Identification and Consequences of a Guanosine-15 to Adenosine-15 Change in the Yeast Mitochondrial $tRNA^{Ser}_{UCX}$ Gene†

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ABSTRACT: We have characterized a mutation affecting the yeast mitochondrial $tRNA^{Ser}_{UCX}$. The mutation is a single nucleotide substitution located within the structural portion of the $tRNA^{Ser}_{UCX}$ gene which causes the strain to be respiratory deficient. The substitution is a G → A transition located in the dihydrouridine arm. The $tRNA^{Ser}_{UCX}$ transcripts from the mutant gene are present in the same amount and are the same size as transcripts from the wild-type gene. The mutant $tRNA^{Ser}_{UCX}$ can be charged in vitro with mitochondrial aminoacyl-tRNA synthetase. Mitochondrial protein synthesis does occur in the mutant, but the amount of cytochrome oxidase subunit I is significantly decreased relative to other mitochondrial translation products.

The yeast mitochondrial genome codes for two distinctly different classes of gene product. One class is those protein subunits involved in oxidative phosphorylation, and the other is those RNAs and proteins involved in the mitochondrial translation system [for a review, see Borst & Grivell (1978)]. Genes coding for enzymes involved in oxidative phosphorylation are called *mit* genes, and strains with mutations in these genes are called *mit*⁻ mutants (Flury et al., 1974; Tzagoloff et al., 1975a–c). Genes coding for products essential for mitochondrial translation are called *syn* genes, and *syn*⁻ mutants are strains with lesions in these genes (Tzagoloff et al., 1976; Coruzzi et al., 1979). Originally, these two types of mutation were distinguished by an analysis of mitochondrial protein synthesis. Strains with *mit*⁻ mutations continue to carry out mitochondrial protein synthesis but are deficient in a respiratory enzyme activity due to a mutation in the structural gene for one of the mitochondrially encoded enzyme subunits. Strains with *syn*⁻ mutations were selected as respiratory deficient, mitochondrial protein synthesis negative since the lack of mitochondrial protein synthesis was part of the original selection regimen.

Subsequent studies have used deletion mapping to localize mutations in or near *syn* genes (Bolotin-Fukuhara et al., 1977; Faye et al., 1976; Trembath et al., 1977; Berlani et al., 1980; Zassenhaus & Perlman, 1982; M. Nichols and P. Perlman, unpublished experiments) but have not always analyzed their effects on mitochondrial protein synthesis directly. Both nonconditional and temperature sensitive *syn*⁻ mutations map in the area of tRNA genes, and some are known to affect the aminoacylation of specific tRNAs. A *syn*⁻ strain producing mitochondrial tRNA deficient in aspartic acid accepting activity has been shown to have a single base pair substitution within the structural gene for $tRNA^{Asp}$ (Miller et al., 1981). This mutant fits the original description of *syn*⁻ mutants since no mitochondrial protein synthesis was detected in this strain. In a continuing effort to understand mitochondrial tRNA biosynthesis, structure, and function, we have analyzed additional *syn*⁻ strains.

We report here on two independently isolated mutants, PZ204A and MN250, which map near the $tRNA^{Ser}_{UCX}$ gene. Both mutants produce tRNA deficient in accepting serine in a reaction catalyzed by *Escherichia coli* aminoacyl-tRNA synthetase under conditions where only the mitochondrial $tRNA^{Ser}_{UCX}$ is charged. These charging experiments provided preliminary evidence that the mutations afffected $tRNA^{Ser}_{UCX}$. DNA sequence analysis of the $tRNA^{Ser}_{UCX}$ gene from each strain showed that both strains carry an identical lesion, a single base substitution $G_{15} \rightarrow A_{15}$ in $tRNA^{Ser}_{UCX}$. Additional analysis of one of these strains, PZ204A, demonstrated that $tRNA^{Ser}_{UCX}$ is produced in normal amounts and is charged with serine in a homologous in vitro system. An unexpected result was obtained when the mitochondrial protein synthetic ability of PZ204A was examined. A display of mitochondrial translation products from a strain carrying this mutant tRNA gene shows a pattern typical of a *mit*⁻ mutant. Even though the lesion in PZ204A is in a tRNA gene, all mitochondrial translation products except cytochrome oxidase subunit I are made.

Experimental Procedures

Strains and Culture Conditions. The wild-type *Saccharomyces cerevisiae* strain, ID41-6/61, and its *syn*⁻ derivatives, PZ204A and MN250, were grown on galactose media (2% galactose, 0.2% glucose, 1% yeast extract, and 1% Bactopeptone) at 28 °C. These *syn*⁻ strains reverted to respiratory sufficiency as judged by their ability to grow on nonfermentable substrates at a frequency of less than 10^{-7}.

DNA Isolation and Sequence Analysis. Mitochondrial DNA was isolated by using published procedures (Martin et al., 1977). The *Hpa*II fragment containing the $tRNA^{Ser}_{UCX}$ gene from each strain was obtained by cloning a subset of genomic *Hpa*II fragments into the *Cla*I site of pBR322 (Miller et al., 1981). An *Hpa*II fragment fraction enriched for this gene was obtained by digesting mitochondrial DNA with *Hpa*II (New England Biolabs) under the recommended conditions and isolating those fragments between 200 and 400 base pair(s) (bp) in length following electrophoresis on a 6% acrylamide gel. These fragments were ligated with phosphatased, *Cla*I-digested pBR322 and used to transform *E. coli* strain HB101. Transformants were screened for the $tRNA^{Ser}_{UCX}$ gene by the procedure of Grunstein & Hogness (1975) using radiolabeled *Hpa*II fragments containing the $tRNA^{Ser}_{UCX}$ gene from strain D273-10B (Miller et al., 1979).

Plasmid DNA containing the $tRNA^{Ser}_{UCX}$ gene was sequenced by the Maxam & Gilbert (1980) technique by using the A > C, A + G, G, C, and C + T reactions. The mito-

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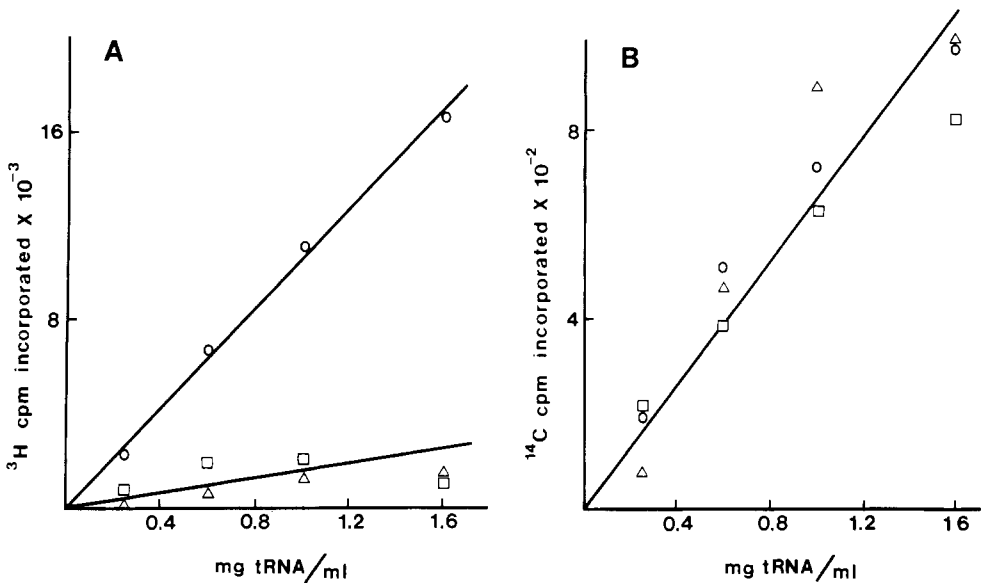

FIGURE 1: Acylation of wild-type and mutant tRNAs, using *E. coli* tRNA synthetase. Increasing amounts of whole cell tRNA were added to otherwise identical in vitro charging reaction containing [^{3}H]serine and [^{14}C]leucine as discussed under Experimental Procedures. (A) [^{3}H]Serine accepted by wild-type (○), PZ204A (□), and MN250 (△) tRNAs. (B) [^{14}C]Leucine accepted by wild-type (○), PZ204A (□), and MN250 (△) tRNAs.

chondrial DNA portion of the plasmid from the wild-type strain and both mutant strains was sequenced by labeling the single *Hin*dIII site of the recombinant plasmid with *E. coli* DNA polymerase I and [α-^{32}P]dATP. Sequence overlap was obtained by labeling the single *Eco*RI site with DNA polymerase I and [α-^{32}P]dATP. Cleavage products were separated on 87-cm, 6% polyacrylamide–7 M urea thin (0.5 mm) gels.

RNA Isolation and Analysis. Total mitochondrial RNA was prepared from isolated mitochondria by a single phenol extraction of sodium dodecyl sulfate ($NaDodSO_4$) lysates as described by Locker (1979). The RNA was separated by electrophoresis in 1.5% agarose–urea or 10% acrylamide–urea gels and transferred to DBM (diazobenzyloxymethyl) paper (Alwine et al., 1979) by electroblotting (Stellwag & Dahlberg, 1980). $tRNA^{Ser}_{UCX}$ gene transcripts were detected by autoradiography following hybridization in 50% formamide, 5 × SSC (standard saline citrate), 1 × Denhardt's buffer, 0.2% $NaDodSO_4$, and 50 μg/mL calf thymus DNA by using a nick-translated DNA fragment containing the $tRNA^{Ser}_{UCX}$ gene. Mitochondrial tRNA was isolated as described in Hopper et al. (1982).

Total cellular tRNA was isolated from whole cells by suspending each gram wet weight into 2 mL of 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, 1 mM ethylenediaminetetraacetic acid (EDTA), and 2 mL of phenol and shaking overnight at room temperature. The aqueous phase was recovered following centrifugation at 10000*g* and passed over a 3 × 3 cm DEAE-cellulose column which had been equilibrated with 250 mM NaCl–50 mM ammonium acetate, pH 5.2. The column was washed with the same buffer until the A_{260nm} of the eluate was less than 0.020. The tRNA was eluted from the column by the addition of 0.8 M NaCl–50 mM ammonium acetate, pH 5.2, and precipitated overnight at −20 °C with 2 volumes of cold 100% ethanol.

tRNAs were acylated in 25 μL of 100 mM Tris-HCl, pH 7.5, 3 mM ATP, 5 mM $MgCl_2$, and 0.2 mM dithiothreitol (DTT) containing 0.5 μCi of [^{14}C]leucine (New England Nuclear, 31.5 mCi/mmol), 2 μCi of [^{3}H]serine (New England Nuclear, 10 Ci/mmol), and 3.5 μg of *E. coli* aminoacyl-tRNA synthetase (Muench & Berg, 1966). The mixture was incubated at 37 °C for 10 min, and the trichloroacetic acid (Cl_3CCOOH) precipitable counts were determined.

Mitochondrial Protein Synthesis. Labeling and isolation of mitochondrial proteins with ^{35}S were done in the presence of cycloheximide as described by Douglas & Butow (1976). Mitochondrial proteins were electrophoresed on a 10% $NaDodSO_4$–polyacrylamide gel with a 5% stacking gel. Following electrophoresis, the gel was fixed, dried, and autoradiographed.

Results

Perlman and his colleagues have isolated several respiratory-deficient yeast strains whose lesions map to a small area of the mitochondrial genome which contains the $tRNA^{Ser}_{UCX}$ gene (personal communication). We reasoned that these lesions were probably located in or near the $tRNA^{Ser}_{UCX}$ gene and resulted in respiratory-deficient strains because of a disruption of mitochondrial protein synthesis.

Syn$^-$ mutants with lesions that map near other tRNA genes make mitochondrial tRNA deficient in accepting aspartic acid (Faye et al., 1976; Trembath et al., 1977), threonine (Trembath et al., 1977), cysteine, and histidine (Berlani et al., 1980). In vitro charging assays using *E. coli* aminoacyl-tRNA synthetase were carried out to compare the serine-accepting activity of tRNA from wild-type, PZ204A, and MN250 strains. *E. coli* aminoacyl-tRNA synthetase was used because it will charge neither cytoplasmic $tRNA^{Ser}$ nor the mitochondrial $tRNA^{Ser}_{AGY}$ with serine (Baldacci et al., 1976). Since *E. coli* aminoacyl-tRNA synthetase also charges mitochondrial tRNA with leucine (Casey et al., 1974), [^{14}C]leucine was included in the reactions to provide an internal standard for comparing the mutant and wild-type tRNAs. The results of these experiments showed that even at elevated tRNA concentrations, little [^{3}H]serine was accepted by tRNA from PZ204A and MN250 (Figure 1). However, at the same time, [^{14}C]leucine was accepted at wild-type levels by tRNA in the mutant strains.

To determine the molecular basis for this difference between wild-type and mutant tRNAs, we sequenced the $tRNA^{Ser}_{UCX}$ gene in these strains. The *Hpa*II fragment containing the mitochondrial $tRNA^{Ser}_{UCX}$ gene was isolated and ligated into the *Cla*I site of pBR322, and the mitochondrial portions of the plasmids were sequenced. A comparison of the mutant

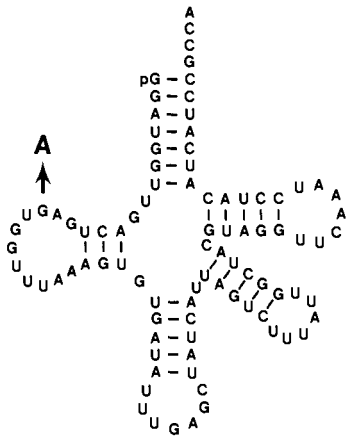

FIGURE 2: Cloverleaf structure of $tRNA^{Ser}_{UCX}$. Since the sequence is deduced from the DNA sequence, modified bases are not shown. The CCA end which is not encoded in the gene is added. The G substitution at A_{15}, found in PZ204A and MN250, is indicated.

sequences with the wild-type sequence showed that the lesion in each mutant was a single base substitution within the $tRNA^{Ser}_{UCX}$ gene. The two strains considered here, PZ204A and MN250, have an identical mutation, an adenosine residue at position 15 instead of the guanosine residue present in the wild-type strain (Figure 2). The $tRNA^{Ser}_{UCX}$ gene sequence from strain ID41-6/61 is, as expected, identical with the sequence from the same gene in strain D273-10B. There are some differences in the flanking sequences (Figure 3).

Since a decrease in charging could be due to a decrease in the number of transcripts as well as to a decrease in the suitability of the tRNA as an amino acid acceptor, an electrophoretic analysis of $tRNA^{Ser}_{UCX}$ transcripts in the wild-type and mutant strains was done to determine if $tRNA^{Ser}_{UCX}$ was made in comparable amounts in the two strains as well as to detect any difference in transcript migration patterns. Mitochondrial RNA from both the wild-type strain and the mutant strain PZ204A was isolated, separated by electrophoresis on a 10% acrylamide gel, transferred to DBM paper by the electroblotting technique of Stellwag & Dahlberg (1980), and hybridized with labeled DNA containing the $tRNA^{Ser}_{UCX}$ gene. As shown in Figure 4, hybridization of the probe with 4S RNA is as strong in the mutant strain as it is in the wild-type strain, indicating that the mutant contains the $tRNA^{Ser}_{UCX}$ transcript at about the same level as the wild-type strain. Further, the migration of these two transcripts was identical on both gel systems.

To determine the effects of this mutation on mitochondrial protein synthesis, we compared the ability of strain PZ204A and the wild-type strain to synthesize mitochondrial proteins by incubating each in the presence of $^{35}SO_4$ and cycloheximide as described under Experimental Procedures. Mitochondrial proteins were isolated and separated by electrophoresis on 10% $NaDodSO_4$–polyacrylamide gels with a 5% stacking gel (Figure 5). Surprisingly, PZ204A makes all of the expected translation products except subunit I of cytochrome oxidase. The mitochondrial DNA of PZ204A carries a single point mutation that has been located within 300 bases of the $tRNA^{Ser}_{UCX}$ gene by deletion mapping (Zassenhaus & Perlman, 1982) and within the $tRNA^{Ser}_{UCX}$ gene by sequence analysis (this work). Therefore, its effect cannot be due to a defect in the cytochrome oxidase subunit I gene but to an alteration in the tRNA gene which ultimately has an effect on cytochrome oxidase subunit I synthesis. Further, the overall level

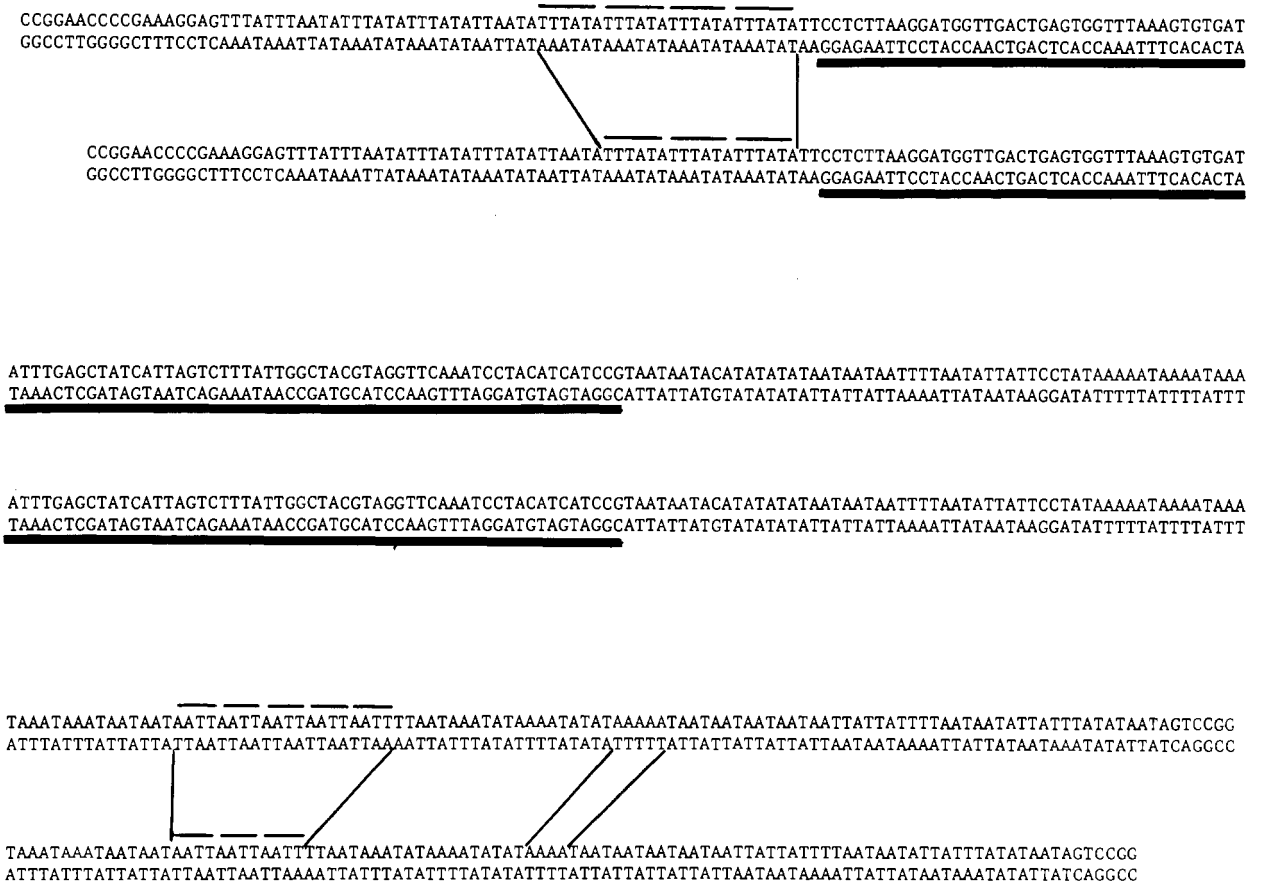

FIGURE 3: Comparison of the sequence of the *Hpa*II fragment containing the $tRNA^{Ser}_{UCX}$ gene from the two wild-type strains, ID41-6/61 (top line) and D273-10B (bottom line). The $tRNA^{Ser}_{UCX}$ gene is underlined. Differences in the flanking sequence of the two strains are indicated. The bars above the sequence indicate the repeating units associated with these differences.

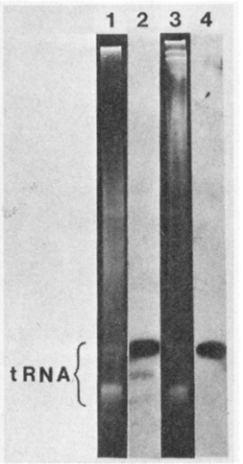

FIGURE 4: $tRNA^{Ser}_{UCX}$ gene transcripts from wild-type and mutant cells. An equal amount of mitochondrial RNA from ID41-6/61 (lanes 1 and 2) and PZ204A (lanes 3 and 4) was separated electrophoretically on a 10% acrylamide–urea gel, stained with ethidium bromide (lanes 1 and 3), transferred to DBM paper, and hybridized with a DNA fragment containing the $tRNA^{Ser}_{UCX}$ gene (lanes 2 and 4). The $tRNA^{Ser}_{UCX}$ gene migrates toward the top of the 4S tRNA region of the gel because it has a long extra arm.

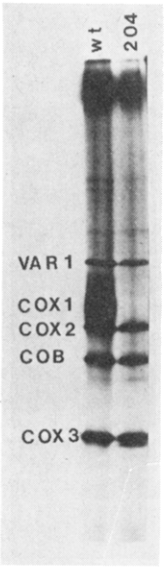

FIGURE 5: Mitochondrial protein synthesis in the wild-type ID41-6/61 strain and in the mutant strain PZ204A. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed as described under Experimental Procedures. VAR1, COX1, COX2, COB, and COX3 indicate the positions of *var1* protein, cytochrome oxidase subunit I, cytochrome oxidase subunit II, cytochrome *b*, and cytochrome oxidase subunit III, respectively. Autoradiograph of the labeled proteins from the wild-type strain ID41-6/61 (wt) and from the mutant strain PZ204A (204).

of protein synthesis appeared roughly equivalent in these two strains.

Since mitochondrial protein synthesis occurs in vivo, the mutant tRNA should be charged with mitochondrial aminoacyl-tRNA synthetase in vitro. A tRNA which accepts [^{3}H]serine and migrates in the same position as does wild-type $tRNA^{Ser}_{UCX}$ on RPC-5 columns has been identified in PZ204A mitochondrial tRNA preparations charged by using mitochondrial aminoacyl-tRNA synthetases in vitro (data not shown). A rigorous quantitative comparison of serine-accepting activity between wild-type and mutant tRNA has not been accomplished. Several tRNA preparations from PZ204A were tested and found to accept 60–80% of the [^{3}H]serine accepted by wild-type tRNA. These results must be interpreted with caution since mitochondrial $tRNA^{Ser}_{AGY}$ as well as any contaminating cytoplasmic $tRNA^{Ser}$ will be charged by mitochondrial aminoacyl-tRNA synthetase. Nonetheless, the mutant tRNA is not as deficient in its ability to accept serine from mitochondrial aminoacyl-tRNA synthetase as it is in its ability to accept serine from *E. coli* aminoacyl-tRNA synthetase. Detailed and rigorous comparisons of the serine-accepting activity of wild-type and mutant tRNAs can only be accomplished if those tRNAs are isolated individually.

Discussion

We report here the characterization of two strains with respiratory deficiencies caused by mutations that map near the $tRNA^{Ser}_{UCX}$ gene of yeast mitochondrial DNA. Both mutants have the identical change of G → A at position 15. To date, we have analyzed one other mutant in the $tRNA^{Ser}_{UCX}$ gene. The mitochondrial genome of this mutant undergoes spontaneous deletions, and the base change in the $tRNA^{Ser}_{UCX}$ gene is different than that reported here. (D. L. Miller and N. C. Martin, unpublished experiments). It is surprising to find that two of the three sequenced mutants in this gene have the same base change. Until the sample size is increased, it cannot be determined if this reflects a statistical quirk, whether position 15 is a "hot spot" for mutations, or whether only changes in a few positions in the $tRNA^{Ser}_{UCX}$ gene can result in stable *syn*$^-$ strains.

The sequence of $tRNA^{Ser}_{UCX}$ in the wild-type strain ID41-6/61 is identical with the sequence of the same gene in the previously sequenced wild-type strain, D273-10B (Miller et al., 1979) even though the mitochondrial genomes of these two wild-type strains differ in genome size and structure (Morimoto & Rabinowitz, 1978; Sanders et al., 1977). The (A + T)-rich sequences flanking the $tRNA^{Ser}_{UCX}$ gene, however, differ in the two wild-type strains. ID41-6/61 contains three small insertions of 6, 8, and 1 bp relative to D273-10B (Figure 3). This increases the size of the 318 bp *Hpa*II fragment to 333 bp and is consistent with the observation that the *Hpa*II fragment from ID41-6/61 that contains the $tRNA^{Ser}_{UCX}$ gene is slightly larger than the fragment which contains this gene in D273-10B (data not shown). The larger two insertion/deletions have an interesting structure. In both cases, the larger form can be made by inserting an exact copy of the basic unit of a series of direct repeats at the boundary of the repeat. The six-base insertion $^{5'}$TTTATA$^{3'}$ goes from three direct repeats in D273-10B to four in ID41-6/61, and the eight-base insertion goes from three direct repeats of $^{5'}$AATT$^{3'}$ in D273-10B to five in ID41-6/61. Alternatively, of course, the smaller form can be made by deletion of the basic units of the direct-repeat series. Since the relationship of these two wild-type strains is not known, we are unable to state which has occurred. A similar observation has been made by Hudspeth et al. (1982) for strains having different alleles of the yeast mitochondrial locus *var1*. They observe that the variation in the size of the *var1* peptide in the two strains is due to the insertion of repeats of $^{5'}$AAT$^{3'}$ into a series of $^{5'}$AAT$^{3'}$ direct repeats.

The base substitution in PZ204A and MN250 is a guanosine to adenosine transition at position 15. There is ample evidence that base substitutions can affect the transcription of tRNA genes (DeFranco et al., 1980; Koski et al., 1980; Ciampi et al., 1982) and processing of tRNA gene transcripts (Altman, 1971; McClain et al., 1975). The G → A transition does not appear to affect transcription or processing since comparable levels of $tRNA^{Ser}_{UCX}$ are present in the mutant and wild-type strains.

It might be expected that the G → A transition will significantly change the secondary structure of $tRNA^{Ser}_{UCX}$ and

thus affect its function. In those tRNAs which have been studied by X-ray crystallography, a guanosine at position 15 invariably hydrogen bonds with a cytosine at position 48 [numbering rules followed are found in Sprinzel & Gauss (1982)], and there is a cytosine at position 48 in mitochondrial $tRNA^{Ser}_{UCX}$. In the $tRNA^{Ser}_{UCX}$ from PZ204A and MN250, the hydrogen bond would be unable to form. An altered tRNA structure could affect any of the steps of protein biosynthesis from aminoacylation to codon recognition. The dramatic difference in serine-accepting activity observed with *E. coli* aminoacyl-tRNA synthetase first suggested that the mutation precluded charging of $tRNA^{Ser}_{UCX}$ in vivo. Since protein synthesis occurs in the mutant, this cannot be the case. Subsequent experiments with mitochondrial aminoacyl-tRNA synthetase demonstrated that the mutant tRNA can, in fact, be charged in vitro. Abelson et al. (1969) have characterized an *E. coli* tyrosine suppressor revertant which also has a G → A transition at position 15. Aminoacylation of the mutant tRNA in this *E. coli* strain is normal.

The pattern of mitochondrial translation products in PZ204A is particularly unusual in that only cytochrome oxidase subunit I is missing. This would be easy to explain if that peptide were the only one requiring $tRNA^{Ser}_{UCX}$ for its synthesis. However, all the known mitochondrial protein genes require $tRNA^{Ser}_{UCX}$ for translation of their mRNAs (Bonitz et al., 1980a). Although the mutant tRNA can be charged with serine, we have not eliminated the possibility that the mutant $tRNA^{Ser}_{UCX}$ sometimes accepts amino acids other than serine. Such substitutions might interfere with several mitochondrial processes which appear normal in the mutant. For example, it is likely that a ribosomal protein is a mitochondrial translation product (Terpstra & Butow, 1979), and if it is not produced in active form, mitochondrial proteins might not be made. The open-reading frames within the introns of cytochrome oxidase subunit I and cytochrome *b* genes contain codons requiring $tRNA^{Ser}_{UCX}$ for their translation. If a protein product is coded by these sequences and is necessary for RNA splicing (Lazowska et al., 1980; LaSalle et al., 1982; Anziano et al., 1982), then it functions in these mutant strains since cytochrome *b* is made normally.

We have examined the context of UCX codons in mitochondrial genes since it is now recognized that the context affects the efficiency of translation of particular codons (Bossi & Roth, 1980). The $tRNA^{Ser}_{UCX}$ in yeast mitochondria can read all four codons in the UCX codon family either by using a novel wobble mechanism or by reading only two out of three bases (Bonitz et al., 1980b). The codon UCU is preceded by a G only once in a mitochondrial gene, and this is in an exon of cytochrome oxidase subunit I. UCU is also followed by a C only in cytochrome oxidase subunit I gene exons or introns. Therefore, it is possible that a context might be the cause of poor cytochrome oxidase subunit I production in PZ204A. With the exception of the above two examples, all four codons of the UCX serine codon family are preceded or followed by all bases in all of the other genes on yeast mitochondrial DNA.

Cytochrome oxidase subunit I synthesis is more sensitive to perturbations of the mitochondrial system than is the synthesis of the other mitochondrially encoded peptides. For example, mitochondria treated with nalidixic acid (Mahler & Johnson, 1979) show reduced synthesis of cytochrome oxidase subunit I compared to the synthesis of other peptides. Synthesis of cytochrome oxidase subunit I in isolated mitochondria is decreased relative to the synthesis of other mitochondrial translation products as if the synthesis of that peptide is more sensitive to the isolation and subsequent treatments of mitochondria (Steinkeller & Mahler, 1980). In vivo, cytochrome oxidase subunit I synthesis is particularly susceptible to regulatory influences. Finally, cytochrome oxidase subunit I synthesis is more sensitive to catabolite repression than is any other mitochondrial gene product (Pajot et al., 1976). Many mitochondrial mutations outside of the cytochrome oxidase subunit I gene but not in tRNA genes disrupt synthesis of cytochrome oxidase subunit I (Claisse et al., 1978; Mahler et al., 1978; Slonimski et al., 1978; Alexander et al., 1979; Haid et al., 1979; Linnane et al., 1979; Murphy et al., 1980).

Mutants other than PZ204A which have decreased levels of cytochrome oxidase subunit I synthesis and map as if they could be in tRNA genes have been described (Mahler et al., 1978; H. P. Zassenhaus and P. S. Perlman, personal communication). Since the synthesis of cytochrome oxidase subunit I is so sensitive, it is not unreasonable to expect certain tRNA mutants which allow mitochondrial translation but not subunit I synthesis. Mutants somewhat deficient in amino acid accepting activity could support near-wild-type levels of most mitochondrial translation products but be unable to make subunit I. Alternatively, mutant tRNAs could show amino acid accepting activity comparable to wild-type tRNAs but be less efficient in a subsequent step in protein synthesis. Abelson et al. (1969) have shown that a G → A transition at position 15 of an *E. coli* tyrosine suppressor does not affect aminoacylation but does abolish the ability of the tRNA to suppress nonsense codons without significantly altering global protein synthesis.

The phenotype of PZ204A described here is interesting in that it raises the question of how a mutation in a single tRNA gene affects the synthesis of only one mitochondrial protein. These results demonstrate that *syn*$^-$ mutants can result in a partial disruption of protein synthesis. Many potentially interesting mutants will be missed if selection regimens for *syn*$^-$ mutants eliminate strains that make mitochondrial proteins.

Acknowledgments

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Registry No. Aminoacyl-tRNA synthetase, 9028-02-8; cytochrome oxidase, 9001-16-5; DNA (*Saccharomyces cerevisiae* mitochondria serine-specific $tRNA_{UCX}$ gene coding region), 84582-71-8; guanosine, 118-00-3; adenosine, 58-61-7.

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