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Suppression of a Defect in the 5′ Untranslated Leader of Mitochondrial *COX3* mRNA by a Mutation Affecting an mRNA-Specific Translational Activator Protein

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Translation of the *Saccharomyces cerevisiae* mitochondrial *COX3* mRNA, encoding subunit III of cytochrome *c* oxidase, specifically requires the action of the nuclear gene products PET54, PET122, and PET494 at a site encoded in the 612-base 5′ untranslated leader. To identify more precisely the site of action of the translational activators, we constructed two large deletions of the *COX3* mRNA 5′ untranslated leader. Both deletions blocked translation without affecting mRNA stability. However, one of the large deletions was able to revert to partial function by a small secondary deletion within the remaining 5′ leader sequences. Translation of the resulting mutant (*cox3-15*) mRNA was still dependent on the nuclear-encoded specific activators but was cold sensitive. We selected revertants of this mitochondrial mutant at low temperature to identify genes encoding proteins that might interact with the *COX3* mRNA 5′ leader. One such revertant carried a missense mutation in the *PET122* gene that was a strong and dominant suppressor of the cold-sensitive defect in the mRNA, indicating that the PET122 protein interacts functionally (possibly directly) with the *COX3* mRNA 5′ leader. The *cox3-15* mutation was not suppressed by overproduction of the wild-type PET122 protein but was very weakly suppressed by overproduction of PET494 and slightly better suppressed by co-overproduction of PET494 and PET122.

Translation of several, and perhaps all, *Saccharomyces cerevisiae* mitochondrial mRNAs requires activation by mRNA-specific nuclear-encoded proteins in addition to the general mitochondrial translation machinery (1, 7, 12, 24, 43, 44). Translation of the mitochondrial *COX3* mRNA, encoding cytochrome *c* oxidase subunit III (coxIII), specifically requires at least three activator proteins: PET54, PET122, and PET494 (3, 5, 6, 9, 30). All three activator proteins are localized to the mitochondrion (5, 10, 34, 41) and act through a site or sites within the *COX3* mRNA 5′ untranslated leader (6). However, their mechanism of action is largely unknown.

We have taken a genetic approach to detecting the protein-protein and protein-mRNA interactions that occur during translational activation in this system. We found previously that mutations truncating the carboxy terminus of the PET122 protein could be allele-specifically suppressed by mutations in any of three different genes encoding proteins of the small subunit of mitochondrial ribosomes, demonstrating that PET122 functionally interacts with the ribosome (19, 21, 22, 35).

Here we present genetic data indicating that the PET122 protein interacts functionally with the *COX3* mRNA as well as with the mitochondrial ribosome. As a first step in localizing the mRNA site of translational activation, two deletions were made in the *COX3* mRNA 5′ untranslated leader-coding region. Although both mutations blocked translation, one was able to revert to partial function (cold-sensitive respiration) by an additional alteration of the leader-coding region. A dominant nuclear mutation that improved translation of this partially functional *COX3* mRNA, selected to allow cold-resistant respiration, was found to be a missense mutation in the *PET122* gene. This indicates that PET122 probably contacts the *COX3* mRNA and may function to mediate an interaction between the mRNA and the ribosomal small subunit.

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MATERIALS AND METHODS

Yeast strains, media, and genetic methods. *S. cerevisiae* strains used in this study are listed in Table 1. All strains were isogenic or congenic to the wild-type strain D273-10B (ATCC 25657), except MCC109rho^0. Strain MCC109rho^0 was derived from the wild-type strain DBY947 (38), whose wild-type *KAR1* gene was replaced by the *kar1-1* mutant allele by using the "pop-in/pop-out" strategy (48) with the integrating plasmid pMR723 (obtained from M. Rose). A *rho*^{0} derivative, lacking mitochondrial DNA (mtDNA), of the resulting strain, MCC109, was made by growth in ethidium bromide as described previously (15).

Media and genetic methods used were described previously (50). Respiratory growth was assessed on YPEG medium (3% ethanol, 3% glycerol, 1% yeast extract, 2% Bacto Peptone, 2% agar).

In vivo labeling of mitochondrial translation products. In vivo labeling at 30°C was performed as described previously (13, 15). For labeling at 16°C, incubation times were extended to compensate for slower growth as follows: after growth to saturation, cultures were diluted twofold in the same medium and incubated for 9 h rather than 2.5 h; cells were then washed, resuspended in minimal galactose-containing medium, and incubated for 10 h rather than 30 min; incubation with ^{35}S-labeled protein hydrolysate was done for 4 h rather than 1 h; incubation with Chase solution was done for 45 min rather than 10 min.

Strategy for replacement of the wild-type *COX3* 5′-untranslated leader region with mutant derivatives. Mitochondrial transformation was performed by high-velocity microprojec-

TABLE 1. Strains used in this study

Strain	Genotype[a]	Source or reference
DBY947	*MATα ade2-101 ura3-52 [rho⁺]*	38
MCC109rho⁰	*MATα ade2-101 ura3-52 kar1-1 [rho⁰]*	This study
MCC118	*MATa lys2 [rho⁺ cox3-5]*	14
MCC125	*MATa lys2 [rho⁺ cox3-10]*	This study
MCC129	*MATa lys2 [rho⁺ cox3-11]*	This study
MCC200	*MATα ade2 [rho⁺ cox3-15]*	This study
MCC200RA1	*MATα ade2 PET122-L195 [rho⁺ cox3-15]*	This study
MCC204	*MATα ade2 ura3-Δ PET122-L195 [rho⁺ cox3-15]*	This study
MCC206	*MATα ade2 ura3-Δ PET122-L195 [rho⁺]*	This study
MCC208	*MATa ade2 ura3-Δ pet122-6 [rho⁺ cox3-15]*	This study
TF210	*MATa ura3-52 leu2-3 leu2-112 [rho⁺ cox3-15]*	This study
DA1	*MATα ade2 [rho⁺]*	36
DL2	*MATa lys2 [rho⁺]*	14
DL2rho⁰	*MATa lys2 [rho⁰]*	L. S. Folley

[a] Mitochondrial genes are in brackets; genes not in brackets are nuclear.

tile bombardment (16, 28) as previously described (15), except that the host strain, MCC109rho⁰, carried the *kar1-1* mutation to allow transfer of the newly introduced mtDNA via cytoduction (4).

To replace the *COX3* 5′ untranslated leader-coding region with in vitro-made derivatives, we designed a method based on the homologous recombination strategy previously described (14, 15). To serve as a convenient recipient for the insertion of mutations altering the 5′ leader, a complete deletion of the *COX3* 5′ untranslated leader-coding region and part of the structural gene was constructed and integrated into the mitochondrial chromosome. This deletion was constructed by cloning into the plasmid pBluescript (−) (Stratagene) two fragments from the *COX3* region: a 2.9-kb fragment from the upstream region and a 1.1-kb fragment carrying the 3′ part of the coding sequence and 3′ flanking sequences. The resulting plasmid, pMC237, had a deletion from positions −605 (the 5′ end of the leader is at −612 [8]) to +228, where +1 represents the first nucleotide of the coding sequence; the deleted region was replaced with 63 bp of polylinker sequences. This deletion plasmid was introduced into the mitochondria of strain MCC109rho⁰ as described above, and a mitochondrial transformant was crossed to a wild-type strain (DL2) to allow replacement of the wild-type *COX3* gene by the deletion via homologous recombination. A nonrespiring haploid cytoductant, MCC118 (14), carried the deletion in the otherwise wild-type (*rho⁺*) mitochondrial genome, as confirmed by genetic crosses and DNA gel-blot analysis. It failed to synthesize detectable amounts of the *COX3* mRNA (Fig. 1) or the coxIII protein (Fig. 2). The name of this *cox3* allele, previously *cox3-Δ5* (14), has been changed to *cox3-5*.

Mutations altering the structure of the *COX3* 5′ leader. Two deletions within the *COX3* 5′ untranslated leader-coding region were constructed by oligonucleotide-directed in vitro mutagenesis (32) by using the reagents and strains provided in the Muta-Gene Phagemid in vitro mutagenesis kit (Bio-Rad). The plasmid used for mutagenesis was pMC241, carrying the *COX3* region on a 2.9-kb stretch of DNA from a *Hae*III site upstream of the promoter to an *Xba*I site downstream of the gene, in the plasmid pBluescript (−). One

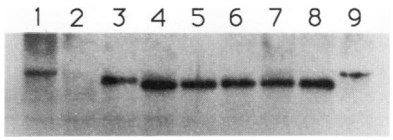

FIG. 1. *COX3* mRNA levels in several deletion mutants and a revertant. Total nucleic acids (primarily RNA) were isolated (53) from yeast strains grown at the indicated temperature in galactose-containing complete medium. Equal amounts (50 μg) were subjected to electrophoresis, blotted to a filter, and hybridized with a radioactively labeled plasmid (pLSF600; [14]) carrying the *COX3* structural gene. Lanes: 1, wild-type (DL2) grown at 30°C; 2, a *cox3-5* mutant (MCC118), lacking all of the 5′ leader-coding region and the 5′ part of the structural gene, grown at 30°C; 3, a *cox3-10* mutant (MCC125), lacking nucleotides −329 to −1 of the 5′ leader-coding region, grown at 30°C; 4, a *cox3-11* mutant (MCC129), lacking nucleotides −473 to −1 of the 5′ leader-coding region, grown at 30°C; 5, a *cox3-15* mutant (MCC200), lacking nucleotides −437 to −359 and −329 to −1 of the 5′ leader-coding region, grown at 30°C; 6, a revertant of the *cox3-15* mutant strain carrying the suppressor *PET122-L195* (MCC200RA1), grown at 30°C; 7, the *cox3-15* mutant (MCC200), grown at 12°C; 8, the *PET122-L195* [*cox3-15*] revertant (MCC200RA1), grown at 12°C; 9, wild type (DA1), grown at 12°C. The blot was next hybridized with a plasmid carrying the yeast actin gene (pSPACT [33]), and *COX3* and actin mRNA levels were quantitated by using a Betascope 603 Blot Analyzer (Betagen Corp.). Standardized to actin mRNA, the ratio of *COX3* mRNA to that of the wild type in each lane was 1 (lane 1), 0 (lane 2), 2.7 (lane 3), 3.1 (lane 4), 3.3 (lane 5), 2.6 (lane 6), 3.8 (lane 7), 7.6 (lane 8), and 0.9 (lane 9).

oligonucleotide, 5′-CTTTCTAAATGTGTCATATTTGTAT TGAATATAATT, was used to delete nucleotides −329 to −1, creating plasmid pMC241Δ1. Another, 5′-CTTT CTAAATGTGTCATGATAATATCTTCTT, was used to

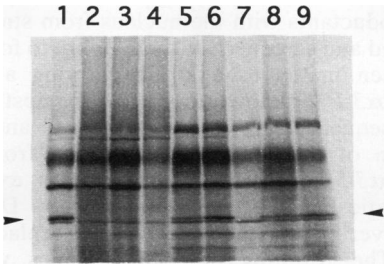

FIG. 2. CoxIII protein synthesis in several deletion mutants and a revertant. Cells were grown in galactose-containing complete medium and mitochondrial translation products were radioactively labeled in the presence of cycloheximide as described in Materials and Methods. Crude mitochondria were subjected to electrophoresis on a sodium dodecyl sulfate-containing 9 to 15% polyacrylamide gel, and the gel was dried and autoradiographed. The position of the coxIII protein is indicated (arrows). In this gel, cytochrome *b* was not resolved from coxII. Lanes: 1, wild-type (DL2) grown at 30°C; 2, a *cox3-5* mutant (MCC118), lacking all of the 5′ leader-coding region and the 5′ part of the structural gene, grown at 30°C; 3, a *cox3-10* mutant (MCC125), lacking nucleotides −329 to −1 of the 5′ leader-coding region, grown at 30°C; 4, a *cox3-11* mutant (MCC129), lacking nucleotides −473 to −1 of the 5′ leader-coding region, grown at 30°C; 5, a *cox3-15* mutant (MCC200), lacking nucleotides −437 to −359 and −329 to −1 of the 5′ leader-coding region, grown at 30°C; 6, a revertant of the *cox3-15* mutant strain carrying the suppressor *PET122-L195* (MCC200RA1), grown at 30°C; 7, the *cox3-15* mutant (MCC200), grown at 16°C; 8, the *PET122-L195* [*cox3-15*] revertant (MCC200RA1), grown at 16°C; 9, wild type (DA1), grown at 16°C.

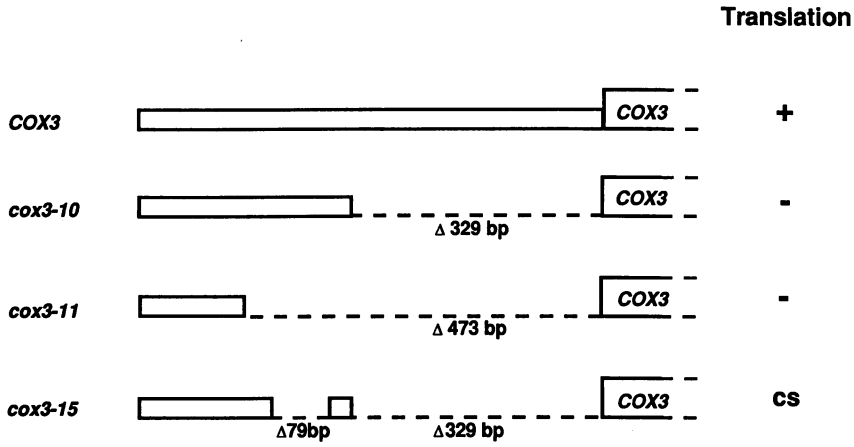

FIG. 3. The *COX3* 5′ untranslated leader-coding region. Smaller boxes, 5′ untranslated leader-coding regions; larger boxes, the structural gene; dashed lines, deleted regions. From top to bottom, the wild-type *COX3* gene encodes a 5′ untranslated leader approximately 612 nucleotides in length (8). The in vitro-made *cox3-10* allele deletes nucleotides −329 to −1 of the leader-coding region, and the in vitro-made *cox3-11* allele deletes nucleotides −473 to −1 of the leader-coding region. Both mutations block respiration by preventing coxIII translation. The *cox3-15* allele, selected as a spontaneous revertant of *cox3-10*, deletes an additional 79 bp between −437 and −359 and causes cold-sensitive (cs) respiration.

delete nucleotides −473 to −1, creating plasmid pMC241Δ2. The deletions were verified by DNA sequence analysis (47).

pMC241Δ1 and pMC241Δ2 were each introduced into the mitochondria of strain MCC109rho^0 as described above. The mitochondrial transformants were mated to strain MCC118, carrying the complete leader deletion (*cox3-5*) in the otherwise wild-type mitochondrial genome, to allow integration of the partial leader deletions and reconstitution of the wild-type *COX3* coding sequence by homologous recombination. None of the progeny from either cross were able to respire, indicating that both partial leader deletions blocked *COX3* expression. To identify strains carrying the partial leader deletions in the otherwise wild-type mitochondrial genome, haploid cytoductants with the nucleus from strain MCC118 were selected and screened for their ability to form respiring diploids when mated to a strain carrying a *cox3* point mutation (*cox3-V76*) known to map to the upstream part of the coding sequence, between positions +1 and +228 (49). This portion of the sequence is missing from the large deletion (*cox3-5*) of the recipient. Strains exhibiting the correct genetic behavior were analyzed by DNA gel-blot analysis to verify that the desired gene replacements had occurred. The resulting new *cox3* alleles were named *cox3-10* (nucleotides −329 to −1 deleted) and *cox3-11* (nucleotides −473 to −1 deleted) (Fig. 3).

Plasmids for overproduction of translational activator proteins. All plasmids used to overproduce the three translational activator proteins carried the *ADC1* promoter and terminator derived from the plasmid pAAH5 (2). *PET494* overexpression was directed by pMC210 (5). *PET54* overexpression was directed by pTM110A (34), which carries the *PET54* gene, from position −5 relative to the start of the coding sequence to the *Hin*dIII site downstream of the gene (10), in pAAH5, or by pAF001 (17), which carries a *Bam*HI fragment excised from pTM110A, consisting of the *ADC1* promoter, the *PET54* gene, and the *ADC1* terminator, in the vector pRS316 (52). *PET122* overexpression was directed by pEAD122 (34), which carries the *PET122* gene on a fragment that extends from −55 to +1500 relative to the structural gene, between the *ADC1* promoter and terminator derived from pAAH5, in the vector YEp352 (23).

Cloning and nucleotide sequence analysis of a *PET122* mutation. The *PET122* gene of strain MCC204 (Table 1) was isolated by gap repair (42). Plasmid pPHY4 (20), which carries *PET122* and flanking sequences on a 2.3-kb fragment in the vector YCp50 (46), was linearized by cleavage with *Sna*BI and *Xba*I. The gel-purified plasmid backbone, lacking the *PET122* structural gene, was used to transform the strain MCC204.

A 1.3-kb *Hin*cII fragment carrying the entire *PET122* coding sequence except for the first two codons was subcloned from the gap-repaired plasmid into the plasmid pBluescript (−) (Stratagene). The nucleotide sequence of the entire structural gene was determined, by the dideoxynucleotide chain termination method (47).

RESULTS

Deletions within the *COX3* mRNA leader that block translation. We have previously demonstrated that the mRNA site required for translational activation lies within the 5′-untranslated leader of the mitochondrial *COX3* mRNA between position −173 and the 5′ end of the mRNA at −612 (where +1 represents the first nucleotide of the *COX3* coding sequence [6]). As a first step towards localizing the site more precisely, two deletion mutations in the *COX3* gene were constructed in vitro, introduced into mitochondria, and integrated into the otherwise wild-type (*rho*$^+$) mitochondrial genome (see Materials and Methods). One mutation, *cox3-10*, deleted nucleotides −329 to −1 of the *COX3* mRNA 5′ untranslated leader; the other mutation, *cox3-11*, deleted nucleotides −473 to −1 (Fig. 3). Strains bearing either mutation failed to respire. Although both mutant strains accumulated greater-than-wild-type steady-state levels of the deleted *COX3* mRNAs (Fig. 1), the coxIII protein was specifically absent from their mitochondrial translation products, radioactively labeled in vivo in the presence of cycloheximide (Fig. 2). Therefore, the respiratory block in the *cox3-10* and *cox3-11* mutant strains was due to the fact that these deletion mutant mRNAs could not be translated.

An alteration in the *COX3* leader causing cold-sensitive translation. Deletions in the *COX3* mRNA leader could have

prevented translation for any of several reasons. They could slightly alter a site required for specific translational activation or create a mutant secondary structure that blocks translation. In either of these cases it might be possible to restore translation by secondary mutations. On the other hand, deletions in the leader could completely remove either the specific translational activation site or some generally required feature of the mRNA, such as a ribosome binding site. To see whether the deleted mRNAs might still contain the site for translational activation, we attempted to select respiring revertants of each mutant. A strain carrying the larger deletion, *cox3-11*, was never seen to revert (except, very infrequently, by heteroplasmic gene rearrangements that completely bypass the *COX3* translational activation system [5, 37]). However, a strain carrying the smaller deletion, *cox3-10*, did revert, both spontaneously and after UV mutagenesis, to respiratory competence by alterations within the remaining portion of the *COX3* leader-coding region (8). Thus, the *cox3-10* mutation appears to have either damaged the site required for translational activation in an easily repairable way or generated an aberrant RNA structure that masks the functional site.

One of the respiring revertants of *cox3-10* isolated after UV mutagenesis had a secondary deletion of nucleotides −437 to −359 in addition to the original deletion of nucleotides −329 to −1 (Fig. 3). This new allele is named *cox3-15*. After transferring the revertant mitochondrial genome by cytoduction (4) to a [*rho*^{0}] strain with an unmutagenized nucleus, creating strain MCC200 (Table 1), we analyzed the effects of the *cox3-15* mutation on respiratory growth, *COX3* mRNA levels, and coxIII protein synthesis. The *cox3-15* allele, selected to allow respiration at 30°C, was found to cause a cold-sensitive respiratory defect. Strains bearing this mutation grew moderately well (more slowly than the wild type) on nonfermentable carbon sources at 30 and 37°C, very poorly at 16°C, and not at all at 13.5°C (Fig. 4 and 5). (*cox3-15* mutant strains grew identically to the wild type on glucose-containing medium at all temperatures [data not shown].)

Two lines of evidence confirmed that the cold sensitivity resulted from the changes in the *cox3-15* leader region, rather than from changes elsewhere in the mitochondrial genome. First, cold-resistant recombinant diploids were formed in a cross between a *cox3-15* strain and a *rho*$^-$ mutant strain carrying only the wild-type *COX3* region of mtDNA. Second, crossing a *cox3-15* strain to a strain carrying a completely wild-type mitochondrial genome except for the larger in vitro-generated *cox3* leader deletion (*cox3-11*) did not relieve the cold sensitivity.

The cold-sensitive respiratory defect of strains bearing the *cox3-15* allele was not due to altered stability of the *cox3-15* mRNA. RNA gel-blot experiments showed that a *cox3-15* strain actually accumulated greater-than-wild-type mRNA levels, whether grown at restrictive or permissive temperatures (Fig. 1). However, the *cox3-15* mutation did block coxIII protein synthesis at low temperatures. In vivo labeling of mitochondrial translation products in the presence of cycloheximide (13, 15) showed that at 16°C, a *cox3-15* strain did not synthesize detectable levels of the coxIII protein (Fig. 2). At 30°C, a *cox3-15* strain did synthesize the coxIII protein, at a lower level than did a wild-type strain (Fig. 2). This result is consistent with the observation that the mutant grew more slowly than the wild type at this temperature on medium requiring respiration.

The doubly deleted *cox3-15* mRNA contains a site for specific translational activation, since expression was dependent on *PET54*, *PET122*, and *PET494*. This was demonstrated by crossing a nuclearly wild-type, *cox3-15* strain to strains lacking mtDNA (*rho*^{0}) that carried null mutations in each of the three nuclear activator genes. In each case the haploid meiotic progeny containing *cox3-15* mtDNA but lacking functional *PET54*, *PET122*, or *PET494* genes (one-half of the total progeny of each cross) failed to respire. This result established that a partially functional site(s) for translational activation was within the remaining 204 nucleotides of the *cox3-15* 5′ untranslated leader.

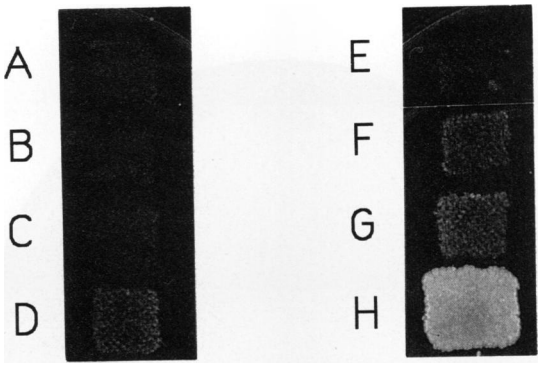

FIG. 4. The *cox3-15* cold-sensitive respiratory defect is weakly suppressed by overproduction of some wild-type translational activator proteins and strongly suppressed by a missense mutation in *PET122*. Cells were patched onto a glucose-containing minimal medium plate with added adenine; the plate was grown at 30°C for 3 days and then printed to YPEG. The YPEG print was grown at 13.5°C for 12 days and reprinted to YPEG, and the second print was grown for an additional 12 days at 13.5°C. Patches A through G are the nuclearly wild-type, mitochondrially *cox3-15* mutant strain TF210, transformed as follows (in each patch the strain was transformed with plasmids bearing the *URA3* and *LEU2* genes, so that all transformants were prototrophic): A, carrying only plasmid vectors (pAAH5 and pRS316); B, overproducing PET54 (pAF001 and pAAH5); C, overproducing PET122 (pEAD122 and pAAH5); D, overproducing PET494 (pMC210 and pRS316); E, overproducing both PET54 and PET122 (pTM110A and pEAD122); F, overproducing both PET54 and PET494 (pAF001 and pMC210); G, overproducing both PET122 and PET494 (pEAD122 and pMC210); H, the *PET122-L195*, [*cox3-15*] strain MCC200RA1.

Effects of overproduction of the translational activator proteins on the cold-sensitive phenotype of the *cox3-15* mutation. If the *cox3-15* respiratory phenotype were due to weaker binding to one or more of the translational activator proteins, then overproduction of the limiting protein or proteins might suppress *cox3-15*. We tested this possibility by transforming strain TF210, a nuclearly wild-type strain carrying the *cox3-15* mitochondrial allele (Table 1), with plasmids carrying each of the translational activator genes *PET54*, *PET122*, and *PET494* under control of the strong *ADC1* promoter (see Materials and Methods). In addition, TF210 was cotransformed with pairs of plasmids to simultaneously overproduce each of the three possible pairs of activators. Respiratory growth of the transformants was monitored by streaking on YPEG medium and comparing colony size with that of the untransformed strain (data not shown) and by printing patches from selective medium to YPEG (Fig. 4). In no case was there strong suppression of the cold sensitivity. Neither PET54 nor PET122 alone had any effect on growth. However, overproduction of PET494 allowed extremely weak growth at 13.5°C, and co-overproduction of both PET494 and PET122 together allowed

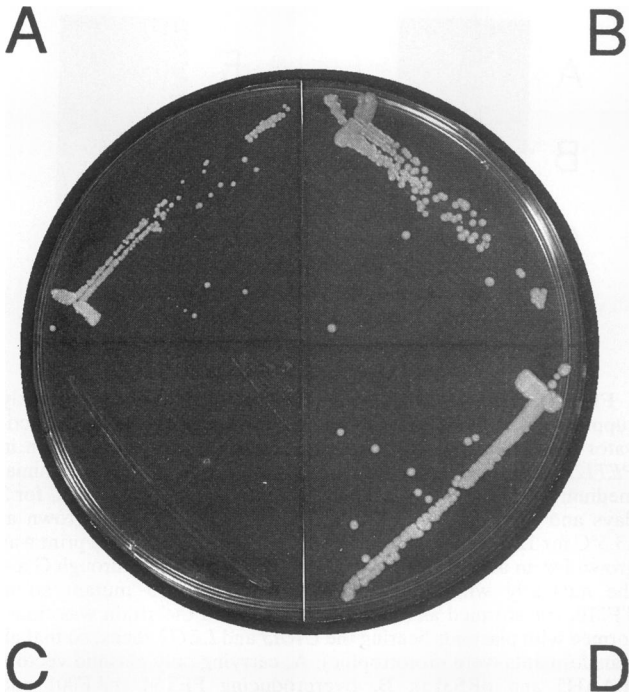

FIG. 5. Suppression of the *cox3-15* cold-sensitive respiratory defect by the *PET122-L195* mutation. Strains were streaked on a YPEG plate (see Materials and Methods) and incubated at 13.5°C for 2 weeks. A, *PET122-L195* [*rho*$^+$ *cox3-15*] (the suppressor strain MCC200RA1); B, *PET122-L195* [*rho*$^+$ *COX3*] (the suppressor mutation combined with the wild-type *COX3* gene; strain MCC206); C, *PET122* [*rho*$^+$ *cox3-15*] (the cold-sensitive mutant MCC200); D, *PET122* [*rho*$^+$ *COX3*] (wild type; strain DL2).

slightly better growth. (Overproduction of PET494, PET122, or both proteins together had no effect on the respiratory growth of a wild-type strain.) Co-overproduction of PET54 and PET122 had no effect on growth. Co-overproduction of PET494 and PET54 allowed the same weak growth in the cold seen for the transformants overproducing PET494 alone. We confirmed that the proteins were indeed overproduced in the appropriate transformants by Western blotting with antisera against each of the three activator proteins (data not shown) (5, 10, 34). These results suggest that PET494 levels may be limiting for translation of the *cox3-15* mRNA at low temperatures, and that once PET494 levels are increased, PET122 levels may also be limiting.

Selection and characterization of second-site suppressors of the cold-sensitive *cox3-15* mutation. To identify genes encoding proteins that could be altered to compensate for the defect of the *cox3-15* mRNA leader, we isolated revertants of a *cox3-15* strain. Six spontaneous cold-resistant revertants of the strain MCC200 (Table 1) were selected at 13.5°C on ethanol-glycerol medium. Revertants were first tested to determine whether their suppressor mutations had occurred in the nuclear or mitochondrial genomes by crossing to a wild-type strain lacking mtDNA (DL2rho^0; Table 1), sporulating the diploids, and analyzing tetrads. Four of the six suppressors segregated 2:2, indicating that they were nuclear mutations, while two showed 4:0 segregation, suggesting mitochondrial mutations. The nuclear suppressors were next crossed to null mutants in each of the known *COX3* translational activators, *PET54*, *PET122*, and *PET494*, to determine whether the suppressors were linked to any of these three genes. Three of the nuclear suppressors were not linked to any of the known translational activator genes; but one suppressor, that of strain MCC200RA1, was tightly linked to *pet122* (no recombinants in 47 tetrads). This suppressor greatly improved the respiratory growth of the *cox3-15* mutant, in contrast to the extremely weak suppression described above resulting from overproduction of the wild-type PET494 or PET494 and PET122 proteins (Fig. 4). The *PET122*-linked suppressor of strain MCC200RA1 not only allowed the *cox3-15* mutant strain to grow on YPEG medium at 13.5°C (Fig. 4 and 5) but also restored nearly wild-type respiratory growth at 30°C (data not shown). A diploid formed by crossing the suppressor strain (MCC200RA1) to a wild-type, *rho*^{0} strain was cold resistant, indicating that the suppressor is dominant.

The suppressor mutation had little or no effect on levels of the *cox3-15* mRNA, which were already severalfold greater than wild-type levels, at either restrictive or permissive temperatures (Fig. 1). However, the suppressor strain was able to translate the *cox3-15* mRNA in the cold: the coxIII protein was synthesized in the suppressor strain at 16°C whereas no coxIII synthesis was detectable in the mutant strain at 16°C (Fig. 2). The suppressor strain also showed increased levels of coxIII synthesis at 30°C relative to that of the mutant (Fig. 2), consistent with the observation that the suppressor improved respiratory growth of a *cox3-15* strain at all temperatures.

The *PET122*-linked suppressor of *cox3-15* is a missense mutation in *PET122*. To determine whether the *cox3-15* suppressor linked to *PET122* affected the structure of the PET122 protein, the *PET122* gene was cloned from the suppressor strain by gap repair and its nucleotide sequence was determined (see Materials and Methods). A single nucleotide difference was found between the wild-type (21, 40) and suppressor *PET122* genes: an A to T change at nucleotide 985 of the coding sequence in the suppressor gene that would substitute leucine for the wild-type glutamine at codon 195.

To confirm that this *PET122* allele, termed *PET122-L195*, was in fact responsible for suppression of *cox3-15*, an approximately 1.7-kb *Sna*BI-*Xba*I fragment carrying the *PET122-L195* gene was used to integratively transform a strain (MCC208) carrying a partial *pet122* deletion and *cox3-15* to respiratory competence. All of the respiring transformants obtained were able to respire at 13.5°C, forming colonies identical in size to those of the original revertant strain MCC200RA1, confirming that *PET122-L195* was indeed the suppressor of the *cox3-15* cold-sensitive phenotype.

Allele specificity of *PET122-L195*. We tested the allele specificity of the *PET122-L195* suppressor by combining it with wild-type and various mutant alleles of *COX3*. Strains with the desired combination of mutations were constructed by cytoduction (4) or by sporulation. Respiratory growth of a *PET122-L195* [*rho*$^+$, *COX3*] strain was indistinguishable from that of the wild type at 13.5 (Fig. 3), 16, 30, and 37°C.

PET122-L195 did not suppress the nonfunctional *cox3* 5′ leader deletion alleles *cox3-10* or *cox3-11*. Thus, the *PET122-L195* suppressor does not simply bypass the translational activation system. However, it partially suppressed an in vitro-made mutation that changes the ATG initiation codon of the *COX3* gene to ATA (*cox3-1*) and causes a leaky respiration-defective phenotype (14). Thus, in addition to strongly suppressing the *cox3-15* mutant mRNA leader, the *PET122-L195* mutation appears to slightly enhance translation of a partially functional *COX3* mRNA with a wild-type leader.

DISCUSSION

Elucidation of the mechanism by which translation of yeast mitochondrial mRNAs is activated will require an understanding of the interactions among the activator proteins, mRNAs, and general components of the translation system. Here, we have used a mutation affecting the 5′ untranslated leader region of the *COX3* mRNA as the starting point for a genetic selection that identified the product of a previously known activator gene as a protein functionally interacting with the mRNA. The mutant *cox3-15* gene specifies an mRNA with a 204-nucleotide 5′ leader, roughly one-third the length of the wild-type 5′ leader. This mutation was isolated as a revertant of a nonfunctional *cox3* leader deletion mutation (*cox3-10*) that had been generated in vitro and inserted into the mitochondrial chromosome. Since translation, at permissive temperature, of the *cox3-15* mRNA requires all three specific activators, a site of action for the activators must be retained in this mutant leader. However, the mutant site of action is either intrinsically weak or partially masked by aberrant structures in the mutant mRNA since it functions more poorly than the wild type at permissive temperatures and is nonfunctional at low temperatures. Comparison of the nucleotide sequences and predicted RNA secondary structures of the 5′ untranslated leaders of the nonfunctional *cox3-10* mutant and the partially functional *cox3-10* revertant, *cox3-15*, with that of the wild type failed to provide an obvious rationalization of the effects of these *cox3* mutations on translation.

We used a *cox3-15* mutant strain to select spontaneous revertants that might carry alterations in proteins interacting with the *COX3* mRNA 5′ untranslated leader (26, 27). One such cold-resistant strain carried a suppressor that mapped to the nuclear *PET122* gene. The *PET122* suppressor allele, named *PET122-L195*, was found to carry a missense mutation that would change residue 195 from glutamine to leucine. This nuclear mutation is a very strong, and dominant, suppressor of the defects caused by the mutated *COX3* mRNA leader. Since overproduction of the wild-type PET122 protein did not suppress *cox3-15*, we believe it unlikely that *PET122-L195* merely increased the stability of the protein without altering its activity. The *PET122-L195* mutation did not suppress either of two nonfunctional in vitro-generated *cox3* leader deletion mutations, one of which was the parent of the suppressible *cox3-15* mutation. Thus, the *PET122-L195* suppressor appears to compensate specifically for the altered mRNA leader structure specified by *cox3-15* to restore an almost fully functional interaction.

Genetic dissection of protein-DNA interactions has provided detailed information on how repressors bind to operators (25, 31, 56) and how RNA polymerase σ factors recognize promoters (11, 18, 29, 51, 54, 55, 57). Although the work reported here does not provide information on the individual amino acid-nucleotide contacts, the functional interaction between the PET122 protein and the *cox3-15* mRNA strongly suggests that they may be in direct contact during the process of translational activation or at least are components of the same complex.

The *PET122-L195* mutation has no effect on the growth of an otherwise wild-type strain. However, the mutation may slightly increase the affinity of PET122 for the wild-type *COX3* leader, since it partially suppresses a leaky initiation codon mutation whose phenotype is affected by PET122 levels (14). Missense mutations that strengthen the binding of bacteriophage λ repressor to its operator site have been reported (39).

The residue affected by the *PET122-L195* mutation is located 59 residues from the PET122 carboxy terminus. The carboxy-terminal region of PET122 is required for function in an otherwise wild-type strain, since mutations that delete either the final 67 or 24 amino acids block respiration (21). However, partial function of both truncated forms of PET122 can be restored by suppressor mutations in any of three genes encoding proteins of the small subunit of the mitochondrial ribosome (19, 21, 22, 35). In addition to demonstrating a functional interaction between the PET122 protein and the mitochondrial ribosome, these findings indicate that residue 195 of PET122 cannot be absolutely required for recognition of the *COX3* mRNA leader. Thus, if residue 195 is in a region that contacts the *COX3* mRNA directly, it is not the only region that does so. Alternatively, the *PET122-L195* mutation could alter the conformation of the protein such that an RNA-binding domain located elsewhere better recognizes the *COX3* mRNA leader altered by the *cox3-15* mutation. In either case, PET122 appears to mediate an interaction between the *COX3* mRNA and the ribosome. Interestingly, PET122 appears to be a membrane-bound protein (34), suggesting that it may play a role in localizing synthesis of the highly hydrophobic coxIII protein to the mitochondrial inner membrane.

Suppression of a mutant defect by overproduction of a protein can also indicate functional interactions (45). Although the effects seen here were very weak, overproduction of the PET494 protein partially suppressed the cold sensitivity. Co-overproduction of PET122 and PET494 strengthened this suppression, while overproduction of PET122 alone had no effect. Overexpression of PET54, either alone or in combination with either of the other two activators, also had no effect. These results suggest that PET494 levels may be limiting for translation of the *COX3* mRNA and also that the PET494 and PET122 proteins may interact with each other.

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