A G43 to U43 mutation in *E. coli* tRNA$^{Tyr}_{su3^+}$ which affects processing by RNase P

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**ABSTRACT**

A novel mutation in the anticodon stem of *E. coli* tRNA$^{Tyr}_{su3^+}$ (G43 to U43) has been characterized. The gene coding for the mutant tRNA, carried by phage $\phi$80DHA61.3 a derivative of $\phi$80psu$_3^+$su$_0^-$, produces only 20% of mature suppressor tRNA as compared with $\phi$80psu$_3^+$. Both the mutant tRNA precursor and mature tRNA have an altered conformation. The precursor tRNA coded for by $\phi$80DHA61.3 is processed by RNase P more slowly than the su$_3^+$ precursor and does not form as stable an enzyme-substrate complex as does su$_3^+$ precursor. $\phi$80DHA61.3 also contains a large deletion which begins in the spacer region between the su$_3^+$ gene and the su$_0^-$ gene, extends through the su$_0^-$ gene and includes most of the repeated region following the tRNA genes.

**INTRODUCTION**

In *Escherichia coli*, the *tyr T* locus contains two copies of the gene which codes for the minor tyrosine accepting tRNA species$^{1-2}$. The suppressor mutation su$_3^+$ results from the substitution of a single base in the anticodon (G35 to C35) of the first copy of the gene in this locus$^3$. This gene has been studied extensively to elucidate many steps in the biosynthesis of tRNA$^{1-9}$. One of these steps, maturation at the 5' terminus of the tRNA sequence, is accomplished by the action of RNase P, a ribonucleoprotein which removes extra 5' proximal nucleotides of the transcript by a single endonucleolytic cleavage. RNase P is an essential enzyme in the maturation of all tRNA molecules in *E. coli*$^{10}$, and a thorough examination of the interaction between this enzyme and its substrates is important for an understanding of tRNA biosynthesis.

$\phi$80psu$_3^+$su$_0^-$ carries the entire *tyr T* locus and $\phi$80psu$_3^+$ carries the su$_3^+$ gene but is missing the su$_0^-$ gene and the spacer between the two genes$^{15}$. Both point and deletion mutations (which affect RNase P processing) have been previously identified in the su$_3^+$ gene sequence of these phage$^{11-14}$ (see Fig. 1). We have examined several mutant derivatives of $\phi$80psu$_3^+$su$_0^-$ which affect its ability to produce a functional tRNA$^{Tyr}_{su3^+}$. In this report we describe a
Figure 1. tRNA\textsuperscript{Tyr} \textsubscript{su\textsubscript{3}+} in the standard cloverleaf secondary structure. Some nucleotide substitutions found in mutants which are not efficiently processed by RNase P are shown. The mutation described in this report (G43 to U43) is also indicated in the figure.

tRNA\textsuperscript{Tyr} weak suppressor mutant isolated following hydroxylamine mutagenesis. This mutant codes for a tRNA\textsuperscript{Tyr} transcript with a G to U transversion at position 43 of the mature tRNA sequence. Both precursor and mature tRNA produced from this mutant appear to have an altered conformation. The precursor RNA does not form as stable an enzyme-substrate complex with RNase P as does the su\textsubscript{3}+ precursor.

Materials and Methods
Isolation of Ø8ODHA61.3

Hydroxylamine mutagenesis of Ø8opsu\textsubscript{3}+su\textsubscript{0}− (doublet) was performed as described by Tessman\textsuperscript{16}.

Suppressor activity of phage derivatives was assayed by plating phage on the E. coli strain CA274 (HfrC lac\textsuperscript{−} 125 amber trp\textsuperscript{−} amber su\textsuperscript{−}) in LB top agar containing 0.4% 5-bromo 4-chloro 3-indole \(\beta\)-galactopyranoside (XG) and 0.4 mM isopropyl-thio \(\beta\)-galactopyranoside (IPTG). Phage which directed the production of functional suppressor tRNA gave blue plaques of intensity proportional to the level of suppression. After mutagenesis of Ø8opsu\textsubscript{3}+su\textsubscript{0}−, plaques with less intense blue color than the parent phage appeared with a frequency of about \(10^{-4}\). One of these was designated Ø8ODHA61.3 and is the
mutant we describe here. In the terminology used in ref. 11, this phage could also be called $\theta 80psu^{-3}U43$.

**Properties of phage DNA**

Phage were prepared by obtaining high titer stocks according to the procedure of Miller\textsuperscript{17}. Phage DNA was isolated by phenol extraction followed by chloroform extraction and ethanol precipitation. Restriction endonuclease digestions were performed at 37°C in 50 mM NaCl, 10 mM MgCl\textsubscript{2}, 100 $\mu$g/ml BSA, and 10 mM dithiothreitol. DNA restriction fragments were purified by electrophoresis in low gelation temperature (LGT) agarose vertical slab gels and isolated from the gel slices by two hot (70°C) phenol extractions followed by chloroform extraction and ethanol precipitation.

**Cloning of Mutant tRNA\textsubscript{TYR} Gene Carried by $\theta 80DH\textsubscript{A61.3}$**

Phage DNA from $\theta 80DH\textsubscript{A61.3}$ was digested with Eco RI and Bam HI and separated in a 1% LGT agarose gel. A 6.4 Kb Bam HI/Eco RI fragment was isolated and ligated to an Eco RI/Bam HI double digestion of pBR328\textsuperscript{18}. The ligation mix was used to transform *E. coli* HB101 cells\textsuperscript{19}. Single colonies were isolated on LB plates containing ampicillin (100 $\mu$g/ml) and those colonies containing recombinants were screened for sensitivity to tetracycline on LB plates containing tetracycline (25 $\mu$g/ml). One candidate from 58 colonies was rescreened and found to contain the appropriate $\theta 80$-derived DNA fragment. This clone was called p61.3 and DNA was purified according to the procedure of Meagher, et al\textsuperscript{20}. An Msp I digestion of p61.3 and $\theta 80DH\textsubscript{A61.3}$ produced an 802 bp fragment which has been shown by Southern blotting and hybridization to in vitro labeled tRNA\textsubscript{TYR} to contain the tRNA\textsubscript{TYR} gene (data not shown). The 802 bp Msp I fragment was isolated from p61.3 in preparative quantities and used for restriction mapping and nucleotide sequence analysis.

**Restriction Mapping and Nucleotide Sequence Analysis of p61.3**

The Msp I fragment from p61.3 containing the su\textsubscript{3} gene was extensively mapped using the procedure of Smith and Birnstiel\textsuperscript{21}. Ava I, Dde I, Hae II, Hae III, Xho I, and Hind III were used to produce a restriction map for comparison with $\theta 80psu^{-3}su_{0}^{-}$ and to develop a strategy for sequencing (data not shown). Selected DNA fragments were labeled with the appropriate $^{32}$P labeled deoxynucleotide and digested again or strand separated to obtain fragments labeled at one end only. DNA sequencing was carried out according to Maxam and Gilbert\textsuperscript{22}.

**Isolation and analysis of precursor and mature tRNA\textsubscript{TYR}**

$^{32}$P labeled tRNA\textsubscript{TYR} coded for by $\theta 80psu^{-3}$ and by $\theta 80DH\textsubscript{A61.3}$ were prepared by in vivo labeling during infection\textsuperscript{12} of the strain BP266\textsuperscript{6}. 

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\(^{32}\)P labeled tRNA\(^{\text{Tyr}}\) precursor RNA was prepared by in vivo labeling during infection of the strain A49\(^{23}\) by either \(\Phi 80\text{DHA61.3}\) or \(\Phi 80\text{psu}^+\). RNA was isolated from polyacrylamide gel slices by overnight electroelution at 5°C into dialysis membranes. The RNA was further purified on a CF-11 cellulose column\(^{24}\) and fingerprinted according to the procedure of Platt and Yanofsky\(^{25}\).

Secondary digests of the RNase T1 generated oligonucleotides were carried out with RNases A and T2\(^{26}\). Tertiary digests of RNase A oligonucleotides were carried out with RNase T2.

**RESULTS**

**Suppressor properties of \(\Phi 80\) derivatives**

\(\Phi 80\text{DHA61.3}\) was isolated after hydroxylamine mutagenesis of \(\Phi 80\text{psu}^+\text{su}^-\) as a plaque showing less intense blue color than the parent phage in the blue plaque assay (see Materials and Methods). \(\Phi 80\text{DHA61.3}\) was further tested for suppression by infecting CA274 in the presence of IPTG and assaying for \(\beta\)-galactosidase activity using o-nitro-phenyl \(\beta\)-galactopyranoside (ONPG) in a standard assay\(^{35}\) (data not shown). Under these conditions, \(\Phi 80\text{DHA61.3}\) infected cells were found to produce about 20% the level of \(\beta\)-galactosidase found in \(\Phi 80\text{psu}^+\text{su}^-\) or \(\Phi 80\text{psu}^+\) infected cells.

**Analysis of the transcript from \(\Phi 80\text{DHA61.3}\)**

The tRNA containing transcript from \(\Phi 80\text{DHA61.3}\) was compared with the corresponding transcript from \(\Phi 80\text{psu}^+\) in an attempt to explain the difference in the level of suppression manifested by these two phages. \(\Phi 80\text{DHA61.3}\) produces about 20% of the amount of both precursor and mature tRNA compared to the amount made by \(\Phi 80\text{psu}^+\). Both precursor and mature tRNA produced by \(\Phi 80\text{DHA61.3}\) migrated slightly slower in non-denaturing gels than the corresponding species from \(\Phi 80\text{psu}^+\) (Fig. 2).

Isolated precursor tRNAs from \(\Phi 80\text{DHA61.3}\) and from \(\Phi 80\text{psu}^+\) were processed in vitro with an excess of RNase P\(^{27}\) and analyzed again in a 10% polyacrylamide non-denaturing gel (Fig. 3, left panel). The 'mature' tRNA product and the substrate precursor tRNA from \(\Phi 80\text{DHA61.3}\) again showed differences in electrophoretic mobilities compared to their \(\Phi 80\text{psu}^+\) counterparts. The oligonucleotides 43 bases long, processed from the 5' end of the precursor from either \(\Phi 80\text{DHA61.3}\) or \(\Phi 80\text{psu}^+\), had more similar mobilities. (The 5' leader sequence of the precursor tRNA\(^{\text{Tyr}}\) originally was determined by RNA sequencing mutants to be 41 nucleotides long. However, analysis of the DNA sequence of this gene revealed one extra A, embedded in a series of A's, and one extra C, embedded in a run of C's\(^{14}\).) When these RNA
Figure 2 Properties of transcripts from Ø80psu⁺ and Ø80DHA61.3. Left). Autoradiograph of 10% polyacrylamide gel separation of RNA extracted from the strain A49 (temperature sensitive for RNase P function) infected at the restrictive temperature with Ø80psu⁺ (C35) (left lane) or Ø80DHA61.3 (C35-U43) (right lane). The position of the origin, the dye marker bromphenol blue, and various RNAs are shown. Right). Autoradiograph of a 10% polyacrylamide gel separation of RNA extracted from BF266 after infection in the presence of 50 μg/ml of chloramphenicol of Ø80psu⁺ (left lane) or Ø80DHA61.3 (right lane). The position of the origin, the mature tRNA Tyr and bulk tRNA are shown. Precursor and mature tRNA coded for by each phage were isolated as described in Materials and Methods.

The amount of precursor tRNA produced by Ø80DHA61.3 was compared to that produced by Ø80psu⁺ as follows: gel slices corresponding to the mobilities of 5S RNA, M3 RNA (a Ø80 encoded transcript), and precursor tRNA produced by both Ø80DHA61.3 and Ø80psu⁺ were excised from the gel. The radioactivity in each band was determined by counting Cerenkov radiation. The amount of radioactivity in the 5S RNA and M3 RNA bands from Ø80DHA61.3 and Ø80psu⁺ served as an internal standard to compare the amount of radioactivity incorporated in each precursor tRNA and the degree of phage infection. The ratio of Ø80DHA61.3 precursor tRNA to Ø80psu⁺ precursor tRNA was calculated after subtracting the background from Ø80-infected and uninfected cell controls (lanes not shown) carried out in the same experiment.

Species were electrophoresed in a denaturing 5% polyacrylamide-7M urea gel, no differences in mobility were apparent (Fig. 3, right panel). This observation indicates that differences in migration in native gels were probably not a
result of an increase in the length of the mutant transcript or mature tRNA but rather to a change in sequence which affected the conformation of the RNAs.

To begin an analysis of the mutant transcript, the precursors from \( \text{o}80\text{psu}_3^+ \) and from \( \text{o}80\text{DHA61.3} \) were digested with RNase T1 (Fig. 4) and the resulting fingerprints compared. This comparison showed that one spot (Fig. 4, no. 5), corresponding to CCG found in the \( \text{s}u_3^+ \) transcript, was missing from the \( \text{o}80\text{DHA61.3} \) precursor. In addition, spot no. 15' in the U43 fingerprint, which contains an oligonucleotide including the anticodon and which immediately precedes CCG in the \( \text{s}u_3^+ \) gene sequence, appeared to migrate more slowly in the second dimension than the corresponding oligonucleotide from \( \text{s}u_3^+ \). These differences could also be detected by analyzing the same RNase T1 digests on a 25% polyacrylamide-7M urea gel. In the digest of the \( \text{o}80\text{DHA61.3} \) precursor tRNA, the band corresponding to the trinucleotide CCG was missing (data not shown) and the band containing the anticodon, which is 12 nucleotides in length in the digest of the \( \text{s}u_3^+ \) transcript, migrated to the position of an oligonucleotide of approximately 15 bases. In addition, about...
Figure 4 RNase T1 generated fingerprints of precursor tRNA\textsuperscript{\text{TYR}}
Top), intact pre-tRNA\textsuperscript{\text{TYR}}\textsubscript{\text{C35}} from \text{\textit{O}80psu\textsuperscript{+}} (C35).
Bottom), intact pre-tRNA\textsuperscript{\text{TYR}}\textsubscript{\text{C35-U43}} from \text{\textit{O}80DHA61.3} (C35-U43).
First dimension: electrophoresis on cellogel, pyridine acetate buffer, pH 3.5.
Second dimension: homochromatography on PEI plates. The differences between
the two fingerprints are shown by arrows. Spot no. 5, found in the C35
fingerprint is missing in the C35-U43 fingerprint and spot no. 15' from
C35-U43 precursor migrates more slowly in the second dimension than spot no.
15 from C35 precursor. Oligonucleotides were eluted from the PEI plates and
analyzed as described in Materials and Methods.

30\% of the largest oligonucleotide, which contains the 3' terminal sequence of
the tRNA precursor, has one extra or one fewer nucleotide reflecting some
imprecision in processing (and is discussed further below). Secondary
analysis of all the RNase T1 oligonucleotides from the \textit{O}80DHA61.3 transcript
showed that none other than no. 15 had an altered sequence and that in this
oligonucleotide, the sequence had been changed from ACUCUAAA\textsubscript{CUG} to
Figure 5 Time course of processing of precursor tRNA$^{\text{Tyr}}$ by RNase P. 10 µl of RNase P purified through the Sepharose 4B step and diluted 800-fold with assay buffer was added (on ice) to 290 µl of assay mix containing precursor tRNA$^{\text{Tyr}}_{\text{su}}$ from either Ø80psu$_3$ or Ø8ODHA61.3. 30 µl were withdrawn immediately and this was considered as the t=0 sample. The mixture was incubated at 37°C and further 30 µl aliquots, taken at 0.5, 1, 2, 4, 6, 8, 10, 15, and 20 minutes were added to 10 µl of a stop solution. The samples were run on a 10% polyacrylamide gel in 89 mM Tris-Borate, 2 mM EDTA and exposed to X-ray film. The bands corresponding to intact precursor, processed tRNA, and 5' fragment were excised and the radioactivity in each determined to facilitate calculation of the percentage of cleavage by RNase P. —o—o- RNase P cleavage of precursor tRNA from Ø80psu$_3$. —x—x— RNase P cleavage of precursor tRNA from Ø8ODHA61.3.

ACUCUAADACUUCG. This alteration is a result of a G to U change in the RNA at position twelve of this oligonucleotide (or position 43 of the mature tRNA sequence). Further analysis of this oligonucleotide by two dimensional thin layer chromatography showed that the nucleotide at position nine is an unmodified C instead of the $\Psi$ found in the corresponding position in the Ø80psu$_3$ transcript. Similar effects of point mutations on nucleotide modification have been described previously.

**Kinetics of processing of precursor tRNAs**

Since the low amount of mature tRNA produced by Ø80ODHA61.3 could be due to a defect in the processing of the precursor tRNA, a comparison of the in vitro processing of the parent and mutant precursors was performed. A very low enzyme to substrate ratio was used in the standard RNase P assay to insure that measurements took place on the linear part of the kinetic curve. The results show that the mutant precursor tRNA was processed much less efficiently than the parent precursor tRNA (Fig. 5). This difference in the
Figure 6 Isolation of RNase P enzyme-substrate complex using Cs$_2$SO$_4$ buoyant density gradients. 5 µl of E. coli RNase P purified through the Sepharose $+4B$ step$^*$ were mixed (on ice) with $^3$P labeled precursor tRNA$_{\text{Tyr}}$ from $\theta$80psi$^+$ (Panel A) or from $\theta$8ODHA61.3 (Panel B) in 10 µl of standard RNase P assay buffer. The mixture was immediately diluted with 40 µl of 50 mM Tris-Cl pH 7.5, 500 mM NH$_4$Cl, 10 mM EDTA, 60 mM β-mercaptoethanol and 50 µl were then loaded onto a preformed Cs$_2$SO$_4$ step gradient. The gradient was made in cellulose nitrate tubes of 0.65 ml capacity by layering 190 µl each of 45%, 37% and 31% (w/w) Cs$_2$SO$_4$ solutions made in the dilution buffer noted above. Centrifugation was carried out in the Beckman SW65 rotor equipped with tube adaptors for 6 hours at 3°C and 59000 rpm. One drop fractions (35-40 µl) were collected after puncturing the bottom of the tube with a 27 gauge hypodermic needle. 2 µl of selected fractions were assayed for RNase P activity in standard fashion (Panel C). Selected fractions were also phenol extracted, ethanol precipitated, the pellet resuspended and loaded on a standard 10% polyacrylamide gel used for RNase P assays (Panel C2). Position of intact precursor tRNA, the RNase P cleavage fragment containing the mature tRNA sequence (tRNA) and the 5' fragment are identified. BPB indicates the position of the bromophenol dye marker. (*) = distribution of radioactivity in the gradient. E-S = Enzyme-Substrate complex. P = Precursor tRNA. The numbered lanes in Cl and C2 correspond to the analysis of the same numbered fractions shown in A and B respectively.

Efficiency of processing may be due to the formation of an unstable enzyme-substrate complex between RNase P and the mutant tRNA$_{\text{Tyr}}$ precursor or to the inability of RNase P to cleave the substrate once a complex has been formed. We have succeeded in detecting enzyme-substrate complexes of RNase P with precursor tRNA by analysis of suitable mixtures in Cs$_2$SO$_4$ gradients (Fig. 6). Precursor tRNA alone has a relatively low buoyant density reflecting tertiary structure and nucleotide modification in the molecule. This material shifts to a higher buoyant density when complexed with enzyme presumably due to a significant loss of tertiary and possibly some secondary structure of the precursor tRNA (Fig. 6A). Material found in the peak labeled E-S (Fig. 6A) consists of intact precursor tRNA if analyzed directly after phenol extraction.
Figure 1 Autoradiograph of a DNA sequencing gel comparing the sequence at position 43 of the tRNA\textsubscript{tyr}~\textsubscript{suc} gene from \textsc{08opsu}~\textsuperscript{+} and from \textsc{08odha61.3} (The T43 mutation is shown by an arrow.) An Ava I/Msp I restriction fragment was labeled using P-deoxy-CTP followed by restriction digestion with Dde I to generate an Ava I/Dde I fragment labeled at one end. Sequencing reactions were performed according to the procedure of Maxam and Gilbert \cite{Maxam} and electrophoresis on an 8% polyacrylamide-7 M urea, 20 cm x 80 cm sequencing gel according to Smith and Calvo \cite{Smith}. The portion of the tRNA sequence shown contained the only difference between the mutant and its parent.

(Fig. 6C2), but is cleaved by RNase P activity in the peak if it is merely diluted into standard assay buffer (Fig. 6C1). Under conditions which allow about 50% of precursor tRNA\textsubscript{tyr}~\textsubscript{suc} to form an enzyme-substrate complex, only trace amounts of precursor tRNA\textsubscript{tyr} from \textsc{08odha61.3} are found in such a complex (Fig. 6B). The conditions we used for complex formation correspond to the earliest time (about 10 seconds) on the kinetic curve shown in Fig. 5 (see Materials and Methods). These results suggest that the conformation of the tRNA precursor, which is altered in a significant way by the G43 to U43 change, plays an important role in the initial interaction between RNase P and its substrate.

**DNA sequencing**

To confirm the results of the RNA analysis, portions of the mutant and parent tRNA genes were obtained on DNA restriction fragments and sequenced. An 802 bp Msp I fragment (see Materials and Methods) was digested with Ava I and isolated as a singly labeled Ava I/Dde I fragment as described in the legend of Fig. 7. The DNA sequence analysis of this Ava I/Dde I fragment
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**Figure 8** Structural organization of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \), \( \theta \theta \theta \text{psu}^+ \), \( \theta \theta \theta \text{DHA61.3} \). The \( \text{tvr}^+ \) locus contained in three \( \theta \theta \theta \) derivatives is shown. This includes the \( \text{su}^+ \) and \( \text{su}_0^- \) genes, the spacer between the two genes, and the three terminal 178 bp repeats. In \( \theta \theta \theta \text{psu}^+ \), a derivative of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \), the spacer and the \( \text{su}_0^- \) gene are missing as a result of an unequal crossover event. In \( \theta \theta \theta \text{DHA61.3} \), a derivative of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \), the amount of DNA deleted is shown i.e. 652 bp. \( \text{T} = \rho \)-dependent terminator.

showed that a transversion-transition from \( \text{G} \) to \( \text{T} \) had occurred at position 43 in the \( \text{su}_3^+ \) gene of \( \theta \theta \theta \text{DHA61.3} \) (Fig. 7).

**Comparison of phage restriction patterns**

Since we have found mutants with large deletions downstream from the \( \text{tRNA}^{\text{Ty}} \) genes in other derivatives of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \), we examined \( \theta \theta \theta \text{DHA61.3} \) for changes which might reflect substantial rearrangements of DNA. Restriction patterns of purified DNA from \( \theta \theta \theta \text{DHA61.3} \) were compared to digests of \( \theta \theta \theta \text{psu}^+ \) DNA. This comparison was aided by a detailed restriction map previously constructed from the DNA sequence of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \). A Sau 3A/Pst I double digest of \( \theta \theta \theta \text{psu}^+ \) DNA produced a 1384 bp Sau 3A fragment. This fragment was replaced in a similar digest of \( \theta \theta \theta \text{DHA61.3} \) DNA by a 1025 bp Sau 3A fragment, indicating the presence of a deletion of 359 bp in \( \theta \theta \theta \text{DHA61.3} \) (data not shown). This result was confirmed by a Sau 3A/Pvu II double digest which produced a 1098 bp fragment from \( \theta \theta \theta \text{psu}^+ \) and a corresponding 739 bp fragment from \( \theta \theta \theta \text{DHA61.3} \). In addition to this deletion, \( \theta \theta \theta \text{DHA61.3} \), which was originally a derivative of \( \theta \theta \theta \text{psu}^+ \text{su}_0^- \), has lost a portion of the spacer and the \( \text{su}_0^- \) gene as indicated by DNA sequencing (data not shown) resulting in the sum total of 652 bp of deleted DNA. These results and the structure of the region downstream from the \( \text{tRNA}^{\text{Ty}} \) gene in \( \theta \theta \theta \text{DHA61.3} \) are illustrated schematically in Fig. 8.

**DISCUSSION**

We have shown that \( \theta \theta \theta \text{DHA61.3} \), in comparison to \( \theta \theta \theta \text{psu}^+ \), is a weak suppressor as judged by its ability to suppress an amber mutation in the
β-galactosidase gene. We propose that the phenotype of this phage results from a G to T base substitution at position 43 in the mutant tRNA\textsuperscript{Tyr} su\textsubscript{3} gene which it carries. This mutation, as determined by electrophoretic mobility differences, alters the conformation of both the precursor and the mature tRNA. (Since the only difference in nucleotide modification between mutant and parent tRNA is in a \psi adjacent to the anticodon, it is unlikely that modification differences solely account for the change in electrophoretic mobility of the mutant RNA.) As a consequence of this proposed change in conformation, the mutant precursor forms a less stable enzyme-substrate complex compared to the precursor from tRNA\textsuperscript{Tyr} su\textsubscript{3} and is not efficiently processed. The reduction in the amount of precursor and mature tRNA seen in the \textit{in vivo} labeling experiments, along with the absence of any changes in the tRNA\textsuperscript{Tyr} gene promoter from O8ODHA61.3 (sequencing data not shown), suggest that the slow maturation of the tRNA precursor and/or the differences seen in secondary structure may render this RNA transcript more susceptible to degradation than the transcript from the su\textsubscript{3} gene. A similar explanation has been proposed for the phenotypes of other mutant derivatives of su\textsubscript{3}.

We are aware that we cannot definitively extrapolate \textit{in vitro} results regarding rates of processing and structures in solution of precursor tRNA to events \textit{in vivo}. However, the phenotype of O8ODHA61.3 and other similar mutants seems consistent with our interpretation.

A change of G to U at position 43 in the tRNA\textsuperscript{Tyr} would disrupt the second G–C base pair in the anticodon stem (Fig. 1) of this molecule. This alteration appears to destabilize the tRNA structure or to promote the stabilization of different conformation\textsuperscript{13}. An analogous mutant is A31 in which a G to A transition at position 31 of the mature tRNA\textsuperscript{Tyr} sequence disrupts the fourth G–C base pair in the anticodon stem\textsuperscript{31}. Mutant A31 produces only 15% of the amount of mature tRNA\textsuperscript{Tyr} compared to O8Opsi\textsubscript{3} \textit{in vivo} and accumulates precursor tRNA\textsuperscript{Tyr}. Revertants have been isolated containing a C to U transition at position 41 which restores the base pairing of the A31 mutation and increases the production of mature tRNA to 67% of wild type. Second site mutations in other regions of the tRNA\textsuperscript{Tyr} sequence also affect the production of mature tRNA\textsuperscript{Tyr} in this mutant. In all cases, the destabilization of the cloverleaf structure or the stabilization of alternate secondary structures seems to be a likely explanation for the phenotypes of these mutants.

It has been proposed\textsuperscript{32} recently that RNase P recognizes its cleavage sites in precursor tRNA molecules by hydrogen bonding, via its own RNA moiety, with
the T>C loop of the precursor molecule. Our results are compatible with the possibility that the first step in the formation of the enzyme-substrate complex is likely to be the recognition by RNase P of the 'correct' conformation of the tRNA moiety in solution. The second step, specific positioning of the enzyme on the substrate, may involve the hypothetical hydrogen bonding between the RNA of the enzyme and the substrate.

The mutational event which caused the G to T transversion-transition in Ø80DHA61.3 DNA presumably resulted from treatment of the parent phage with hydroxylamine. Hydroxylamine has been reported to mediate C to T (G to A) transitions and not transversions\(^{33-34}\). Thus, the mechanism responsible for this mutation we have described is as yet unknown. One possibility is that hydroxylamine, or one of its free radicals, was able to stabilize the hydrogen bonding of a G\(_{\text{enol}}\), imino tautomer to an A\(_{\text{syn}}\) isomer and thus facilitate a purine-purine base pair\(^{35}\). We cannot exclude the possibility that the Ø80DHA61.3 was a spontaneous mutant fortuitously present in our phage stock prior to hydroxylamine mutagenesis.

In addition to the point mutation found in the su\(_3^+\) gene of Ø80DHA61.3, restriction mapping and nucleotide sequence analysis (manuscript in preparation) have shown that a large deletion exists downstream from the tRNA sequence. This deletion, which we surmise arose as a secondary effect of the original point mutation, has removed part of the spacer between the su\(_3^+\) and the su\(_0^-\) genes in the parent phage, the su\(_0^-\) gene, and most of the repeated region following this gene (Fig. 8). The initial transcript made from Ø80DHA61.3, although it spans the region of DNA deleted from Ø80psu\(_3^+\)su\(_0^-\) (including the normal site of transcription termination) seems to be efficiently processed to a precursor RNA recognized by RNase P. Presumably, transcription of the tyr T region in Ø80DHA61.3 is terminated efficiently at the \(\sigma\)-dependant termination sequence\(^{36}\) in the third repeat following the tRNA sequence in the DNA. The third repeat region is preserved in Ø80DHA61.3.

One anomaly in processing of precursor tRNA\(_{\text{TYR}}\) from Ø80DHA61.3 is the heterogeneity at the 3' terminus of the precursor resulting in a small portion of species with one more or one less nucleotide as compared to the su\(_3^+\) precursor. This may be a consequence of the downstream deletion and the fact that the sequence following the mutated su\(_3^+\) gene in Ø80DHA61.3 is now different from that following the su\(_3^+\) gene in Ø80psu\(_3^+\). Ø80DHA61.3, which is derived from Ø80psu\(_3^+\)su\(_0^-\), has a portion of the spacer sequence (normally found between the two tRNA genes) following the su\(_3^+\) sequence instead of the repeated region as found in Ø80psu\(_3^+\). This difference in the downstream
sequence may affect both exo- and endoribonuclease lytic processing reactions. The fraction of precursor lacking the correct 3' terminus is not sufficient to account for the weak suppressor properties of 80ODHA61.3. The genetic properties of the downstream deletion and its possible relationship to the U43 mutation will be discussed elsewhere.

The tRNA mutant we have described again illustrates the importance of substrate conformation in the processing of tRNA precursors. The study of such mutants will lead to a clearer understanding of the features important in the formation of RNase P-substrate complexes, one of the key steps in the biosynthesis of E. coli tRNA.

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