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Genetics

Specific translational activation by nuclear gene products occurs in the 5′ untranslated leader of a yeast mitochondrial mRNA

(translational control/*Saccharomyces cerevisiae*/cytochrome oxidase subunit III/*oxi2* gene)

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ABSTRACT **Translation of the yeast mitochondrial mRNA encoding cytochrome *c* oxidase subunit III (coxIII) is specifically activated by the products of at least three nuclear genes, *PET494*, *PET54*, and *PET122*. To investigate whether the target site for translational activation is within the 5′ untranslated leader of the coxIII mRNA, we asked whether translation of another mitochondrial protein, apo-cytochrome *b*, from a chimeric mRNA bearing the coxIII mRNA leader required *PET494*, *PET54*, or *PET122*. Mutations in any of these three genes abolished translation of cytochrome *b* from an mRNA bearing the 5′ two-thirds of the coxIII mRNA 5′ untranslated leader, showing that all three gene products are required for translation of the chimeric mRNA and must act within the 5′ two-thirds of the coxIII mRNA leader. Our data suggest that in wild-type cells, the specific activation of coxIII translation by *PET494*, *PET54*, and *PET122* occurs by the action of these three gene products at a site or sites in a region of the 5′ untranslated leader at least 172 nucleotides upstream of the initiation codon.**

Among the many nuclear genes necessary for mitochondrial gene expression in *Saccharomyces cerevisiae* is a particularly interesting class of genes required for the translation of specific mitochondrial mRNAs (reviewed in ref. 1). For example, mutations in the nuclear gene *PET111* prevent accumulation of cytochrome *c* oxidase subunit II by abolishing translation of its mRNA (2, 3). Mutations in either of the nuclear genes *CBS1* and *CBS2* (4, 5) are known to block translation of the mitochondrial *cob* mRNA encoding apocytochrome *b*, and another gene, *CBP6*, may be required as well (6). Thus translation of at least some mitochondrial transcripts is a surprisingly complex process, specifically requiring one or several nuclear gene products in addition to the normal translation machinery.

The products of three nuclear genes, *PET494*, *PET54*, and *PET122*, are all specifically required for translation of the mitochondrial *oxi2* gene transcript to yield subunit III of cytochrome *c* oxidase (coxIII) (7–14). [We formerly referred to *PET122* as *PET55* (13).] *pet494*, *pet54*, and *pet122* mutant cells are unable to respire because the coxIII protein fails to accumulate, despite the presence of coxIII mRNA at levels comparable to those in wild-type cells. The respiratory defect in all three nuclear mutant strains is suppressible by mitochondrial gene rearrangements, carried on deletion derivative (suppressor *rho*$^-$) genomes, that fuse the 5′ flanking sequences of other mitochondrial genes to the intact coxIII coding sequence (10). In the suppressed mutant strains these suppressor *rho*$^-$ genomes coexist unstably with the wild-type (*rho*$^+$) mitochondrial genome. The altered coxIII mRNAs transcribed from the suppressor genes, bearing the 5′ untranslated leaders of other transcripts, are translatable in a *pet494*, *pet54*, or *pet122* mutant background to yield wild-type coxIII (10, 11, 13). This evidence established that the nuclear gene products act to promote translation of the coxIII mRNA and are not required for coxIII protein stability or activity.

Although the fact that the suppressor genes were altered in the coxIII 5′ leader-encoding region suggested that *PET494*, *PET54*, and *PET122* might act in the 5′ leader, we could not draw any firm conclusions about the site or sites of action of the nuclear gene products from the structures of these bypass mutations. To investigate directly whether the nuclear gene products act on the coxIII mRNA leader, we asked whether attachment of the leader to the structural gene transcript of a different mitochondrial gene would put translation of the other gene product under control of *PET494*, *PET54*, or *PET122*. Since mutations in *CBS1* were known to be suppressible by a mitochondrial gene rearrangement that resulted in the substitution of the *oli1* 5′ untranslated leader on the *cob* mRNA (4), we reasoned that it might also be possible to select such a substitution of the coxIII 5′ untranslated leader on the *cob* mRNA as a *cbs1* suppressor. We report here that we have isolated a *cbs1* suppressor whose transcript carries the 5′ two-thirds of the coxIII mRNA 5′ leader fused to the cytochrome *b* structural gene transcript and have demonstrated that translation of cytochrome *b* from this chimeric mRNA requires *PET494*, *PET54*, and *PET122*.

MATERIALS AND METHODS

Selection and Genetic Characterization of *cbs1* Suppressors. The *S. cerevisiae* strains used in this study are described in Table 1. The wild-type strain was D273-10B (*MATα*; ATCC25657). Media and most genetic methods were as described by Sherman *et al.* (16). To construct *cbs1* mutant strains that were Gal$^+$ and isomitochondrial to D273-10B, a *rho*° derivative of the *cbs1::TRP1* disruption mutant strain GDV1 was mated with DA1 (a derivative of D273-10B); *cbs1* mutant strains MCC60 and MCC62 were meiotic progeny of this cross. Respiring revertants of MCC60 were selected as described (10). The ability to respire was mitotically unstable in all 70 strains selected. Suppressor *rho*$^-$-carrying segregants were identified by their ability to form respiring diploids with a *cbs1* mutant, *rho*$^+$ strain (MCC62) but not with a nuclearly wild-type, *rho*° strain (DA1rho°).

Construction of Parent Strains for Mass Mating. Standard genetic methods (16) were used, except that in crosses involving mitochondrially heteroplasmic strains, cells were transferred directly from selective conditions (YPEG plates) to sporulation medium. Yeast strains were transformed by the method of Ito *et al.* (17). Three sets of strains were constructed, one set each to determine whether the nuclear

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Abbreviations: nt, nucleotide(s); coxIII, cytochrome *c* oxidase subunit III.

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Table 1. Yeast strains used in this study

Strain	Genotype: nuclear [mitochondrial]	Source or ref.
GDV1	*MATa, cbs1::TRP1, his3-Δ1, leu2-3, leu2-112, trp1-289, ura3-52, [rho⁺]*	G. Rödel
DA1	*MATα, ade2, [rho⁺]*	9
MCC60*	*MATa, cbs1::TRP1, ade2, his3-Δ1, leu2-3, leu2-112, [rho⁺]*	This study
MCC62*	*MATα, cbs1::TRP1, his3-Δ1, [rho⁺]*	This study
MCC60R2-16*	*MATa, cbs1::TRP1, ade2, his3-Δ1, leu2-3, leu2-112, [rho⁺, MSUcbs1-2]*	This study
TF136rho°	*MATα, cbs1-1, pet494-41, trp1, [rho°]*	15
DAU2	*MATa, ade2, ura3-Δ, [rho⁺]*	Unpublished
MCC64	*MATa, cbs1-1, pet494-41, ade2, his3-Δ1, trp1, [MSUcbs1-2]*	This study
MCC66	*MATα, cbs1-1, pet494-41, trp1, ura3-Δ, [rho⁺]*	This study
MCC67†	*MATα, cbs1-1, trp1, ura3-Δ, [rho⁺]*	This study
TF154rho°	*MATα, pet54-1, ura3-52, [rho°]*	Unpublished
MCC68*	*MATα, cbs1::TRP1, pet54-1, ade2, his3-Δ1, ura3-52, [rho⁺]*	This study
MCC69*	*MATa, cbs1::TRP1, pet54-1, his3-Δ1, [MSUcbs1-2]*	This study
MCC71*‡	*MATα, cbs1::TRP1, ade2, his3-Δ1, ura3-52, [rho⁺]*	This study
TF180	*MATα, pet122-5, ade2, ino1, ino4, ura3-Δ, [rho⁺]*	Unpublished
MCC97*§	*MATα, cbs1::TRP1, pet122-5, ade2, his3-Δ1, ura3-Δ, [rho⁺]*	This study
MCC98*§	*MATα, cbs1::TRP1, ade2, ura3-Δ, [rho⁺]*	This study
MCC99*§	*MATa, cbs1::TRP1, pet122-5, ade2, his3-Δ1, leu2-3, leu2-112, ura3-Δ, [MSUcbs1-2]*	This study

All strains constructed for this study were isomitochondrial to D273-10B and were Gal⁺.
*May carry the *trp1-289* mutation.
†Carries the *URA3* gene at the *PET494* locus.
‡Carries the *URA3* gene at the *PET54* locus.
§May carry one or both of the mutations *ino1* and *ino4*.

genes *PET494*, *PET54*, and *PET122* were required for cytochrome *b* synthesis from the coxIII–*cob* fusion gene. For *PET494*, strains MCC64 and MCC66 were constructed by crosses involving strains TF136rho°, DAU2, and MCC60R2-16. To create a strain isogenic to MCC66 but with a wild-type copy of *PET494*, MCC66 was transformed with the integrative plasmid pMC217, carrying *PET494* under control of the *ADC1* promoter (18) on a 3.9-kilobase-pair (kb) *Bam*HI fragment derived from pMC210 (10), ligated into the *Bam*HI site of YIp5 (19). The resulting strain was termed MCC67. For *PET54*, a similar strategy was followed, starting with strains TF154rho°, MCC60, and MCC60R2-16. MCC68 was transformed to *PET54* with the integrative plasmid pMC215, carrying *PET54* on a 2.9-kb *Eco*RI–*Bgl* II fragment inserted into *Eco*RI–*Bam*HI-cleaved YIp5 (unpublished), to create strain MCC71. For *PET122*, strains were derived from crosses involving TF180, MCC60, and MCC60R2-16. MCC97 and MCC98 were meiotic progeny of one cross.

Synchronous Mating and Radioactive Labeling of Mitochondrial Proteins. The procedure of Rogers and Bussey (20) for synchronous mating was followed closely, except that galactose was substituted for glucose in all media. A 4:1 ratio of mated to unmated cells was achieved. Mitochondrial translation products of mated or haploid cells were radioactively labeled and subjected to electrophoresis as described (9, 21).

RESULTS

Selection and Characterization of Mitochondrial Mutations Suppressing the Nuclear *cbs1* Mutation. The parent strain for selection of *cbs1* mitochondrial suppressors, MCC60 (see Table 1), was mutagenized by growth in medium containing $MnCl_2$, and respiring revertants were selected. Like the previously isolated mitochondrial suppressors of *cbs1* and *pet494* (4, 9, 10), the revertant strains were mitochondrially heteroplasmic, carrying both wild-type and suppressor rho^- genomes. Suppressor rho^--carrying segregants from each strain were crossed to tester strains to determine which portions of the mitochondrial genome were retained on the rho^- molecules. We predicted from the known arrangement of genes on the yeast mitochondrial genome (22) that a recombination event fusing the coxIII 5′ flanking sequences to the *cob* structural gene should delete the *oxi2* (coxIII) and *oxi3* genes, while retaining *oxi1*. The suppressor rho^- genomes of two of the MCC60 revertants, MCC60R2-16 and MCC60R2-23, showed this pattern, suggesting that they might carry the desired mitochondrial gene rearrangement.

Physical Analysis of *cbs1* Suppressors Joining the 5′ Flanking Sequences of the coxIII Gene to the *cob* Structural Gene. Restriction mapping of the suppressor rho^- genomes of MCC60R2-16 and MCC60R2-23 indicated that both strains carried identical or very similar alterations near the 5′ end of the coxIII gene. MCC60R2-16 was chosen for further study and its suppressor rho^- genome was designated *MSUcbs1-2* (*m*itochondrial *su*ppressor of *cbs1*).

MCC60R2-16 mitochondrial DNA had a newly generated *Msp* I fragment of about 1.35 kb that hybridized to both coxIII- and *cob*-specific probes (not shown). This fragment was cloned into bacteriophage M13mp19 and the nucleotide sequences of the regions around both ends were determined by using the Sanger dideoxy chain-termination method (23). As expected, the sequence immediately adjacent to one end corresponded to the known sequence of the *cob* gene near the *Msp* I site at +1224 (24). The sequence at the other end was identical to that downstream of the coxIII *Msp* I site at −293 (25) until −173, where it joined *cob* sequences at −5 (Fig. 1). Since the 5′ end of the coxIII mRNA is encoded at −610 relative to the coxIII ATG codon (9), the fusion gene carried the 5′ two-thirds of the coxIII leader-encoding region attached to the *cob* structural gene. Inspection of the sequences of both wild-type genes (24, 25) revealed a 10-bp region of identity between coxIII and *cob* 5′ flanking sequences (−182 to −173 in the coxIII gene; −15 to −6 in *cob*) where recombination took place to generate the fusion gene.

The coxIII-*cob* Fusion Gene Is Transcribed to Yield a *cob* mRNA Carrying the 5′ Two-Thirds of the coxIII mRNA Leader. The structure of the mRNA transcribed from the coxIII–*cob* fusion gene was first investigated by RNA gel-blot analysis (not shown). A single-stranded DNA probe complementary to the predicted fusion mRNA hybridized to a 1.55-kb RNA in MCC60R2-16 RNA that was absent from the wild-type and *cbs1* mutant RNAs. This estimated size

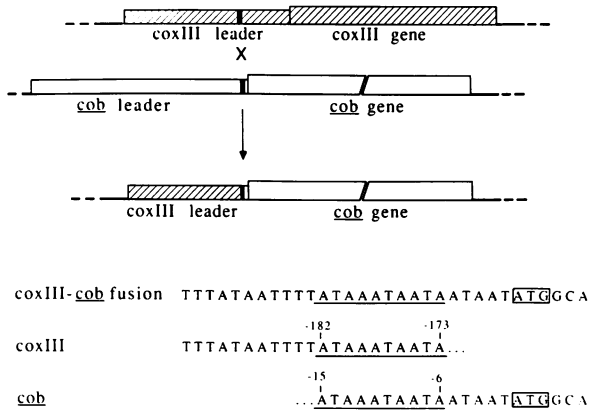

FIG. 1. Diagram of the recombination event generating *MSUcbs1-2* and nucleotide sequence of the fusion gene. (*Upper*) Schematic diagram of the formation of the coxIII–*cob* fusion gene (not to scale). Large boxes represent structural genes; smaller boxes, 5′ untranslated leader-encoding regions; black area, 10-bp region of nucleotide sequence identity between the wild-type coxIII and *cob* leader-encoding regions. (*Lower*) Nucleotide sequences of the leader-encoding regions of the coxIII–*cob* fusion gene *MSUcbs1-2*, coxIII (25), and *cob* (24). Negative numbers indicate distance (in nucleotides) upstream of the translational initiation codon of each gene. The 10-bp region where recombination took place is underlined, and the *cob* translational start codon is boxed.

agrees well with that predicted (1.6 kb) for a coxIII–*cob* fusion mRNA with the same 5′ end as that of the normal coxIII mRNA.

The 5′ end of the coxIII–*cob* fusion mRNA was next examined in more detail by S1 nuclease mapping (26). A 1.75-kb fragment spanning the coxIII–*cob* junction of the fusion gene, singly end-labeled within the first exon of the *cob* structural gene and extending into coxIII 5′ flanking sequences, was prepared as a probe. The probe was hybridized to total RNA isolated from a wild-type strain (D273-10B), the *cbs1* mutant strain (MCC60), or the respiring *cbs1* mutant carrying the coxIII–*cob* fusion gene *MSUcbs1-2* (MCC60R2-16). The hybrids were treated with S1 nuclease to digest single-stranded regions and the sizes of the protected DNA fragments were determined by electrophoresis and autoradiography (Fig. 2).

All three RNA samples protected a fragment of about 335 nucleotides (nt) from digestion with S1 nuclease. This fragment is approximately the size expected (320 nt) to be protected by the wild-type *cob* transcript, which is known to be present in all three strains (4). In addition, MCC60R2-16 RNA protected a fragment of 750 nt whose estimated size agrees well with the predicted size, 763 nt, for a coxIII–*cob* fusion mRNA with the same 5′ end as the wild-type coxIII mRNA. Thus a large proportion of the *cob* mRNA in MCC-60R2-16 carries the 5′ two-thirds of the wild-type coxIII mRNA leader.

Three fragments larger than the expected 750-nt fragment were also protected by MCC60R2-16 RNA. These larger RNA species could result from inefficient RNA processing (28) in the suppressed *cbs1* mutant strain. Protected fragments comigrating with the two major fragments protected by MCC60R2-16 RNA, 750 and 810 nt, were seen very weakly but reproducibly in the wild-type and *cbs1* mutant samples (they are nearly invisible in the experiment of Fig. 2). Since a chimeric gene corresponding to the probe does not exist in either of these two strains, this protection presumably results from the formation of trimolecular hybrid structures (29). A relatively large proportion of the entire probe was protected by wild-type, *cbs1* mutant, and MCC60R2-16 RNAs. This protection was probably due to

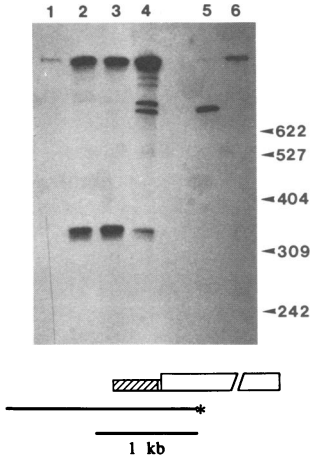

FIG. 2. S1 nuclease mapping of the coxIII–*cob* fusion transcript. Total nucleic acid (primarily RNA) (100 μg) prepared as described (27) from various strains was hybridized at 40°C with a DNA probe complementary to the coxIII–*cob* mRNA, radioactively end-labeled within *cob* sequences and extending about 1.45 kb upstream of the *cob* structural gene. (*Lower*) Diagram of the probe in relation to the coxIII–*cob* fusion gene (represented as in Fig. 1). The asterisk represents the radioactively labeled end. Hybridization conditions and digestion of the hybrids with S1 nuclease were as performed by Sharp *et al.* (26). The protected DNA fragments were subjected to electrophoresis on a 5% polyacrylamide/7 M urea gel. The gel was dried and exposed to x-ray film with an intensifying screen at −70°C. RNAs used in S1 nuclease protection were as follows: *Escherichia coli* tRNA, lane 1; D273-10B RNA, lane 2; MCC60 RNA, lane 3; MCC60R2-16 RNA, lane 4. DNA size standards were the DNA probe cut with *Xba* I (763 bp) (lane 5) and the uncut DNA probe (1.75 kb) (lane 6). Radioactively labeled *Msp* I fragments of pBR322 were also subjected to electrophoresis on the same gel as size standards (positions indicated by arrowheads with sizes in bp). A DNA fragment of about 335 nt was protected by RNA from all three yeast strains; in addition, two major fragments of 750 and 810 nt and two larger minor fragments that cannot be sized accurately in this gel were protected by MCC60R2-16 RNA. The two major fragments protected by MCC60R2-16 RNA were also protected extremely weakly by D273-10B and MCC60 RNAs (almost invisible in this experiment).

the presence of mitochondrial DNA in the RNA preparations and in any case was not specific to MCC60R2-16 RNA.

The *PET494*, *PET54*, and *PET122* Gene Products Are Required for Cytochrome *b* Translation from the Chimeric mRNA. To determine whether the *PET494*, *PET54*, and *PET122* products activate cytochrome *b* translation from the coxIII–*cob* mRNA, we tested the effects of mutations in each of these three genes on cytochrome *b* synthesis. Since *rho*$^-$ genomes do not support mitochondrial protein synthesis, cytochrome *b* synthesis from the coxIII–*cob* mRNA had to be tested in strains carrying both *rho*$^+$ and suppressor *rho*$^-$ genomes. However, the mitotic instability of the heteroplasmic combination of genomes in the suppressor strain posed a problem in the design of these experiments. The heteroplasmic state can only be maintained under selection, by demanding respiration, but a *cbs1* mutant strain carrying both *rho*$^+$ and suppressor *rho*$^-$ genomes and also mutant at *pet494*, *pet54*, or *pet122* could not respire because it would lack coxIII.

To avoid this problem, we tested transient expression of cytochrome *b* in zygotes with the desired genotype generated by mass mating, an approach developed by Strausberg and Butow (21). To determine whether *PET494* is required for cytochrome *b* expression, a *MAT***a**, *cbs1*, *pet494* [*MSUcbs1-2*] strain, MCC64, was mated with a *MATα*, *cbs1*, *pet494* [*rho*$^+$] strain, MCC66, to create diploid zygotes that were homozygous mutants in the *cbs1* and *pet494* genes and were mitochondrially heteroplasmic. As a control, the *MATα*, *cbs1*, *pet494* [*rho*$^+$] strain MCC66 was transformed

to wild-type *PET494* by using the cloned gene, and the resulting transformant, MCC67, was also mass-mated with the double mutant, *MSUcbs1-2* strain MCC64. Mitochondrial translation products of the mass-mated cells were radioactively labeled and inspected for the presence of cytochrome *b* (Fig. 3). There was no cytochrome *b* synthesis from unmated cells in this experiment, since the strains carrying only the suppressor *rho*$^-$ genome could not carry out any mitochondrial protein synthesis, whereas the *cbs1 pet494* [*rho*$^+$] strain, as expected, failed to synthesize either cytochrome *b* or coxIII (Fig. 3, lane 2).

As shown by the experiment of Fig. 3, a wild-type copy of *PET494* was necessary for cytochrome *b* expression from the chimeric mRNA. Zygotes that were homozygous mutants at the *cbs1* gene but had one wild-type copy of *PET494* and carried both *rho*$^+$ and *MSUcbs1-2* mitochondrial genomes synthesized cytochrome *b* (Fig. 3, lane 4). In contrast, isogenic zygotes that differed only in that they were homozygous mutant at *pet494* did not synthesize cytochrome *b* (Fig. 3, lane 3). Thus *PET494* was required to activate translation of cytochrome *b* from the chimeric mRNA bearing the 5′ two-thirds of the coxIII mRNA leader.

Analogous mating and labeling experiments were performed to test whether *PET54* or *PET122* was similarly required for cytochrome *b* expression from the chimeric mRNA. Mitochondrially heteroplasmic zygotes that were homozygous mutant at *cbs1* and at *pet54* or *pet122* failed to synthesize cytochrome *b* (Fig. 3). These experiments showed that all three nuclear genes required for coxIII translation—*PET494, PET54*, and *PET122*—were required for cytochrome *b* translation from the chimeric mRNA bearing part of the coxIII 5′ leader.

DISCUSSION

The three yeast nuclear genes *PET494, PET54*, and *PET122* were previously shown to be required for translation of the mitochondrial coxIII mRNA (10, 11, 13). We demonstrate here that translation of a mitochondrial gene product not normally under *PET494, PET54*, or *PET122* control, apocytochrome *b*, required the activity of these three nuclear genes when the 5′ untranslated leader of its mRNA was replaced with part of the coxIII mRNA 5′ untranslated leader.

These experiments place the site of action of *PET494, PET54*, and *PET122* within the coxIII mRNA leader between its 5′ end, at position −610 relative to the coxIII structural gene, and position −173. Thus in the wild-type coxIII mRNA the nuclear gene products must act at a site located at least 172 nt from the AUG codon. Furthermore, they need not act at a fixed distance from the AUG codon, since their presumed site of action must be 172 nt closer to the AUG in the coxIII–*cob* fusion transcript than it is in the wild-type coxIII mRNA. This may suggest that translational activation can occur at a variable distance from the target site of the activator proteins; alternatively, RNA secondary structure might compensate for differences in the placement of a site of action in one dimension along an mRNA. The actual secondary structure of the coxIII mRNA leader is unknown, but computer analysis suggests that it may be extensively base-paired (unpublished observations).

None of the evidence accumulated to date on the mechanism of coxIII translational activation has distinguished between the functions of *PET494, PET54*, and *PET122*. Mutations in these three unlinked genes cause essentially identical phenotypes and are suppressible by the same mitochondrial suppressor mutations (7–14). The experiments reported here show that these three nuclear gene products all act on the same region of the coxIII mRNA, consistent with the idea that they may function together, perhaps as a complex, to promote the same step of translation (Fig. 4). This model is also supported by the fact that both the PET494 and PET54 proteins are imported into mitochondria (ref. 10; unpublished results). (It is unknown at present whether the *PET122* gene encodes a mitochondrially located protein.)

Another mitochondrial translational activator, the product of the *CBS1* gene, also acts on the 5′ untranslated leader of its target mRNA and appears to be able to function at varying distances from the AUG codon (4). Translation of the wild-type *cob* mRNA requires another nuclear gene, *CBS2* (5), and possibly also the *CBP6* gene (6), in addition to *CBS1*. Therefore, the translational activation of two mitochondrial proteins, coxIII and cytochrome *b*, occurs by at least superficially similar pathways: the products of several

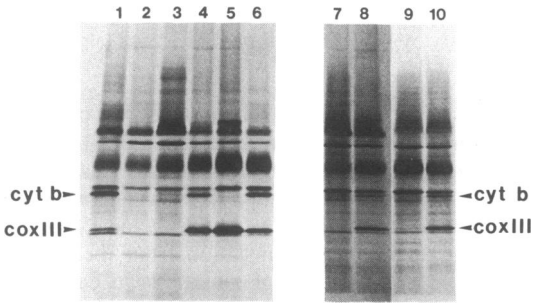

FIG. 3. Dependence of zygotic cytochrome *b* expression on the *PET494, PET54*, and *PET122* genes. Mitochondrial translation products were radioactively labeled and subjected to electrophoresis on $NaDodSO_4$/polyacrylamide gels. The positions of cytochrome *b* (cyt b) and coxIII are indicated. Mitochondrial translation products of the following haploid strains were labeled as controls: the *cbs1* [*rho*$^+$, *MSUcbs1-2*] strain MCC60R2-16, lane 1; the *cbs1 pet494* [*rho*$^+$] strain MCC66, lane 2; the *cbs1* [*rho*$^+$] strain MCC67 derived from MCC66 by transformation with the wild-type *PET494* gene, lane 5; the wild-type strain DA1, lane 6. Transient expression of cytochrome *b* in mass-mated populations is shown in lanes 3, 4, and 7–10. All zygotes were homozygous mutant at *cbs1* and carried both *rho*$^+$ and *MSUcbs1-2* mitochondrial genomes. In addition, the zygotes carried the following mutations: *pet494/pet494*, lane 3; *PET494/pet494*, lane 4; *pet54/pet54*, lane 7; *PET54/pet54*, lane 8; *pet122/pet122*, lane 9; *PET122/pet122*, lane 10. A faint band seen in lanes 3 and 7 at approximately the position of cytochrome *b* did not comigrate with cytochrome *b* under different conditions of electrophoresis. The strains used to generate the zygote populations were MCC66 × MCC64, lane 3; MCC67 × MCC64, lane 4; MCC68 × MCC69, lane 7; MCC71 × MCC69, lane 8; MCC97 × MCC99, lane 9; MCC98 × MCC99, lane 10.

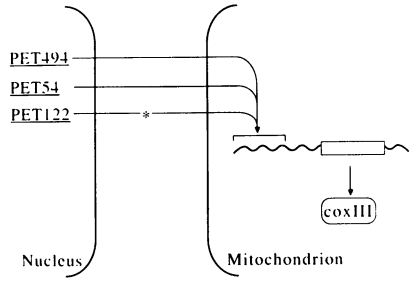

FIG. 4. Model for specific translational activation of the coxIII mRNA by *PET494, PET54*, and *PET122*. Wavy line represents the coxIII mRNA; box, coding region of the mRNA. The bracket indicates the region containing the site of action of the nuclear gene products within the 5′ untranslated leader. The asterisk interrupting the line representing *PET122* action indicates that it is unknown whether *PET122* acts directly within the mitochondrion. Unbroken lines representing *PET494* and *PET54* action indicate that the products of these genes are imported into mitochondria and may interact directly with the 5′ leader of the coxIII mRNA.

nuclear genes act on the 5′ untranslated leaders of specific mRNAs, at sites that need not be in a fixed position relative to the coding sequences.

Some aspects of mitochondrial translational activation are reminiscent of two examples of translational control in eukaryotic nucleocytoplasmic genetic systems. First, the ability of *Drosophila melanogaster* heat-shock mRNAs to be selectively translated at high temperatures has been shown to depend on a site in the 5′ untranslated mRNA leader (30–32). As in the mitochondrial system, the portion of the leader carrying this site in both the hsp70 and hsp22 mRNAs functions when placed at varying distances from the AUG codon (30, 32).

A second type of eukaryotic translational control that may be relevant to mitochondrial translational activation is exemplified in the case of the yeast gene *GCN4* (33, 34). The presence of AUG codons followed by short open reading frames in the *GCN4* mRNA 5′ untranslated leader greatly inhibits translation of the mRNA (33), as predicted by the "scanning" model of translational initiation (35, 36), unless the inhibition is relieved by trans-acting factors in response to environmental conditions (33, 34).

Several mitochondrial mRNAs have long 5′ leaders containing one or more AUG codons followed by short open reading frames. The wild-type coxIII 5′ leader, for example, has one short open reading frame starting with AUG (25); the *cob* leader has six (37). Although it is unknown whether mitochondrial ribosomes act by a scanning mechanism, if these open reading frames were inhibitory for the translational apparatus, a plausible function for the translational activators would be to overcome this inhibition in a transcript-specific manner. However, even if this model held true for coxIII translation, it could not represent the only mechanism for translational activation in mitochondria, since the coxII mRNA, whose translation is specifically controlled by the nuclear gene *PET111* (2, 3), does not contain open reading frames in its 5′ leader (38).

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