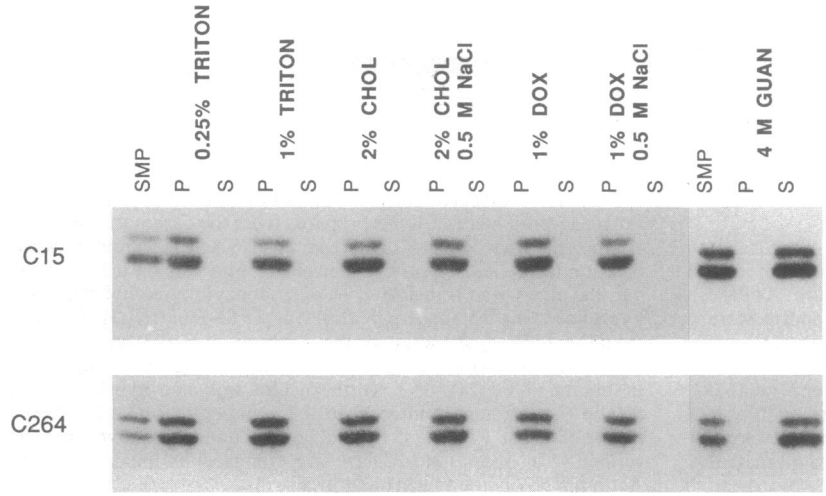

FIG. 4. Extraction of membranes from *atp11* and *atp12* mutants with detergents and guanidine hydrochloride (GUAN). SMP from C15 and C264 were suspended at 6 mg/ml in 0.125 M sucrose/10 mM Tris acetate (pH 7.5). Solutions of 5 M NaCl, 10% (wt/vol) Triton X-100, 10% (wt/vol) sodium deoxycholate (DOX), and of 10% (wt/vol) sodium cholate (CHOL) were added to the final concentrations indicated. Guanidine hydrochloride was added from a 6 M stock solution to SMP at 3.5 mg/ml. Mixtures were incubated on ice for 10 min and centrifuged for 20 min at 50,000 rpm. Pellets were resuspended in the starting volume of 10 mM Tris acetate (pH 7.5) and supernatants were dialyzed against the same buffer to remove excess salt. The original SMP membranes (4 μg) and twice the volume of the pellet and supernatant fractions were run on a 10% polyacrylamide gel and probed with a mixture of antibodies against the α and β subunits of F_1.

The above results suggest that the α and β subunits are not part of an F_1–F_0 complex or of a native F_1 oligomer in the mutant mitochondria. The aberrant properties of the F_1 subunits could be explained were the proteins only partially translocated across the membrane. Alternatively, the subunits could have been transported normally but because of a block in some subsequent step have been polymerized into inactive aggregates.

To test whether the F_1 subunits are only partially translocated, and, therefore, still associated with the membrane fraction, mitochondria prepared from the wild type, a ρ^0 mutant, and the ATPase-deficient strains C15 and C264, were exposed to a brief burst of sonic irradiation sufficient in intensity to release the matrix components but not to cause any extensive damage to the membranes. The mixture of broken mitochondria was applied to a step sucrose gradient with interfaces appropriate for separation of proteins from the less dense membrane fragments. Centrifugation time and speed were chosen such that only proteins or lipoprotein complexes of very high molecular weight would sediment to their equilibrium positions in the gradient. The results of this experiment are shown in Fig. 5. In the wild type most α and β subunits of F_1 cosediment with cytochrome c_1, a marker for the mitochondrial inner membrane. The membranes peak at the 30% sucrose cushion, corresponding to a density of 1.127. The F_1 associated with the mitochondria of the ρ^0 strain separate from the membrane marker, and most of it is recovered in the upper part of the gradient. This result was expected because the native F_1 oligomer present in ρ^0 mitochondria has a M_r of 340,000 and is, therefore, too small to reach its equilibrium position during the short time of centrifugation. The α and β subunits of C15 and C264 mitochondria also separate from the membrane fraction. In contrast to the F_1 of the ρ^0 strain, subunits in both mutants sediment to the 60–80% sucrose interface with a density of 1.39, corresponding to the equilibrium position of large protein aggregates. The α and β subunits of F_1 were the predominant proteins in this region of the gradient as determined by Coomassie staining (data not shown). When intact mitochondria of wild type, C15, and C264 were centrifuged through sucrose gradients, the α and β subunits in all three samples cosedimented with cytochrome c_1 (Fig. 5, *Bottom*), confirming the intramitochondrial location of F_1 in all strains.

The results of the sucrose gradient sedimentations indicate that in atp11 and atp12 mutants neither subunit stably associates with the mitochondrial membrane but rather form part of a large enzymatically inactive aggregate. These results also exclude association of the F_1 subunits with some feature of the transport machinery, such as a pore complex. The conditions of sonic irradiation used to disrupt mitochondria do not solubilize intrinsic membrane components of either the outer or inner membrane. Therefore, the pore complex should remain part of the outer membrane and band at the density of lipoproteins. A partially translocated form of the F_1 subunits is also unlikely in view of their lack of susceptibility to proteolytic digestion when the mutant mitochondria are treated with proteinase K or trypsin (data not shown).

Mutations in *ATP11* or *ATP12* therefore appear to exert their effects at a step(s) subsequent to the translocation of the subunits into the interior of mitochondria. The fact that mutants from both complementation groups have identical phenotypes suggests loss of a common function. The three most likely candidates for this function are folding of the polypeptide chains into native tertiary structure, posttranslational modification of the subunits, or assembly of the F_1 from the native subunits.

As a test of whether aggregation of the subunits is a necessary consequence of improper folding, the physical properties of the α and β subunits were examined in mutants expressing only one of the proteins. C29 is an atp2 mutant

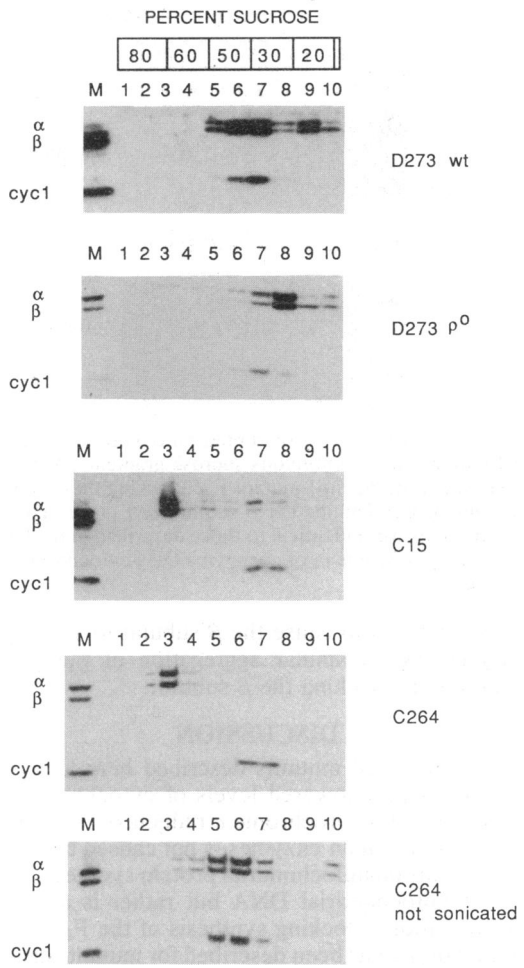

FIG. 5. Centrifugation of intact and disrupted mitochondria from wild-type and atp11 and atp12 mutants on discontinuous sucrose gradients. For experiments shown in the first four panels, mitochondria suspended in 10 mM Tris·HCl (pH 7.5) at a protein concentration of 3–4 mg/ml were sonically irradiated for 10 sec, and 200 μl of the mixture was overlaid on top of a discontinuous gradient of 10 mM Tris·HCl (pH 7.5)-buffered sucrose built from 1.2 ml of 80%, 0.9 ml of 60%, 0.9 ml of 50%, 0.9 ml of 30%, and 0.9 ml of 20% sucrose. The gradients were centrifuged at 50,000 rpm for 3 hr in a Beckman SW-65Ti rotor, and 10 fractions were collected. Equivalent volumes (15 μl) of each fraction and 10 μg of total mitochondrial protein (lane M) were separated electrophoretically on a 12% polyacrylamide gel. The somewhat abberant migration of the ATPase subunits in lane M is from an edge artifact. The proteins were transferred to nitrocellulose paper and treated with a mixture of antibodies against α and β subunits of F_1 and cytochrome c_1 (cyc1) of coenzyme QH_2-cytochrome c reductase complex. The experiment shown in the lowest panel was done in an identical manner, except that the C264 mitochondria were not sonicated. The mitochondria band at a slightly higher density than the SMP in the top four panels because of a higher protein-to-lipid ratio.

lacking the β subunit. Similarly, the *pet* mutant E753 is unable to synthesize the α subunit due to a mutation in *atp1*. Because the only mutations in these strains are in the F_1 structural genes, we reasoned that assembly of the oligomer would be blocked, but folding of either α or β subunit should not be disturbed. Mitochondria prepared from C29 and E753 were analyzed by centrifugation through discontinuous sucrose gradients under the same conditions used for the analysis of the *atp11* and *atp12* mutants. Significantly, most of the α or β subunits in the mutant mitochondria was detected in the region containing high-molecular-weight protein aggregates (Fig. 6). These results indicate that arrest of F_1 assembly because of the absence of the α subunit is a

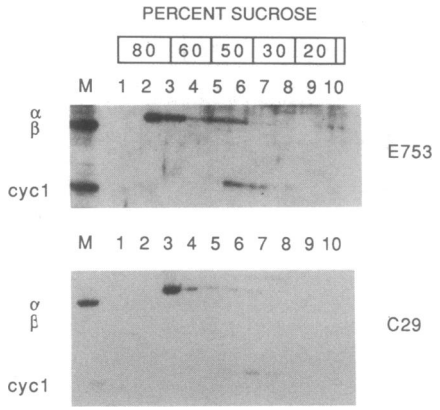

FIG. 6. Centrifugation of disrupted mitochondria from an atp1 and atp2 mutant on discontinuous sucrose gradients. Mitochondria were prepared from the atp1 mutant E753 lacking the α subunit and the atp2 mutant C29 lacking the β subunit of F_1. All other experimental conditions were identical to those described in the legend for Fig. 5. cyc1, Cytochrome c_1 of coenzyme QH_2-cytochrome c reductase complex.

sufficient condition to cause the β subunit to associate into large aggregates. A similar aggregation of the α subunit occurs in mutants lacking the β subunit.

DISCUSSION

The atp11 and atp12 mutants described here lack ATPase activity and display lowered levels of cytochrome oxidase and coenzyme QH_2-cytochrome c reductase. The reduction of the respiratory chain enzymes is not caused by any effect of the mutations on mitochondrial protein synthesis or on the stability of mitochondrial DNA but rather is a secondary effect of mutations blocking synthesis of the F_1-ATPase. A similar phenotype has been described for mutants lacking F_1, because of mutations either in the α and β subunit genes (11, 23, 24) or in a nuclear gene required for translation of F_1 subunits (31, 32).

The ATPase deficiency cannot be attributed to lesions in subunits of F_1 because the primary structures of the proteins encoded by *ATP11* and *ATP12* have no homology with any subunit of mammalian or bacterial F_1. Moreover, antibodies against the ATP11 and ATP12 proteins fail to detect antigenic determinants in purified yeast F_1.

The ATP11 and ATP12 proteins are most likely required after the F_1 subunits have been imported into mitochondria. Consistent with this notion are the observations that mutant mitochondria have near wild-type concentrations of mature size α and β subunits. Neither protein, however, can be isolated as part of a water-soluble F_1 or of a detergent-solubilized F_1–F_0 complex. Furthermore, in atp11 and atp12 mutants both F_1 subunits are present in a high-molecular-weight aggregate that can be released from mitochondria by a brief burst of sonic irradiation and separated from the membrane fraction by isopycnic centrifugation.

The ATP11 and ATP12 proteins could catalyze one of the following steps in the postimport pathway of F_1 synthesis: chemical modification of subunits, folding of the subunits into the native conformation, or assembly of the oligomer from the subunits. In the absence of any evidence for posttranslational chemical modification of F_1 subunits, the first possibility seems unlikely. More difficult to exclude is a role of the proteins in conferring a proper tertiary conformation. Several observations tend to argue against a chaperonin-like function of the ATP11 or ATP12 products in protein folding. Mutants unable to assemble the F_1 oligomer as a result of mutations in the structural genes of either the α or β subunits accumulate the normal subunit in a highly polymerized form, even though the protein-folding machinery is intact. This indicates that the high-molecular-weight polymers of α and β detected in atp11 and atp12 mutants need not necessarily be a consequence of improper folding. It is of interest that cold dissociation of purified F_1 also causes the α and β subunit to aggregate into large inactive polymers (33). Improper folding of α and β subunits in atp11 and atp12 mutants is also difficult to reconcile with the stability of the proteins, as evidenced by their occurrence at near wild-type concentrations in the mutants. These arguments lead us to presently favor a role of both proteins in promoting a correct interaction of the subunits during assembly of the F_1 oligomer. Whether the two proteins may play a similar role in assembly of other oligomeric enzymes is not clear at present. Only the α-ketoglutarate complex has been examined. No difference was found in the amount or sedimentation properties of this enzyme complex between wild-type and atp11 and atp12 mutants (data not shown). Independent of their precise lesions, the atp11 and atp12 mutants reported here identify proteins responsible for catalyzing a late event(s) in F_1 synthesis and, as such, should be useful tools for *in vitro* analysis of this process (34).

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