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A G43 to U43 mutation in *E. coli* tRNA$^{Tyr}su_3^+$ which affects processing by RNase P

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ABSTRACT

A novel mutation in the anticodon stem of E. coli tRNA$_1^{Tyr}su_3^+$ (G43 to U43) has been characterized. The gene coding for the mutant tRNA, carried by phage ø80DHA61.3 a derivative of ø80psu$_{3+}$su$_0^-$, produces only 20% of mature suppressor tRNA as compared with ø80psu$_3^+$. Both the mutant tRNA precursor and mature tRNA have an altered conformation. The precursor tRNA coded for by ø80DHA61.3 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. ø80DHA61.3 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[4-9]. 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.

ø80psu$_3^+$su$_0^-$ carries the entire tyr T locus and ø80psu$_3^+$ 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 ø80psu$_3^+$su$_0^-$ which affect its ability to produce a functional tRNA$^{Tyr}su_3^+$. In this report we describe a

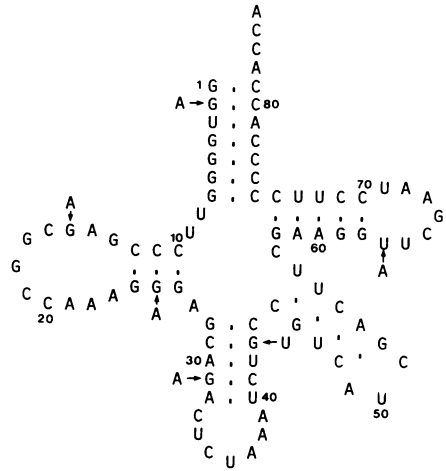

Figure 1 $tRNA_1^{Tyr}su_3^+$ in the standard cloverleaf secondary structure. Some nucleotide substitutions found in mutants which are not efficiently processed by RNase P are shown. The mutation described in this report (G43 to U43) is also indicated in the figure.

$tRNA^{Tyr}$ weak suppressor mutant isolated following hydroxylamine mutagenesis. This mutant codes for a $tRNA^{Tyr}$ 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_3^+ precursor.

Materials and Methods

Isolation of ø80DHA61.3

Hydroxylamine mutagenesis of $ø80psu_3^+su_0^-$ (doublet) was performed as described by Tessman[16].

Suppressor activity of phage derivatives was assayed by plating phage on the E. coli strain CA274 (HfrC $lac^-_{125\ amber}$ trp^-_{amber} su^-) in LB top agar containing 0.4% 5-bromo 4-chloro 3-indole β-galactopyranoside (XG) and 0.4 mM isopropyl-thio β-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 $ø80psu_3^+su_0^-$, plaques with less intense blue color than the parent phage appeared with a frequency of about 10^{-4}. One of these was designated ø80DHA61.3 and is the

mutant we describe here. In the terminology used in ref. 11, this phage could also be called $\phi 80psu_3U43$.

Properties of phage DNA

Phage were prepared by obtaining high titer stocks according to the procedure of Miller[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, 100 µg/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 $tRNA^{Tyr}$ Gene Carried by $\phi 80DHA61.3$

Phage DNA from $\phi 80DHA61.3$ 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[18]. The ligation mix was used to transform E. coli HB101 cells[19]. Single colonies were isolated on LB plates containing ampicillin (100 µg/ml) and those colonies containing recombinants were screened for sensitivity to tetracycline on LB plates containing tetracycline (25 µg/ml). One candidate from 58 colonies was rescreened and found to contain the appropriate $\phi 80$-derived DNA fragment. This clone was called p61.3 and DNA was purified according to the procedure of Meagher, et al[20]. An Msp I digestion of p61.3 and $\phi 80DHA61.3$ produced an 802 bp fragment which has been shown by Southern blotting and hybridization to in vitro labeled $tRNA^{Tyr}$ to contain the $tRNA^{Tyr}$ 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_3^+ gene was extensively mapped using the procedure of Smith and Birnstiel[21]. Ava I, Dde I, Hae II, Hae III, Xho I, and Hind III were used to produce a restriction map for comparison with $\phi 80psu_3^+su_0^-$ 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[22].

Isolation and analysis of precursor and mature $tRNA^{Tyr}$

^{32}P labeled $tRNA^{Tyr}$ coded for by $\phi 80psu_3^+$ and by $\phi 80DHA61.3$ were prepared by in vivo labeling during infection[12] of the strain BF266[6].

^{32}P labeled $tRNA^{Tyr}$ precursor RNA was prepared by *in vivo* labeling during infection of the strain A49[23] by either ø80DHA61.3 or ø80psu_3^+. 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 ø80 derivatives

ø80DHA61.3 was isolated after hydroxylamine mutagenesis of ø80$psu_3^+su_0^-$ as a plaque showing less intense blue color than the parent phage in the blue plaque assay (see Materials and Methods). ø80DHA61.3 was further tested for suppression by infecting CA274 in the presence of IPTG and assaying for β-galactosidase activity using o-nitro-phenyl β-galactopyranoside (ONPG) in a standard assay[35] (data not shown). Under these conditions, ø80DHA61.3 infected cells were found to produce about 20% the level of β-galactosidase found in ø80$psu_3^+su_0^-$ or ø80psu_3^+ infected cells.

Analysis of the transcript from ø80DHA61.3

The tRNA containing transcript from ø80DHA61.3 was compared with the corresponding transcript from ø80psu_3^+ in an attempt to explain the difference in the level of suppression manifested by these two phages. ø80DHA61.3 produces about 20% of the amount of both precursor and mature tRNA compared to the amount made by ø80psu_3^+. Both precursor and mature tRNA produced by ø80DHA61.3 migrated slightly slower in non-denaturing gels than the corresponding species from ø80psu_3^+ (Fig. 2).

Isolated precursor tRNAs from ø80DHA61.3 and from ø80psu_3^+ 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 ø80DHA61.3 again showed differences in electrophoretic mobilities compared to their ø80psu_3^+ counterparts. The oligonucleotides 43 bases long, processed from the 5' end of the precursor from either ø80DHA61.3 or ø80psu_3^+, had more similar mobilities. (The 5' leader sequence of the precursor $tRNA^{Tyr}$ 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

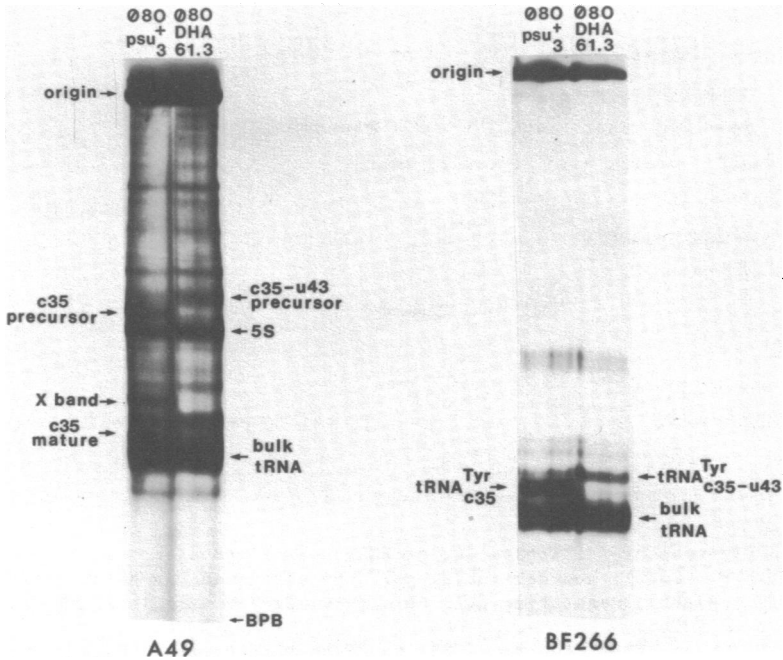

Figure 2 Properties of transcripts from $\phi 80psu_3^+$ and ϕ80DHA61.3. Left). Autoradiograph of 10% polyacrylamide gel separation of ^{23}RNA extracted from the strain A49 (temperature sensitive for RNase P function) infected at the restrictive temperature with $\phi 80psu_3^+$ (C35) (left lane) or ϕ80DHA61.3 (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 $\phi 80psu_3^+$ (left lane) or ϕ80DHA61.3 (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 ϕ80DHA61.3 was compared to that produced by $\phi 80psu_3^+$ as follows: gel slices corresponding to the mobilities of 5S RNA, M3 RNA (a ϕ80 encoded transcript), and precursor tRNA produced by both ϕ80DHA61.3 and $\phi 80psu_3^+$ 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 ϕ80DHA61.3 and $\phi 80psu_3^+$ 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 ϕ80DHA61.3 precursor tRNA to $\phi 80psu_3^+$ precursor tRNA was calculated after subtracting the background from ϕ80-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

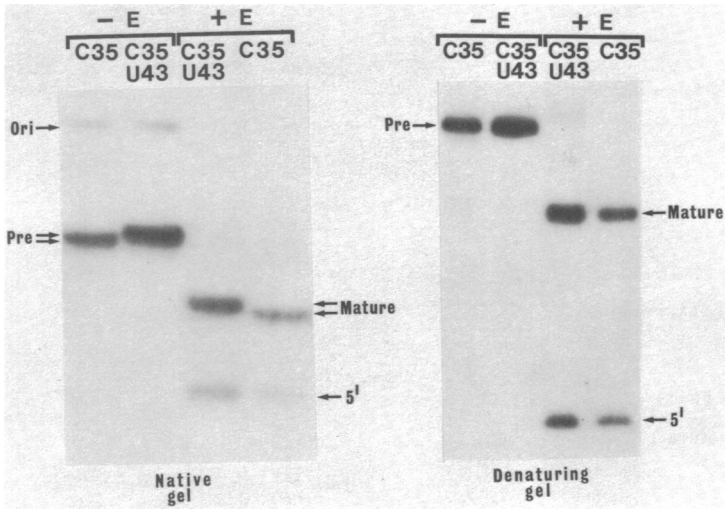

Figure 3 Electrophoretic separation of RNAs before and after cleavage by E. coli RNase P. Left). non-denaturing gel: 10% acrylamide in 89 mM Tris-Borate, 2 mM EDTA. Right). denaturing gel: 5% acrylamide-7 M urea in 50 mM Tris-Borate, 1 mM EDTA
Lane 1: Precursor from ϕ80psu$_3^+$ (C35) no RNase P treatment. Lane 2: Precursor from ϕ80DHA61.3 (C35-U43) no RNase P treatment. Lane 3: Precursor from ϕ80DHA61.3 (C35-U43) after treatment with excess RNase P. Lane 4: Precursor from ϕ80psu$_3^+$ (C35) after treatment with excess RNase P.

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 ϕ80DHA61.3 and from ϕ80psu$_3^+$ 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 su$_3^+$ transcript, was missing from the ϕ80DHA61.3 precursor. In addition, spot no. 15' in the U43 fingerprint, which contains an oligonucleotide including the anticodon and which immediately precedes CCG in the su$_3^+$ gene sequence, appeared to migrate more slowly in the second dimension than the corresponding oligonucleotide from su$_3^+$. 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 ϕ80DHA61.3 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 su$_3^+$ transcript, migrated to the position of an oligonucleotide of approximately 15 bases. In addition, about

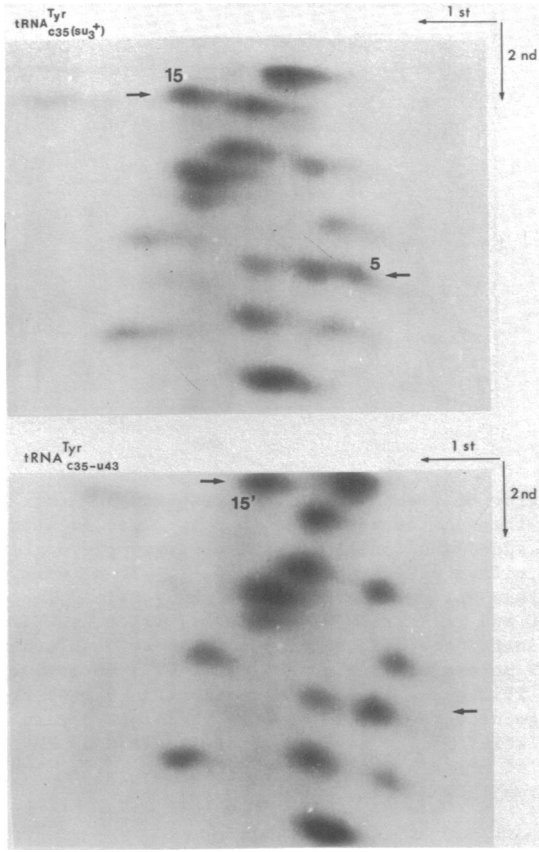

Figure 4 RNase T1 generated fingerprints of precursor tRNA^{Tyr}
Top). intact pre-tRNA^{Tyr} from ø80psu$_3^+$ (C35).
Bottom). intact pre-tRNA^{Tyr} from ø80DHA61.3 (C35-U43).
First dimension: electrophoresis on cellogel, pyridine acetate buffer, pH 3.5. Second dimension: homochromatography on PEI plates. 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 ø80DHA61.3 transcript showed that none other than no. 15 had an altered sequence and that in this oligonucleotide, the sequence had been changed from ACUCUAAAΨCUG to

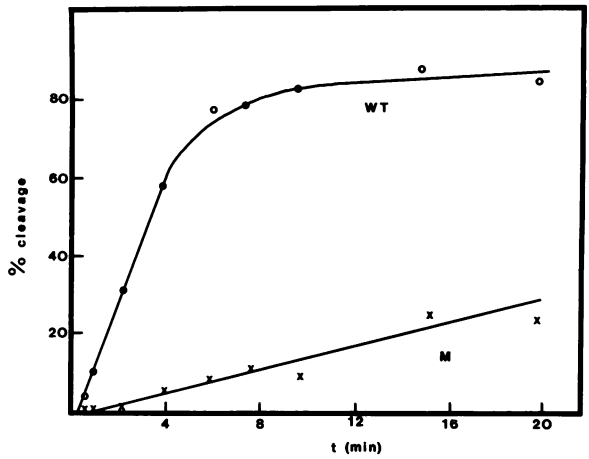

Figure 5 Time course of processing of precursor $tRNA^{Tyr}_{37}$ by RNase P. 10 µl of RNase P purified through the Sepharose 4B step and diluted 800-fold with assay buffer was added (on ice) to 290 µl of assay mix containing precursor $tRNA^{Tyr}su_3^+$ from either ø80psu$_3^+$ or ø80DHA61.3. 30 µl were withdrawn immediately and this was considered as the t=0 sample. The mixture was incubated at 37°C and further 30 µl aliquotes, 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 ø80DHA61.3.

ACUCUAAAUCUUCCG. 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[28] showed that the nucleotide at position nine is an unmodified U instead of the ψ found in the corresponding position in the ø80psu$_3^+$ transcript. Similar effects of point mutations on nucleotide modification have been described previously[29].

Kinetics of processing of precursor tRNAs

Since the low amount of mature tRNA produced by ø80DHA61.3 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

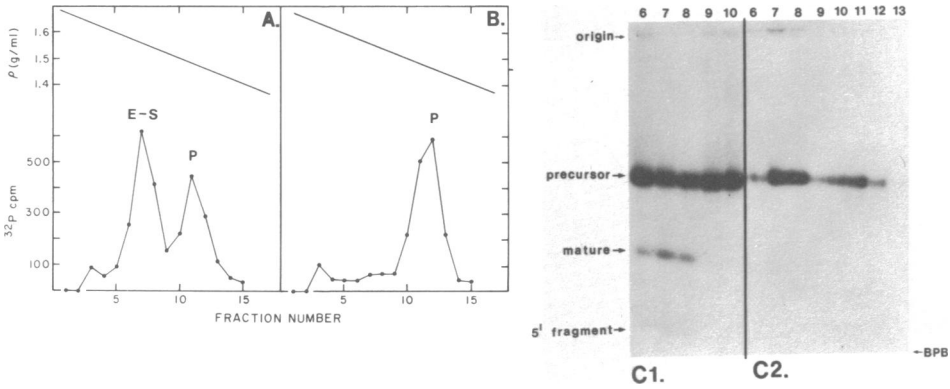

Figure 6 Isolation of RNase P enzyme-substrate complex using Cs_2SO_4 buoyant density gradients. 5 µl of E. coli RNase P purified through the Sepharose 4B step[37] were mixed (on ice) with ^{32}P labeled precursor $tRNA^{Tyr}$ from ϕ80psu$_3$ (Panel A) or from ϕ80DHA61.3 (Panel B) in 10 µl of standard RNase P assay buffer[37]. The mixture was immediately diluted with 40 µl of 50 mM Tris-HCl pH 7.5, 500 mM NH_4Cl, 10 mM EDTA, 60 mM β-mercaptoethanol and 50 µl were then loaded onto a preformed Cs_2SO_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_2SO_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[37] (Panel C1). 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 bromphenol dye marker. (•) = distribution of radioactivity in the gradient. E-S = Enzyme-Substrate complex. P = Precursor tRNA. The numbered lanes in C1 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 $tRNA^{Tyr}$ 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_2SO_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

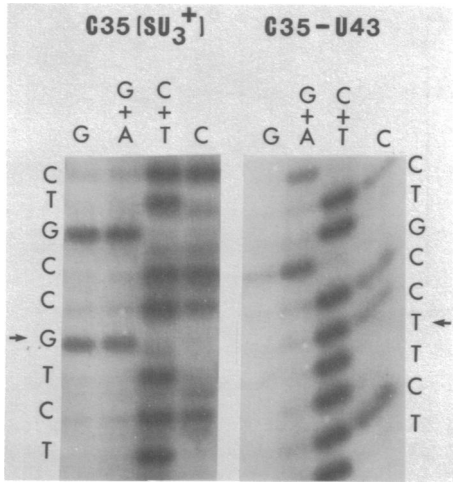

Figure 7 Autoradiograph of a DNA sequencing gel comparing the sequence at position 43 of the $tRNA^{Tyr}$ su_3^+ gene from ϕ80psu$_3^+$ and from ϕ80DHA61.3 (The T43 mutation is shown by an arrow.) An Ava I/Msp I restriction fragment was labeled using α-^{32}P-deoxy-CTP followed by restriction digestion with Dde I to generate an Ave I/Dde I fragment labeled at one end. Sequencing reactions were performed according to the procedure of Maxam and Gilbert[22] and electrophoresis on an 8% polyacrylamide-7 M urea, 20 cm x 80 cm sequencing gel according to Smith and Calvo[36]. 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 $tRNA^{Tyr}su_3^+$ to form an enzyme-substrate complex, only trace amounts of precursor $tRNA^{Tyr}$ from ϕ80DHA61.3 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

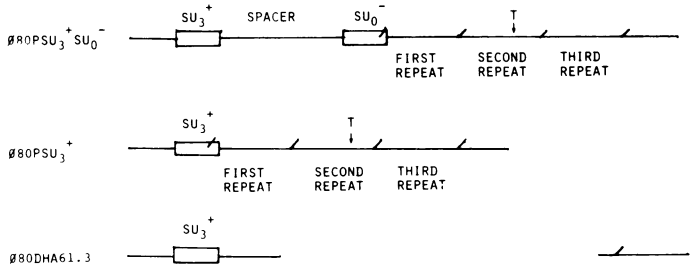

Figure 8 Structural organization of ø80psu$_3^+$su$_0^-$, ø80psu$_3^+$, ø80DHA61.3. The tyr$_+$T locus contained in three ø80 derivatives is shown. This includes the su$_3^+$ and su$_0^-$ genes, the spacer between the two genes, and the three terminal 178 bp repeats[30]. In ø80psu$_3^+$, a derivative of ø80psu$_3^+$su$_0^-$, the spacer and the su$_0^-$ gene are missing as a result of an unequal crossover event[15]. In ø80DHA61.3, a derivative of ø80psu$_3^+$su$_0^-$[34] the amount of DNA deleted is shown i.e. 652 bp. T = ρ-dependent terminator.

showed that a transversion-transition from G to T had occurred at position 43 in the su$_3^+$ gene of ø80DHA61.3 (Fig. 7).

Comparison of phage restriction patterns

Since we have found mutants with large deletions downstream from the tRNA^{Tyr} genes in other derivatives of ø80psu$_3^+$[14], we examined ø80DHA61.3 for changes which might reflect substantial rearrangements of DNA. Restriction patterns of purified DNA from ø80DHA61.3 were compared to digests of ø80psu$_3^+$ DNA. This comparison was aided by a detailed restriction map previously constructed from the DNA sequence of ø80psu$_3^+$[14,30]. A Sau 3A/Pst I double digest of ø80psu$_3^+$ DNA produced a 1384 bp Sau 3A fragment. This fragment was replaced in a similar digest of ø80DHA61.3 DNA by a 1025 bp Sau 3A fragment, indicating the presence of a deletion of 359 bp in ø80DHA61.3 (data not shown). This result was confirmed by a Sau 3A/Pvu II double digest which produced a 1098 bp fragment from ø80psu$_3^+$ and a corresponding 739 bp fragment from ø80DHA61.3. In addition to this deletion, ø80DHA61.3, which was originally a derivative of ø80psu$_3^+$su$_0^-$, has lost a portion of the spacer and the 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 tRNA^{Tyr} gene in ø80DHA61.3 are illustrated schematically in Fig. 8.

DISCUSSION

We have shown that ø80DHA61.3, in comparison to ø80psu$_3^+$, 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 $tRNA^{Tyr}su_3^+$ 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 ψ 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 $tRNA^{Tyr}$ su_3^+ and is not efficiently processed. The reduction in the amount of precursor and mature tRNA seen in the *in vivo* labeling experiments, along with the absence of any changes in the $tRNA^{Tyr}$ gene promoter from ϕ80DHA61.3 (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_3^+ gene. A similar explanation has been proposed for the phenotypes of other mutant derivatives of su_3^+ [11-12]. We are aware that we cannot definitively extrapolate *in vitro* results regarding rates of processing and structures in solution of precursor tRNA to events *in vivo*. However, the phenotype of ϕ80DHA61.3 and other similar mutants seems consistent with our interpretation.

A change of G to U at position 43 in the $tRNA^{Tyr}$ 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 conformations[13]. An analogous mutant is A31 in which a G to A transition at position 31 of the mature $tRNA^{Tyr}$ sequence disrupts the fourth G-C base pair in the anticodon stem[31]. Mutant A31 produces only 15% of the amount of mature $tRNA^{Tyr}$ compared to ϕ80psu_3^+ *in vivo* and accumulates precursor $tRNA^{Tyr}$. 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 $tRNA^{Tyr}$ sequence also affect the production of mature $tRNA^{Tyr}$ 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[32] recently that RNase P recognizes its cleavage sites in precursor tRNA molecules by hydrogen bonding, via its own RNA moiety, with

the TψC 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 ϕ80DHA61.3 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 $G_{enol,\ imino}$ tautomer to an A_{syn} isomer and thus facilitate a purine-purine base pair[35]. We cannot exclude the possibility that the ϕ80DHA61.3 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 ϕ80DHA61.3, 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 ϕ80DHA61.3, although it spans the region of DNA deleted from ϕ80$psu_3^+su_0^-$ (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 ϕ80DHA61.3 is terminated efficiently at the ρ-dependant termination sequence[36] in the third repeat following the tRNA sequence in the DNA. The third repeat region is preserved in ϕ80DHA61.3.

One anomaly in processing of precursor $tRNA^{Tyr}$ from ϕ80DHA61.3 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 ϕ80DHA61.3 is now different from that following the su_3^+ gene in ϕ80psu_3^+. ϕ80DHA61.3, which is derived from ϕ80$psu_3^+su_0^-$, 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 ϕ80psu_3^+. This difference in the downstream

sequence may affect both exo- and endoribonuclease lytic processing reactions[10]. The fraction of precursor lacking the correct 3' terminus is not sufficient to account for the weak suppressor properties of ØBODHA61.3. 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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