Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5592–5596, July 1991
Biochemistry

Splicing-defective mutants of the yeast mitochondrial *COXI* gene can be corrected by transformation with a hybrid maturase gene

(*Saccharomyces cerevisiae*/biolistic transformation/RNA splicing)

PAUL Q. ANZIANO AND RONALD A. BUTOW

Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9038

Communicated by Giuseppe Attardi, March 22, 1991

ABSTRACT **We have developed a recombinant vector, termed pMIT, for transient expression of genes delivered to yeast mitochondria by biolistic transformation. Using that vector, we introduced a hybrid RNA maturase (splicing) gene into mitochondria of ρ^0 petite cells and showed the gene to be functional in crosses. The hybrid maturase is an in-frame fusion between the N-terminal half of the maturase encoded by intron 1 of the *COXI* (cytochrome oxidase) gene and the C-terminal half of a similar maturase encoded by *COXI* intron 2. pMIT transformants can provide a functional maturase in crosses to restore respiration and COXI polypeptide synthesis to a respiratory-deficient strain defective in the synthesis of a maturase encoded by *COXI* intron 1; the transformant will also restore respiration to two splicing-defective cis mutants of *COXI* introns 1 and 3. We detect a 68-kDa polypeptide comparable in abundance to other major mitochondrial translation products as a likely product of the hybrid maturase gene. Transformants containing an internal 218-amino acid deletion mutation of the hybrid maturase gene no longer express a functional maturase in crosses and produce the expected shortened polypeptide of ≈40 kDa; however, those transformants still restore respiration to the *COXI* cis mutants. These studies show the utility of the pMIT transformation system for the expression and reverse genetic analysis of yeast mitochondrial genes.**

Some introns of the mitochondrial genome of *Saccharomyces cerevisiae* can self-splice *in vitro* (1). Within mitochondria, however, splicing is strictly dependent on either the activity of nucleus-encoded splicing factors or a family of proteins encoded by mitochondrial DNA (mtDNA) called maturases (see ref. 2 for a recent review). In most cases, maturases are encoded by the introns that they splice and are synthesized as in-frame fusions between upstream exons and intron reading frames (3–6); thus maturases autoregulate their abundance in mitochondria, since the RNA splicing which they promote will destroy their mRNA. Introns that encode maturases dictate at least two types of splicing-defective mutants: cis-acting, which inactivate the splicing substrate, and trans-acting, which have no effect on the substrate but inactivate the maturase.

Elucidation of the biochemical mechanism of maturase function has been hampered by the lack of an *in vitro* assay for maturase activity and by the low abundance of these proteins in wild-type cells. Thus attempts to identify functional domains of maturases have had to rely on conventional genetic approaches (1). One potentially useful approach to study maturase function is to engineer a maturase gene for expression in the yeast nucleus–cytoplasm and subsequent import of the maturase protein into mitochondria. In this way, a maturase encoded by the fourth intron of the cytochrome *b* gene (bI4) of yeast has been delivered to mitochondria *in vivo* and shown to be functional (7). However, this same approach failed to yield functional expression of the latent maturase activity encoded by intron 4α of the *COXI* (cytochrome oxidase) gene (8). The reasons for this are unclear, though one can imagine a number of possibilities, such as the presence or absence of post-transcriptional or post-translational modifications not duplicated by the ectopic expression of a mitochondrion-encoded protein.

In principle, a more reliable method to study maturase function would be to introduce altered maturase genes back into mitochondria for expression there. However, until recently there has been no method for transforming mitochondria with DNA, so that reverse genetic approaches were not possible. The demonstrations of transformation of mitochondria with exogenous DNA (9–12) using biolistic bombardment (13, 14) now open the way for the application of powerful reverse genetic strategies to the study of maturase structure and function.

In this study we have devised a recombinant vector termed pMIT for transient expression of genes delivered to mitochondria by biolistic transformation. In particular, we have constructed and analyzed recombinant plasmids containing a hybrid maturase gene in which the maturase domains of *COXI* introns 1 and 2 have been fused in-frame to yield a hybrid maturase. We show that a yeast mitochondrial splicing mutant defective in maturase activity encoded by the first intron of the *COXI* gene can be complemented in trans when crossed to a strain whose mitochondria are transformed with the fusion maturase gene. Expression of the plasmid-borne fusion maturase is readily detected in cells as a novel polypeptide. We also show that this construct can correct by recombination cis mutations of the *COXI* gene that also block splicing. Finally, the ability of the pMIT constructs to provide a functional maturase requires integrity of the fusion gene, whereas correction of the cis mutations does not.

MATERIALS AND METHODS

Media and Growth Conditions. The following media were used as specified in the text. YPD: 1% Difco yeast extract (Y), 1% Difco Bactopeptone (P), and 2% dextrose; YPGly: YP + 3.2% (vol/vol) glycerol; YPGal: YP + 2% galactose; YPDifferential: YP + 0.2% dextrose and 2% galactose. For biolistic transformations nuclear transformants of ρ^0 cells of strain 947 (9) were selected on solid (3.5% Difco agar) YNBD medium containing yeast nitrogen base (without amino acids), 2% dextrose, 200 mg of adenine per liter, and 0.75 M each sorbitol and mannitol (YSM medium).

Strains. See Table 1.

Mitochondrial Transformation. A commercial biolistic device (Du Pont) was used to bombard strain 947 ρ^0 cells with 1-μm-diameter tungsten beads coated with a *URA3* plasmid, Yep352 (for nuclear transformants), and the pMIT DNA of interest (9). Briefly, a 2.5-μl sample of CsCl-purified plasmid DNA is added to 30 μl of 1-μm tungsten beads suspended at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. Yeast strains

Strain	Nuclear genotype	Mitochondrial genotype	Ref.
947	*α ade2 ura3-52*	ρ^0	9
ID41-6	**a** *ade1 lys1*		15
161		ρ^+	15
C1036		mit$^-$; *COXI* intron 1 splicing-defective point mutant (trans-acting)	16
TF145		mit$^-$; *COXII* deletion	10
C2126		mit$^-$; *COXI* intron 1 splicing-defective point mutant (cis-acting)	17
AD1		mit$^-$; suppressor of mit$^-$ C1036	16
M44		mit$^-$; *COXI* exon 3 point mutant	18
kar1	**a** *ade lys can^r*	ρ^0	19
kar1/TF145	**a** *ade lys can^r*	mit$^-$; *COXII* deletion	This work

A mit$^-$ genotype is a point mutation or small deletion of ρ^+ mt DNA.

50 mg/ml of 50% (vol/vol) glycerol. To this is added 30 μl of 2.5 M $CaCl_2$ and 5 μl of 1 M spermidine (free base). After a 10-min incubation on ice, the suspension is centrifuged and 55 μl of the supernatant fraction is removed. About 2–3 μl of the suspension is applied to the macroprojectile for each shot. The 947 ρ^0 cells used for transformation are grown to stationary phase in 100 ml of YPD medium containing 100 mg of adenine per liter, diluted 1:2 in fresh YPD + adenine, and grown for 4 hr, pelleted, and chilled on ice for at least 1 hr. To the cell pellet is added 1/10 vol of YSM and YP. About 10^8 cells are spread on chilled YSM plates, which are kept at 4°C for at least 1 hr before shooting. After 7–10 days of incubation at 30°C, *URA3* transformants are pooled in groups of 5–10 into microtiter wells containing YPD, grown overnight, and stamped to a lawn of TF145. The ability to complement the *COXII* deletion in the TF145 mitochondrial genome is scored on YPGly plates. Mitochondrial transformants are subcloned to purity and tested against the various mit$^-$ strains as described in the text.

Plasmid Constructs. *p7Z-COXII.* A 2.5-kilobase (kb) *Pst* I fragment containing the entire *COXII* gene from pMT36 (20) was inserted into the *Pst* I site of Yep352. A 2.5-kb *Sph* I–*Xba* I fragment containing the *COXII* gene from that construct was then inserted into the *Sph* I–*Xba* I sites of pGEM7Z(+) to yield p7Z-*COXII*.

pMIT-aI1/2. To construct the complete *COXI* segment containing the fusion maturase gene, a 0.8-kb *Hpa* II fragment containing the 5′ region of *COXI*, including the transcriptional start site and 300 base pairs (bp) of the 5′ untranslated leader, was filled in with the Klenow fragment of DNA polymerase I and inserted into a filled-in *Eco*RI site downstream of *COXII* in pMIT7Z-*COXII*; this ligation restored the *Eco*RI site. To subclone the *COXI* intron 1–intron 2 fusion maturase region, a 4.2-kb *Hpa* II–*Bam*HI COXI fragment of mtDNA from the mit$^-$ strain AD1 (16) was ligated into the *Cla* I and *Bam*HI sites 20 bp downstream of the 5′ *COXI* segment.

pMIT-aI1/2ΔAccI/SpeI. pMIT-aI1/2 was partially cleaved with *Acc* I and cleaved to completion with *Spe* I, filled in, and ligated. The desired deletion removed 218 codons internal to the fusion maturase, retaining the reading frame downstream of the ligation site.

Plasmid Purification and Northern Blot Analysis. Plasmid DNAs were purified by CsCl/ethidium bromide ultracentrifugation as described by Maniatis *et al.* (21). Northern blot analysis of mitochondrial RNAs was carried out as described by Parikh *et al.* (22) with ^{32}P-radiolabeled probes as noted in the legend of Fig. 4.

Assay of Mitochondrial Gene Expression in Mating Mixtures. Samples (1 ml) of YPD overnight cultures of pMIT transformants and mit$^-$ tester strains were mixed into 1 ml of YPD and shaken for 3 hr at 30°C. Cells in the mating mixture were pelleted and spread onto YPDifferential plates and incubated at 30°C. After 16 hr, the cells were removed from the plate, washed in sterile water, and suspended in 10 ml of YPGal. Mitochondrial translation products were labeled on a sample of cells with $^{35}SO_4^{2-}$ (New England Nuclear) and analyzed on SDS/11% polyacrylamide gels as described by Douglas *et al.* (23).

RESULTS

Mitochondrial Transforming Plasmids. To test whether a functional maturase gene could be delivered to mitochondria by biolistic transformation, we constructed the mitochondrial transforming plasmid pMIT-aI1/2 (Fig. 1*A*). The basic pMIT vector contains bacterial sequences for shuttling in *Escherichia coli* and the wild-type mitochondrial *COXII* gene. The *COXII* transcriptional unit on a recombinant plasmid introduced into mitochondria of ρ^0 cells by biolistic transformation can complement a *COXII* deletion mit$^-$ strain in crosses (10) (see also Fig. 2), thus providing a convenient screen for mitochondrial transformants.

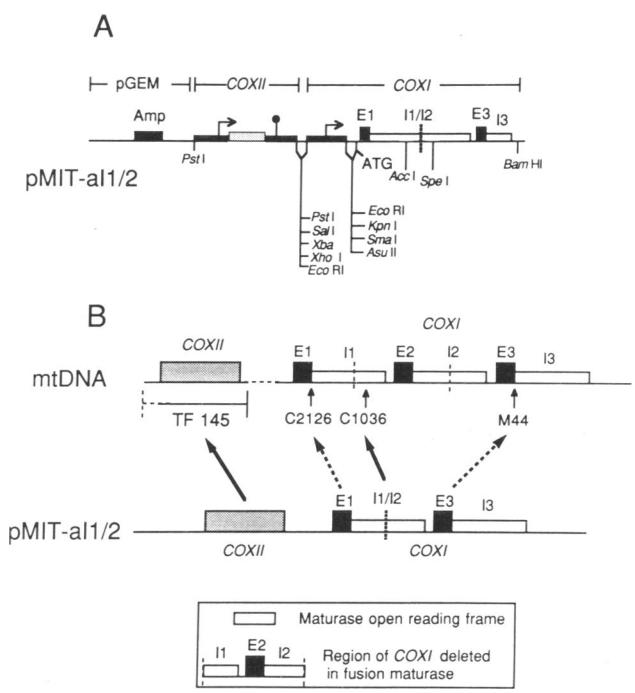

FIG. 1. Schematic of pMIT constructs and *COXI* and *COXII* mit$^-$ mutants. (*A*) Structure of the plasmid recombinant pMIT-aI1/2. The basic expression vector contains the *COXII* gene (stippled box), its transcriptional initiator region (indicated by the upstream rightward arrow over the filled box), and the downstream mitochondrial post-transcriptional processing site (indicated by the ↑). Downstream of *COXII* is the fusion maturase region of introns 1 and 2 of *COXI*. The vertical line at I1/I2 indicates the joint region of the maturase reading frames of introns 1 and 2. Immediately upstream is the *COXI* transcriptional initiator region and 5′ nontranslated leader. (*B*) Mitochondrial mutations rescued by pMIT-aI1/2. The approximate locations of the *COXII* deletion in TF145 (indicated by the bracketed line) and three *COXI* splicing-defective mutations are shown on the same map for clarity. Below is a schematic of pMIT-aI1/2. Bold arrows signify restoration to respiratory sufficiency by complementation; dashed arrows denote restoration by recombination.

The maturase function we examined is encoded by a novel in-frame fusion of the maturase-coding regions of introns 1 (aI1) and 2 (aI2) of the *COXI* gene. The hybrid maturase gene (1400 bp of the 5′ end of aI1 fused in frame to 1030 bp of the 3′ end of aI2) was derived from a mit$^-$ genome, AD1, which can suppress a chain-terminating mutation in the C-terminal part of the aI1-encoded maturase of the mutant strain, C1036 (16). We selected this hybrid maturase for mitochondrial transformation (*i*) because the hybrid protein encoded by this gene retains the RNA splicing (maturase) activity of the full-length aI1-encoded maturase protein, and (*ii*) because the fusion maturase gene cannot correct C-terminal mutations in aI1, such as in C1036, by homologous recombination, since that region of aI1 is absent from the fusion gene; thus complementation of that splicing-defective mutant occurs in trans.

For expression of the fusion maturase, we constructed the vector pMIT-aI1/2 (Fig. 1) as described in *Materials and Methods*. We have also constructed and analyzed a mutant of pMIT-aI1/2, pMIT-aI1/2Δ*Acc*I/*Spe*I, in which a 654-bp *Acc* I–*Spe* I fragment spanning the junction between the aI1 and aI2 fusion maturase gene has been deleted (Fig. 1). The deletion removes 218 internal amino acids of the fusion maturase but retains the remainder of the reading frame.

Selection of Mitochondrial Transformants. These pMIT constructs were used to transform mitochondria of yeast cells by bombarding a ρ^0 derivative of strain 947 with tungsten beads coated with both pMIT and Yep352 DNA. To detect mitochondrial transformants, pools of *URA3* transformants were replica crossed to the *COXII* deletion strain, ID41-6/TF145, and the diploids were scored for growth on glycerol (glycerol$^+$). Haploid mitochondrial transformants were retrieved among the *URA3* transformants and subcloned by crossing to TF145. In some experiments the *URA3* transformants were crossed to a *kar1* strain (19) containing the TF145 mitochondrial genome. Since nuclear fusion is greatly suppressed in zygotes derived from crosses between *KAR1* and *kar1* strains, glycerol$^+$ colonies contain some haploid segregants with the 947 nucleus and pMIT DNA in their mitochondria.

Glycerol Growth of Splicing-Defective *COXI* Mutants Is Restored by pMIT Transformants. Mitochondrial transformants of strain 947 were tested in crosses for their ability to correct three splicing-defective mutations in the *COXI* gene. Two, C2126 and M44, are cis mutations that block the splicing of aI1 and aI3, respectively; the third, C1036, is a mutant defective in aI1 splicing because of a chain-terminating mutation in the aI1 maturase gene. Because of these splicing defects, those mutant strains cannot synthesize COXI protein and thus are unable to grow on glycerol. The location of these *COXI* mutations and the expected pattern of their restoration by pMIT-aI1/2 are summarized in Fig. 1*B*.

Fig. 2 shows the glycerol growth capability of diploids derived from crosses between the pMIT transformants of strain 947 and the mit$^-$ mutants of the *COXII* and *COXI* genes indicated in Fig. 1. As controls, crosses were also carried out with the nontransformed ρ^0 parent of the transformants and with a derivative of the pMIT-aI1/2 that spontaneously had lost all *COXI* sequences but retained *COXII*. None of the diploids issued from crosses between the mit$^-$ tester strains and the 947 ρ^0 petite grew on glycerol. The transformant of strain 947 containing the pMIT derivative with just *COXII* was able to restore glycerol growth to the *COXII* deletion mit$^-$ strain but not to any of the *COXI* mit$^-$ mutants. However, the pMIT-aI1/2 transformant of 947 restored glycerol growth to all of those *COXI* mutant strains. In the crosses between the pMIT-aI1/2 transformant and the cis-dominant *COXI* mutants, C2126 and M44, the diploids retained the glycerol$^+$ phenotype even when grown for many generations on nonselective medium. This is the expected result for

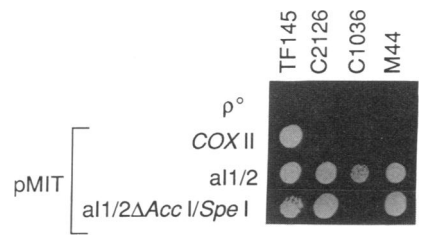

FIG. 2. Glycerol growth capability of diploids between pMIT transformants and *COXI* and *COXII* mit$^-$ strains. Matings between the mit$^-$ strains indicated across the top and the pMIT transformants, as well as the ρ^0 untransformed strain 947 control, indicated on the left were carried out in microtiter wells containing YPD. After overnight incubation at 30°C, the mating mixtures were stamped to YPGly plates and incubated for 3 days at 30°C.

restoration by recombination to yield mit$^+$ mitochondrial genomes. The glycerol$^+$ diploids derived from crosses to TF145 and C1036, however, were unstable and gave rise to pMIT and mit$^-$ segregants (data not shown). That pMIT-aI1/2 complements the aI1 maturase mutations in C1036 provides evidence that a functional maturase gene can be delivered to mitochondria of yeast cells by biolistic transformation.

In these experiments it was important to determine whether integrity of the fusion maturase gene is required for restoration of glycerol growth to C1036, as would be expected for trans complementation by a maturase protein encoded by the pMIT. A corollary is that restoration of glycerol growth to the cis mutants, C2126 and M44, should not require an intact maturase gene when these mutants are crossed to pMIT transformants as long as the pMIT DNA covers the mutant *COXI* sequences in those strains. The results of crosses between the transformants containing pMIT-aI1/2Δ*Acc*I/*Spe*I transformants and the *COXI* mit$^-$ strains bear out these expectations: deleting 218 internal amino acids from the fusion maturase inactivates its ability to complement C1036, while restoration of glycerol growth to C2126 and M44 is unaffected.

pMIT-Encoded Maturase Polypeptides. Since pMIT transformants are equivalent to ρ^- petites (10, 12), they are incapable of mitochondrial protein synthesis. Therefore, to investigate the proteins expressed from the fusion maturase constructs, pMIT transformants were mated to the mit$^-$ strains TF145 and C1036 and the profile of mitochondrial translation products labeled *in vivo* was examined between 16 and 25 hr after mating (Fig. 3). To enrich for respiring—i.e., complemented—diploids, the mating mixture was transferred from YPD to YPDifferential medium 3 hr after mixing of the cells. This step takes advantage of the observation that respiring diploids from these particular crosses quickly acclimate to growth on galactose (within 8–12 hr), whereas the unmated parental mit$^-$ cells require at least 36 hr to begin efficient galactose utilization (P.Q.A., unpublished observation). In addition, those complemented respiring cells incorporate $^{35}SO_4^{2-}$ into their mitochondrial translation products much more robustly than the unmated mit$^-$ cells. To follow the pattern of mitochondrial translation products in these complemented cells, mating mixtures were labeled with $^{35}SO_4^{2-}$ in the presence of cycloheximide (23).

The wild-type (ρ^+) pattern of mitochondrial translation products in strain 161 (Fig. 3*A*, lane 1) shows bands corresponding to cytochrome oxidase subunits I–III, cytochrome *b*, and var1. The significant difference between this pattern and that of the *COXII* deletion strain TF145 (lane 2) is the absence of the COXII polypeptide (COXI polypeptide is also diminished in TF145, most likely because it is degraded in the absence of assembly of a functional cytochrome oxidase); in addition, because of different *var1* alleles, the var1 protein

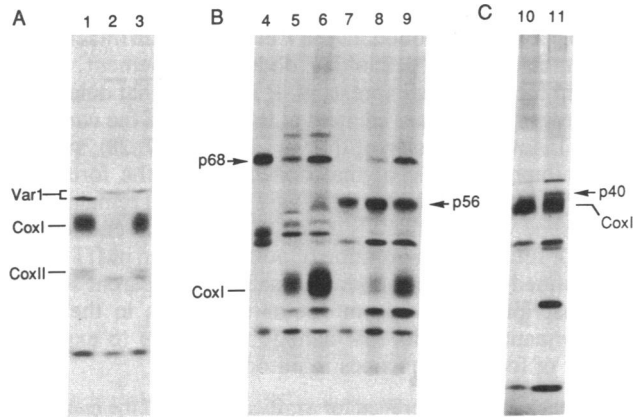

FIG. 3. Mitochondrial translation products labeled *in vivo*. Mitochondrial translation products were analyzed as described by Douglas *et al.* (23). (*A*) Lane 1, ρ^+ 161; lane 2, TF145; lane 3, 947 pMIT*COXII* crossed to TF145. (*B*) Lane 4, mit$^-$ AD1; lanes 5 and 6, pMIT-aI1/2 crossed to TF145, labeled after 16 and 25 hr, respectively, after mating; lane 7, mit$^-$ C1036; lanes 8 and 9, pMIT-aI1/2 crossed to C1036, labeled after 16 and 26 hr, respectively. (*C*) Lanes 10 and 11, pMIT-aI1/2Δ*Acc*I/*Spe*I crossed to TF145, labeled after 18 and 21 hr, respectively.

encoded by the TF145 mitochondrial genome is larger than the protein encoded by the 161 genome. In diploids derived in a cross between TF145 and the 947 pMIT*COXII* transformant, COXII polypeptide is restored (lane 3).

To evaluate the pattern of mitochondrial translation products in crosses with pMIT-aI1/2 transformants, we have also examined the profile in the mit$^-$ strain AD1, the source of the fusion maturase gene. The aI1-aI2 fusion intron is not spliced from the precursor RNA (P.Q.A., unpublished results); consequently the fusion maturase polypeptide accumulates and is detectable as a 68-kDa (p68) species (Fig. 3*B*, lane 4). Fig. 3*B*, lanes 5 and 6, shows that in the cross between pMIT-aI1/2 transformants and TF145, a polypeptide is present that comigrates with p68 of AD1; in addition, COXII is restored in those diploids, as expected. The data also indicate that the relative restoration of COXII and the intensity of the labeling pattern and of p68 are greater at the later time after mixing the cells. However, with continued outgrowth of the diploids, the pMIT and mit$^-$ and mitochondrial genomes segregate, reducing these signals that result from complementation (data not shown).

The profile of mitochondrial translation products in the mit$^-$ strain C1036 (Fig. 3*B*, lane 7) shows the presence of a novel p56 species, and no COXI protein; p56 is the truncated product of the aI1 reading frame containing a premature stop codon 161 codons from the 3′ end of the coding region. In the cross between pMIT-aI1/2 transformants and C1036 (Fig. 3*B*, lanes 8 and 9), COXI polypeptide appears, as does p68. Although our mating protocol selects for respiratory competent diploids, the p56 species from C1036 persists even at the 25-hr time point. This further indicates that restoration of glycerol growth to cells containing the C1036 mit$^-$ mitochondrial genome by pMIT-aI1/2 transformants is the result of trans complementation by the plasmid-encoded fusion maturase, p68, or a derivative of that protein.

The profile of mitochondrial translation products in the diploids from the cross between pMIT-aI1/2Δ*Acc*I/*Spe*I transformants and TF145 (Fig. 3*C*, lanes 10 and 11) shows a novel ≈40-kDa species whose size is consistent with the 218 amino acid internal deletion of the p68 fusion maturase. Although this species is relatively abundant among the mitochondrial translation products, it is evidently incapable of functioning as a maturase. The band that appears in lane 11 in the lower portion of the gel has not been identified, but its synthesis is independent of the presence of the maturase gene (data not shown).

Transcription of pMIT DNAs. The results presented thus far show that synthesis of abundant amounts of the functional fusion maturase protein from pMIT-aI1/2 does not require the complete *COXI* gene or a 3′ conserved dodecamer element (24, 25) immediately downstream of the *COXI* insert. To better understand the expression of these constructs transformed into mitochondria, we have analyzed transcripts from pMIT-aI1/2 transformants and the pMIT derivative containing just the *COXII* gene, using probes specific for *COXI*, *COXII*, and pGEM DNA sequences (Fig. 4). In both pMIT transformants, a major *COXII* species is present that comigrates with the 800-nucleotide COXII mRNA produced in wild-type ρ^+ cells. These results show that the same transcriptional and post-transcriptional signals responsible for generating the *COXII* mRNA from natural mtDNA templates (ρ^+ or ρ^-) also operate efficiently from plasmid sequences transformed into mitochondria. Moreover, pGEM sequences in those same pMIT transformants are also transcribed and appear as large, polydisperse RNAs. Although we do not know whether these transcripts arise as independent initiation events within pGEM DNA or result from run-through from *COXI* or *COXII* initiations, it is likely that most or all of the pMIT DNA is transcribed. The center of Fig. 4 shows that transcripts of the fusion maturase are comparable in size to the pGEM transcripts; for comparison, the mature 1800-nucleotide *COXI* mRNA from ρ^+ cells is also shown. These data suggest that transcripts of the fusion maturase gene also include pGEM sequences; but despite the absence of a mitochondrial post-transcriptional processing site (see Fig. 1) immediately downstream of the COXI insert, those RNAs can be translated to yield relatively abundant amounts of the maturase protein (see Fig. 3).

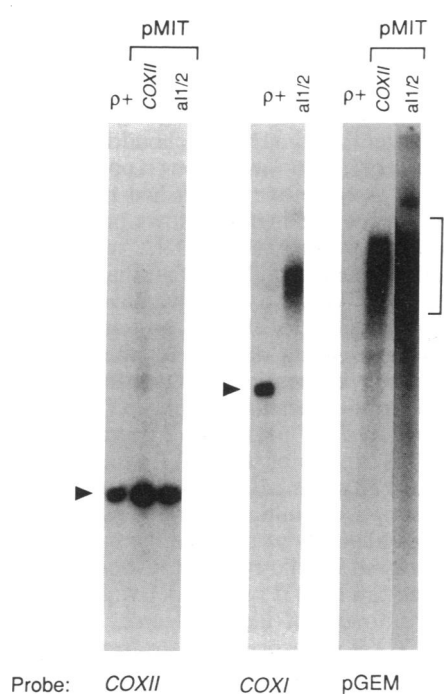

FIG. 4. Northern blot analysis of pMIT transformants of strain 947. Mitochondrial RNAs were analyzed on 1.2% agarose/formaldehyde gels as described by Parikh *et al.* (22). The *COXII* probe is a 2.5-kb *Pst* I *COXII* fragment from pMIT-aI1/2. The *COXI* probe is a spliced exon fragment containing introns 1–3. The pGEM probe is a 0.5-kb *Pvu* II fragment of pGEM 7Zf(+). The arrowheads indicate the *COXI* and *COXII* mRNAs. The bracket indicates a 4- to 6-kb nucleotide region of the gel.

DISCUSSION

This paper describes the functional expression of a hybrid maturase gene transformed on a recombinant plasmid into yeast mitochondria. That gene is an in-frame fusion between the maturase coding regions of the first two introns, I1 and I2, of the *COXI* gene (16), and its product consists of the N-terminal 465 amino acids of the aI1-encoded protein fused to 295 amino acids of the C-terminal part of the aI2 maturase protein. We have inserted this hybrid maturase gene into a mitochondrial expression vector and introduced that recombinant (pMIT) into mitochondria of ρ^0 petite cells by biolistic transformation (9–12). Using well-established procedures of mitochondrial genetics, we find that cells transformed with the fusion maturase in the vector pMIT-aI1/2 can correct in crosses a trans splicing defect of intron 1 of the *COXI* gene in the mutant C1036. In similar crosses, the pMIT-aI1/2 construct will also restore splicing to cis mutants that block splicing of introns 1 and 3 of the *COXI* gene. Restoration of the defective maturase protein of C1036 most likely occurs by trans complementation in heteroplasmic cells containing the pMIT-aI1/2 DNA and the mit$^-$ mitochondrial genome. This transient expression of genes in mitochondria is similar to zygotic gene rescue (26), where genes on petite mitochondrial DNAs can be expressed in zygotes derived from crosses between petite cells and cells competent for mitochondrial protein synthesis.

An important finding from our experiments is that the fusion maturase encoded by pMIT-aI1/2 is readily detected among mitochondrial translation products as a unique p68 species, even under conditions that are not selective for maturase function (Fig. 3, lanes 5 and 6). Further, the deletion mutant, pMIT-aI1/2Δ*Acc*I/*Spe*I, yields an inactive maturase and a shortened polypeptide species, consistent with that deletion, among the products of mitochondrial protein synthesis. These results show the feasibility of using biolistic transformation for the transient expression of proteins in mitochondria; moreover, they suggest that low-abundance proteins, such as maturases, can be detected whether these proteins are functional or not.

Our pMIT system utilizes the *COXII* gene as a convenient selectable marker for primary mitochondrial transformants of ρ^0 haploid cells (10) identified among a population of nuclear transformants. As in other established transformation systems, selection for one marker allows one to assay for the properties of the other gene or DNA sequence of interest present on the same plasmid, whether that gene is functional or not. The pMIT vector system we have described should now make it possible to express proteins in mitochondria without having to select directly for them, or to resort to engineering their expression and subsequent import into mitochondria in the nucleus–cytoplasm (7, 8).

Northern analysis of pMIT-aI1/2 transformants indicates extensive transcription of the pMIT vector sequences, perhaps to include all of the pMIT DNA. Further, the signals for *COXII* transcriptional initiation and 3′ processing appear to be operating efficiently to produce the same *COXII* mRNA species as present in wild-type mitochondria; relative to the amount of mature *COXII* mRNA derived from pMIT-aI1/2, only minor amounts of high molecular weight transcripts containing *COXII* sequences are apparent (Fig. 4). Despite the absence of adjacent 3′ RNA processing signals for the *COXI* sequences (only the 5′ portion of the *COXI* gene is present in pMIT-aI1/2), the large heterogeneous transcripts that contain fusion maturase sequences must be relatively stable and translated efficiently enough to produce amounts of maturase protein comparable to those of other abundant mitochondrial translation products.

Although there is little information on the 3′ end requirements for expression of intron-encoded proteins that are in frame with upstream exon sequences in unspliced or partially spliced pre-mRNAs, our data show clearly that an intact *COXI* gene, including its 3′ flanking dodecamer element, is not required for maturase expression. It is known that deletion of a conserved dodecamer element at the 3′ end of the *var1* gene results in low expression of the var1 protein (27, 28), suggesting an important role of that dodecamer in the formation, stability, or translatability of the *var1* mRNA. It remains to be established whether there is a similar stringent 3′ end requirement for functional expression of other genes on pMIT DNAs transformed into mitochondria. Finally, the apparent stability of transcripts derived from vector sequences in the pMIT transformants suggests that it may be possible to express a variety of foreign sequences in mitochondria.

We thank Philip S. Perlman for a critical review of the manuscript and for making unpublished data and strains available to us. We also thank Tom Fox for kindly providing yeast strain TF145 and the plasmid pMT36. This research was supported by Grant GM41426 from the National Institutes of Health and Grant I-642 from the Robert A. Welch Foundation.

1. Perlman, P. S. (1990) *Methods Enzymol.* **181,** 539–558.
2. Lambowitz, A. & Perlman, P. S. (1990) *Trends Biochem. Sci.* **15,** 440–445.
3. Lazowska, J., Jacq, C. & Slonimski, P. P. (1980) *Cell* **22,** 333–348.
4. Schmelzer, C., Haid, A., Grosch, G., Schweyen, R. J. & Kaudewitz, F. (1981) *J. Biol. Chem.* **256,** 7610–7619.
5. Anziano, P. Q., Hanson, D. K., Mahler, H. R. & Perlman, P. S. (1982) *Cell* **30,** 925–932.
6. Weiss-Brummer, B., Rodel, G., Schweyen, R. J. & Kaudewitz, F. (1982) *Cell* **29,** 527–536.
7. Banroques, J., Delahodde, A. & Jacq, C. (1986) *Cell* **46,** 837–844.
8. Delahodde, A., Goguel, V., Becam, A. M., Creusot, F., Perea, J., Banroques, J. & Jacq, C. (1989) *Cell* **56,** 431–441.
9. Johnston, S. A., Anziano, P. Q., Shark, K., Sanford, J. C. & Butow, R. A. (1988) *Science* **240,** 1538–1541.
10. Fox, T. D., Sanford, J. C. & McMullin, T. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7288–7292.
11. Thorsness, P. E. & Fox, T. D. (1990) *Nature (London)* **346,** 376–379.
12. Butow, R. A. & Fox, T. D. (1990) *Trends Biochem. Sci.* **15,** 465–468.
13. Klein, T. M., Wolf, E. D., Wu, R. & Sanford, J. C. (1987) *Nature (London)* **327,** 70–73.
14. Sanford, J. C., Klein, T. M., Wolf, E. D. & Allen, N. (1987) *J. Part. Sci. Tech.* **5,** 27–37.
15. Alexander, N. J., Vincent, R. D., Perlman, P. S., Miller, D. H., Hanson, D. K. & Mahler, H. R. (1979) *J. Biol. Chem.* **254,** 2471–2479.
16. Anziano, P. Q., Moran, J. V., Gerber, D. & Perlman, P. S. (1990) *Nucleic Acids Res.* **18,** 3233–3239.
17. Mecklenburg, K. (1986) Ph.D. thesis (Ohio State Univ., Columbus).
18. Hoffman, P. W. (1989) Ph.D. thesis (Ohio State Univ., Columbus).
19. Conde, J. & Fink, G. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3651–3655.
20. Poutre, C. & Fox, T. D. (1987) *Genetics* **115,** 637–647.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 86–94.
22. Parikh, V. S., Conrad-Webb, H., Docherty, R. & Butow, R. A. (1989) *Mol. Cell. Biol.* **9,** 1897–1907.
23. Douglas, M. G., Finkelstein, D. & Butow, R. A. (1979) *Methods Enzymol.* **56,** 58–66.
24. Osinga, K. A., de Vries, E., van der Horst, G. & Tabak, H. F. (1984) *EMBO J.* **3,** 829–834.
25. Butow, R. A., Zhu, H., Perlman, P. & Conrad-Webb, H. (1989) *Genome* **31,** 757–760.
26. Lopez, I. C., Farelly, F. & Butow, R. A. (1981) *J. Biol. Chem.* **256,** 6496–6501.
27. Conrad, H. (1987) Ph.D. thesis (Ohio State Univ., Columbus).
28. Conard-Webb, H., Perlman, P. S., Zhu, H. & Butow, R. A. (1990) *Nucleic Acids Res.* **18,** 1369–1376.