MUTANT TYROSINE tRNA OF ALTERED AMINO ACID SPECIFICITY

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1. Introduction

Each set of tRNA molecules accepting a particular amino acid are recognized and acylated by an amino acyl tRNA synthetase. This enzyme must recognize some feature in this set of tRNA molecules which distinguishes them from all other tRNAs. In this communication we describe the isolation and initial characterization of a set of *E. coli* tyr tRNA mutants that have altered amino acid specificity. These mutants should eventually lead to an understanding of which features of the tRNA are recognized by the tyrosine tRNA synthetase.

Sequence analysis of one of the mutants ($Su^+III^{a\text{-}23}$) has revealed that a single base change (A82 → G) near the amino acid acceptor end of the molecule is sufficient to alter its specificity.

2. Results

2.1. *Isolation of the mutants*

The details of the isolation procedure of the mutant Su^+III tRNA having altered amino acid specificity will be described elsewhere [1]. In short, we prepared a hybrid phage $\phi 80pSu^+III\ h^{\phi 80}i^{\lambda}$ *susP3susR216* by successive crosses, replacing the right hand arm of $\phi 80pSu^+III$ with that of λ carrying *susP3* and *susR216*. This phage could not form plaques on non-permissive (Su^-) hosts, because the two amber mutations of λ are not suppressed by Su^+III. After treating with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine, the lysate was plated on *E. coli* 594 (Su^-) and plaques were found at a frequency of $10^{-9}-10^{-10}$ per phage particle. In this way we have isolated some 30 mutants, which were designated as $\phi 80pSu^+III^{a\text{-}1}$, $\phi 80pSu^+III^{a\text{-}2}$, etc. These mutants still maintained the characteristic feature of our $\phi 80pSu^+III$ in spontaneously losing the entire transducing fragment [2] including the suppressor gene. Thus the mutation must be located on the transducing fragment, presumably in the Su^+III gene itself. The suppressor must possess a new ability to suppress *susP3* and *susR216* not evident on the original Su^+III gene. As shown in the next section, these mutants also show altered suppression patterns for various amber mutants of bacteriophage T4.

2.2. *In vivo suppression patterns of the mutant suppressors*

Lysogens of the mutant $\phi 80pSu^+III$ were formed in an Su^- host. The various suppressors were tested for their ability to suppress a set of T4 amber mutants (table 1). We note that there are 2 sets of altered suppressor mutants: one set which can suppress *am* eLA31 and one set which cannot. *am* eLA31 is a mutant of T4 lysozyme in residue 105 (glutamine) [3, 4]. About half of our mutants gained the ability to suppress *am* eLA31. This set of mutants must be inserting some amino acid(s) other than tyrosine in response to the UAG codon. Since the original λ sus mutants used for selection do not grow on Su^+III, it seems likely that both sets of suppressors are inserting amino acid(s) other than tyrosine.

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Table 1
Suppression of T4 amber mutants by different alleles of the SuIII gene*.

	T4 amber mutants						
	lysozyme [3, 4]				rIIA [15, 16]		54
	eM91	eM92	eLA31	eLA41	S116	S24	*am*H21
Amino acid in wild type	trp(138)	trp(158)	glu(105)	glu(141)	trp	trp	?
Suppressor							
Su^-	–	–	–	–	–	–	–
Su^+I (ser)	–	+	+	+	–	+	+
Su^+II (glu)	–	+	+	+	–	+, –42°	+
Su^+III (tyr)	+	+	–	+	+	+	+, –42°
Su^+III^{a-1}	+	+	–	+	+	+	+, –42°
Su^+III^{a-5}	+	+	–	+			
Su^+III^{a-9}	+	+	–	+			
Su^+III^{a-23}	+	+	+	+	+ poor	+, –42°	+ +
Su^+III^{a-26}	+	+	+	+			
Su^+III^{a-30}	+	+	+	+			

* Unless otherwise noted the suppression of T4 amber mutants was done by spot tests at 37°.

Our suppression data with $r_{II}A$ amber mutants and *am* H21 suggest the following conclusions. *am* S116 can only be suppressed by the tyrosine suppressor. Su^+III^{a-1} suppresses *am* S116 equally well indicating that this mutant suppressor inserts an aromatic amino acid. Su^+III^{a-23} suppresses *am* S116 poorly so it must insert an aromatic amino acid but with a much lower efficiency. *am* S24 is temperature sensitive when glutamine is inserted by Su^+II and it is also temperature sensitive on Su^+III^{a-23} but not on Su^+III. *am* H21 of gene 54 is temperature sensitive when plated on Su^+III or Su^+III^{a-1} but not when plated on Su^+III^{a-23}.

These data suggest that Su^+III^{a-23} is capable of inserting an aromatic amino acid at low efficiency and some other amino acid(s) at higher efficiency. None of the T4 *am* mutants allow us to distinguish between Su^+III and Su^+III^{a-1}.

2.3. *Nucleotide sequence change in Su^+III^{a-23} tRNA*

^{32}P tRNA was prepared from cells infected with $\phi 80pSu^+III^{a-23}$. The infection and labeling was performed as described previously [5]. The tyrosine tRNA was purified by benzoylated DEAE-cellulose chromatography, acrylamide gel electrophoresis [6] and DEAE-Sephadex chromatography [7].

The purified ^{32}P tyr tRNA was digested with U1 (equivalent to T1) ribonuclease and the products separated by 2-dimensional electrophoresis [8, 9]. The resulting fingerprint is shown in fig. 1. $\phi 80pSu^+III^{a-23}$ carries 2 tyrosine tRNA genes [10]. One is the mutated SuIII gene and the other is tyr tRNA I. The tyrosine tRNA should therefore be a mixture of altered suppressor tRNA and normal tyrosine tRNA I. This proved to be true. All of the U1 oligonucleotides were eluted and characterized by further digestion with pancreatic ribonuclease or by alkaline digestion. They all proved to have identical composition to those of normal Su^- tyr tRNA I [11] except for spots 3 and 17A and a third spot which is not seen in this fingerprint. Spot 3 contains the anticodon of Su^+III tRNA (CUA) running adjacent to spot 4 which contains the normal Su^- tyr tRNA anticodon G*UA. Spot 17A is similar to 17B, the normal 3′-end of the molecule which has the sequence: $A_2UC_2U_2C_5ACCACCA_{OH}$. 17A is different from 17B in that pancreatic ribonuclease digestions of the oligonucleotide contain a G and 1 mole of AC instead of 2. The third unusual oligonucleotide seen in Su^+III^{a-23} tyr tRNA moves very slow in the first dimension and slightly faster than the blue dye marker in the second dimension. Alkali digestion of this oligonucleotide gives only Cp. Snake venom phospho-

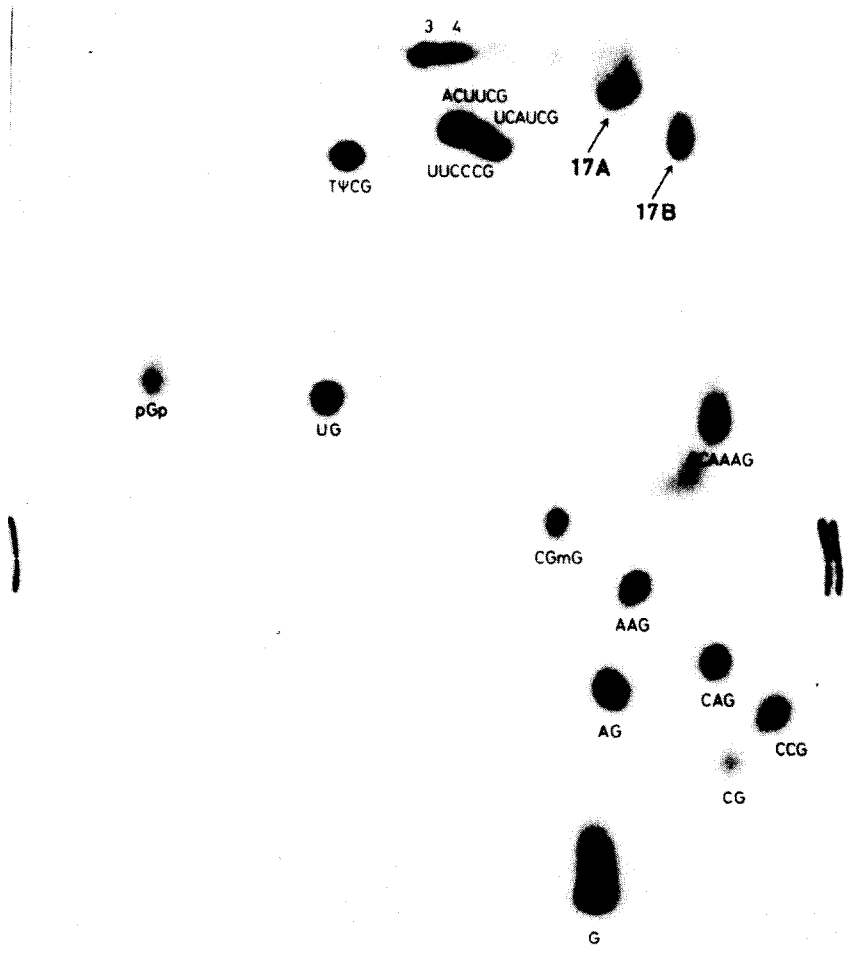

Fig. 1. Two-dimensional oligonucleotide fingerprint of purified, RNase U_1-digested tyrosine tRNA stimulated by ϕ80pSu$^+$III$^{a\text{-}23}$. Separation is by electrophoresis on cellulose acetate at pH 3.5 in 7 M urea (right to left) followed by electrophoresis on DEAE paper in 7% formic acid (v/v).

diesterase digestion of the oligonucleotides gives pC and pA in equal molar yields. These results are consistent with this onligonucleotide having the sequence $CpCpA_{OH}$.

We conclude that the mutation in Su$^+$III$^{a\text{-}23}$ is an A to G transition in position 82 (fig. 2).

Pancreatic ribonuclease digestions of the Su$^+$III$^{a\text{-}23}$ tyr tRNA did not reveal any other changes although we acknowledge that recognition of certain changes could be difficult when one must recognize them against a background of 50% Su$^-$ tyr tRNA. At this point, however, there is no reason to think there is more than one nucleotide difference between the Su$^+$III$^{a\text{-}23}$ tRNA and Su$^+$III tRNA.

2.4. *In vivo suppression assay*

We have done a considerable amount of work to determine the amino acid accepted *in vitro* by Su$^+$III$^{a\text{-}23}$ tRNA. This work is being published elsewhere [1]. Our principal conclusion is that Su$^+$III$^{a\text{-}23}$ tRNA accepts glutamic acid *in vitro*. In this publication we report the results of our initial attempts to deter-

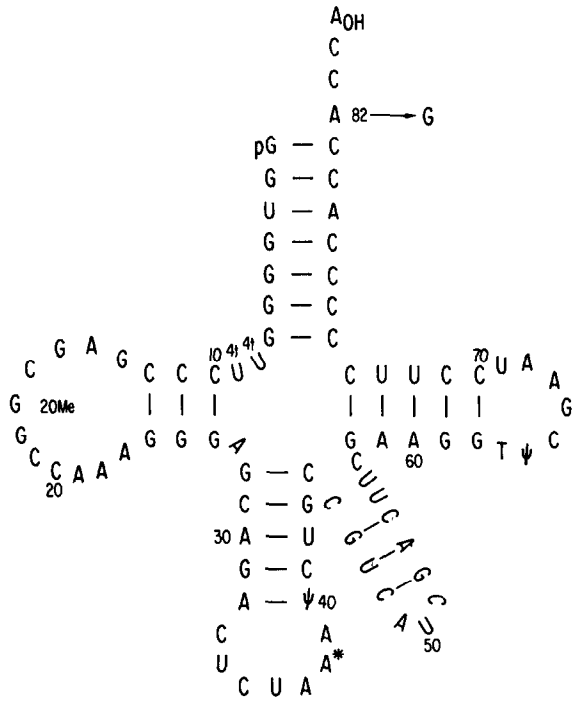

Fig. 2. Nucleotide sequence of Su⁺III tyrosine tRNA [7] showing the base substitution of the mutant Su⁺III$^{a\text{-}23}$ tRNA. A* is 2-methylthio-N^6-(Δ^2-isopentenul)adenosine.

mine which amino acid is inserted by Su⁺III$^{a\text{-}23}$ *in vivo*. For this purpose we have employed the T4 head protein fingerprint assay [12] to determine which amino acid is inserted at the *am* H36 site [13].

Lysogens carrying the wild type and mutant Su⁺III alleles were infected with T4 *am* H36 and the head protein was labelled with ^{14}C-phenylalanine. Tryptic digests of the labelled head proteins produced by *am* H36 were subjected to electrophoresis at pH 6.4 and pH 3.6 and compared with tryptic digests of wild type T4D, and *am* H36R1 (a spontaneous revertant of *am* H36 containing tyrosine at the amber codon site). Wild type T4 contains glutamine in this position [13]. In all cases the mobility of the tryptic peptide PhT12 was monitored for evidence of suppression and of the peptide PhT11 for evidence of fragment production [13]. It was observed that the fragment peptide PhT11 was produced in comparable amounts in cells which contained the Su⁺III, Su⁺III$^{a\text{-}1}$ and Su⁺III$^{a\text{-}23}$ alleles. In addition the mobility and amount of PhT12 was identical when *am* H36 is suppressed by either Su⁺III or Su⁺III$^{a\text{-}1}$.

The suppression pattern in cells containing the Su⁺III$^{a\text{-}23}$ allele is very different. The preliminary results indicate that there are different versions of the suppressed peptide with one class migrating with the tyrosine-containing PhT12 and the other having the same mobility as the glutamine-containing PhT12.

From electrophoretic mobilities alone we cannot decide what amino acid is inserted at the *am* H36 site by the mutant tRNAs. Our results are consistent with the following interpretation: Su⁺III$^{a\text{-}1}$ could be inserting an amino acid that gives PhT12 the same mobility as PhT12 containing tyrosine. Alternatively Su⁺III$^{a\text{-}1}$ could be mainly inserting tyrosine but in addition could insert small amounts of some undetected amino acid. Su⁺III$^{a\text{-}23}$ could be inserting some tyrosine but it also inserts some other amino acid.

Our results preclude the possibility that Su⁺III$^{a\text{-}23}$ could be inserting very much glutamic acid. (The insertion of an acidic amino acid would have a marked effect on the electrophoretic mobility of PhT12.) We cannot exclude the possibility that some glutamic acid is inserted. Clearly more work in characterizing the *in vivo* and *in vitro* specificity of this and other altered tRNAs is needed.

3. Discussion

We have developed a selection procedure which forces the Su⁺III suppressor to insert amino acid(s) different from tyrosine. The *in vivo* suppression patterns of a collection of these mutants suggest that they fall into at least 2 different classes.

The sequence of the tRNA from one of the mutant suppressors, Su⁺III$^{a\text{-}23}$, contained the alteration A82 → G. This is identical to one of the mutants found by Hooper et al. [14].

Apparently this single nucleotide change is sufficient to markedly inhibit the charging of this tRNA by the tyrosine tRNA synthetase and to make the tRNA a much better substrate for some other amino acyl tRNA synthetase. These results taken together suggest that the stem (a) of the clover leaf structure could be playing an important role in the recognition of tRNA by amino acyl tRNA synthetase. This site has also been implicated as the site for UV inactivation of acceptor activity of yeast alanine tRNA by its amino acyl tRNA synthetase [17].

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