MUTANT TYROSINE TRANSFER RIBONUCLEIC ACIDS

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1. Introduction

The su_{III} amber suppressor of *E. coli* is a mutant of a structural gene for tyrosine tRNA in which the anticodon of the tRNA is changed from G*UA to CUA thus enabling the tRNA to translate the amber codon UAG as tyrosine ([1], G* is a modified G residue). We have now isolated mutants of su^{+}_{III} which act as very weak suppressors. Three of these produce mutant tyrosine tRNAs with different single base changes: residue 15, G → A; residue 17, G_m → A; and residue 31, G → A (fig. 1). Some functional properties of two of these defective tRNAs will be described.

2. Materials and methods

Ø80 psu^{+}_{III} is a non-defective transducing phage 80 carrying a single copy of the su^{+}_{III} gene (R.Russell et al., in preparation). It was grown on *E. coli* CA 274 $lac^{-}_{amber\ 125}$ $tryp^{-}_{amber}$ su^{-} [1]. Transfer RNA (either [^{32}P] labelled or non-radioactive) specifically enriched in su_{III} tyrosine tRNA was obtained from CA274 infected with Ø80 psu, and the tyrosine tRNA purified, by methods previously described [1,2]. Nucleotide sequences were determined by the methods of Sanger et al. [3,4], and the assays for tyrosyl tRNA synthetase and non-enzymatic tRNA binding to ribosomes have been described [2]. The T factor was prepared by a modification of the procedure of Ravel [5].

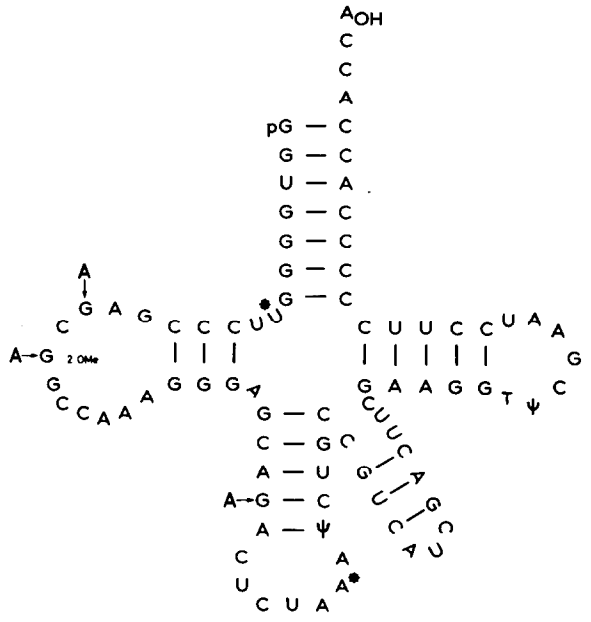

Fig. 1. Nucleotide sequence of su^{+}_{III} tyrosine tRNA showing the base substitutions of each of the three mutant tRNAs. In order from the 5′ end these are mutants 15, 24 and 12. A* is 2-thiomethyl, 6-isopentenyl adenylic acid.

3. Results

3.1. *Su⁻ mutants of* Ø80 psu^+_{III}

Cells infected with Ø80 psu^+_{III} were treated with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine and the progeny phage plated on CA274 lac^- amber in the presence of isopropyl-β-D-thio-galactoside (as inducer for β-galactosidase) and 5-bromo, 4-chloro, 3-indolyl-β-D-galactoside as indicator for the enzyme. On these indicator plates su^+ phages give blue plaques and su^- mutant phages give white plaques. Among the mutants picked up, 12, 15 and 24, gave very faintly blue plaques suggesting only marginal suppressor activity.

3.2. *Sequences of the mutant tRNAs*

[^{32}P]-labelled tyrosine tRNA from CA274 infected with the Ø80 psu^- mutants was purified [2] and the products from T_1 and from pancreatic ribonuclease digestion were separated on the two-dimensional electrophoretic system and compared with those from su^+_{III} tRNA [1]. Reference to the sequence in fig. 1 will explain the origin of the different products obtained from the mutant tRNAs.

In su^-_{15} tRNA guanine residue G15 was changed to A. In the T_1 ribonuclease products (fig. 2) 1 mole of AG was absent and only traces of CGmG and CG, derived from contaminating host-specified tRNA,

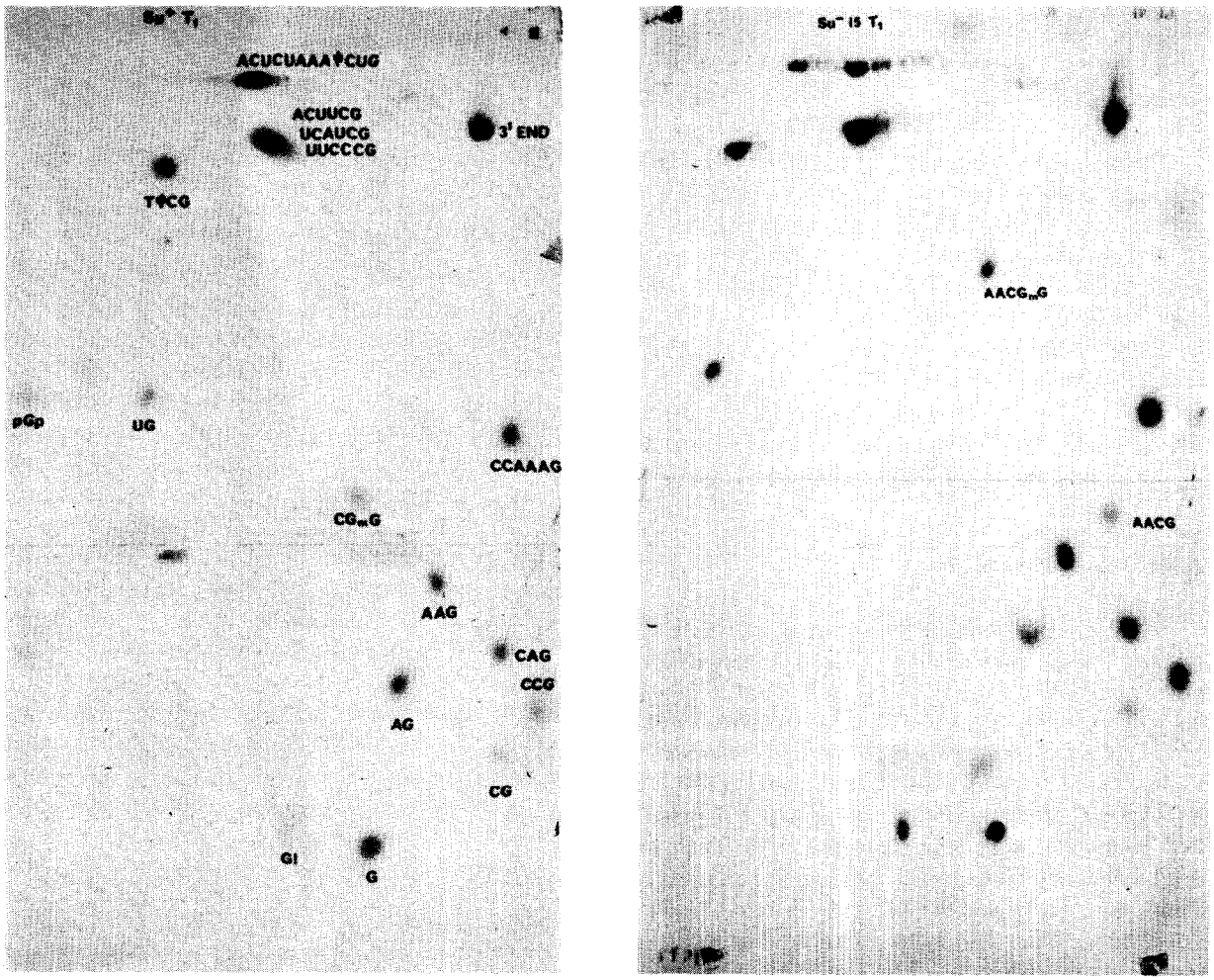

Fig. 2. Comparison of the T_1 ribonuclease products from su^+ and mutant 15 tRNAs. Separation is by electrophoresis on cellulose acetate at pH 3.5 in 7 M urea (right to left) followed by electrophoresis on DEAE paper in 7% formic acid (v/v).

were found. (CG is derived from G17 in molecules where Gm (2′-*O*-methyl G) is not methylated.) Two additional products were found; AACGmG and AACG. These were identified by their pancreatic ribonuclease products (AAC + GmG, and AAC + G, respectively). The AACG is derived from tRNA molecules in which G_m residue 17 is not methylated. In the pancreatic ribonuclease digest of the tRNA GACG was absent and replaced by the additional product GAAC (identified by T_1 ribonuclease digestion to give G and AAC).

In su^-_{24} tRNA G_m residue 17 is changed to A. CGmG was absent from the T_1 ribonuclease products and CG was in trace amounts (this is derived from molecules in which Gm 17 is not methylated). Instead an additional mole of CAG was found. (One mole of CAG is derived from the anticodon stem.) In the pancreatic ribonuclease digest GmGC and GGC were almost entirely absent and replaced by an additional trinucleotide AGC. This was identified by its T_1 ribonuclease products AG and C.

In su^-_{12} G residue 31 is changed to A. The T_1 ribonuclease digestion product, ACUCUAA*AΨCUG derived from the anticodon region was replaced by CAAACUCUAA*AΨCUG. On pancreatic ribonuclease digestion this gave C, U, AAAC, AA*AΨ and G, and did not yield AC. In the pancreatic ribonuclease digest of the tRNA AGAC was replaced by AAAC.

3.3. *Function of 12 and 15 mutant tRNAs in protein synthesis*

Tyrosine tRNA was purified from CA274 infected with the transducing phages [2]. From phage infected cells three species of su_{III} tyrosine tRNA can be separated, differing in the modifications of the 2-thiomethyl, 6-isopentenyl A, residue 38 [2]. All measurements were made on the species containing 6-isopentenyl A because this functions almost as well as the fully modified species in protein synthesis and is the least contaminated with host-specified tyrosine tRNA.

Determinations of apparent K_m values for tyrosyl synthetase are given in table 1. 15 tRNA does not

Table 1

Apparent K_m and V_{max} values of tyrosyl tRNA synthetase for the tRNAs. Su^-_0 is the wild type su^- with the G*UA anticodon.

tRNA	Preparation 1		Preparation 2	
	Apparent K_m (μM)	Apparent V_{max} (mμmoles/ l′/mg)	Apparent K_m (μM)	Apparent V_{max} (mμmoles l′/mg)
su^+	0.066	1.6	0.025	2.4
su^-_0	–	–	0.020	2.2
su^-_{15}	0.052	1.6	–	–
su^-_{12}	0.67	5.3	0.40	2.4

Table 2

T factor stimulated binding of the tRNAs to ribosomes and randum UAG polymer. Binding assays were according to Nirenberg and Leder [6]. The complete system (0.05 ml) contained (in μmoles): ammonium cacodylate buffer pH 7.1, 2.5; KCl, 2.5; $MgCl_2$, 0.2; GTP, 4.8×10^{-3}; and ribosomes 0.43 A_{260} units; poly UAG, 0.03 A_{260} units; supernatant factor, 0.25 μg; [^{3}H]tyrosyl tRNA (2×10^4 cts/min/μμmole).

tRNA	Input (cpm)	Bound complete-polymer (cpm)	Bound complete-factor (cpm)	Bound complete (cpm)	% bound specifically
su^+	8,564	439	549	2,102	19.3
	17,128	881	884	5,188	25
su^+	17,128	778	1,007	4,695	22.8
su^-_0	12,025	707	727	3,586	23.8
$su^+ + SU^-_0$	29,153	1,357	–	6,713	18.4
su^-_{15}	9,800	608	472	2,472	19.0
su^-_{12}	7,500	515	–	1,870	18.0

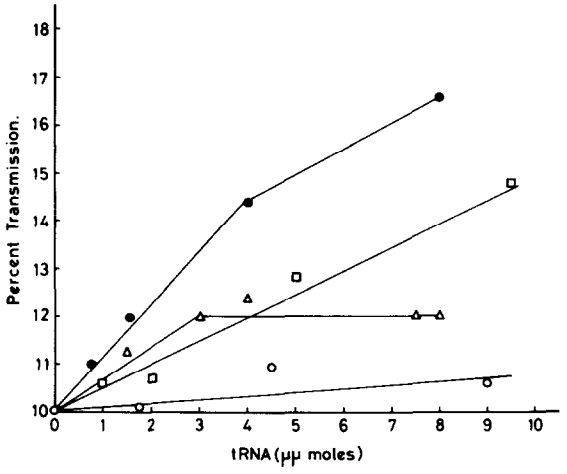

Fig. 3. *In vitro* suppressor activity of the mutant tRNAs. Assay according to Gefter and Russell [2]. Bacteriophage f2 mutant su 4A RNA is added to a complete *E. coli* protein synthesising system containing [^{35}S]-labelled formylmethionyl tRNA together with varying amounts of the mutant tRNA. The f2 mutant has an amber codon corresponding to the 7th amino acid of the coat protein. Transmission is the ratio (^{35}S in complete protein)/(^{35}S in polypeptide fragment and complete protein). ●—● su^+ tRNA; △—△ 15 tRNA; □—□ 12 tRNA; ○—○ su_o^- tRNA.

differ significantly from su^+ or (su_o^-), but 12 tRNA shows a marked decrease in affinity for the synthetase, having a K_m 10–15 times greater than su^+ tRNA.

The Nirenberg and Leder [6] ribosome binding assay was used to measure the stability of complexes of mutant tRNAs with ribosomes and UAG at 20 mM Mg^{++}. 15 tRNA binds as well as su^+ tRNA, but 12 tRNA hardly binds to ribosomes at all under these conditions. By measuring UAU dependent ribosome binding, the extent of contamination of the preparations by host-specified tyrosine tRNA could be measured. This was negligible with 12 tRNA, but amounted to 35% of the tyrosine tRNA in the 15 tRNA preparation.

In protein synthesis, recognition of the messenger RNA-ribosome complex by tRNA involves interaction of tRNA with transfer factor and GTP. Transfer factor-dependent binding of the tRNAs to ribosomes in the presence of a random UAG polymer, at 4 mM Mg^{++} was measured, and both 15 and 12 tRNAs were as effective as su^+ tRNA (table 2).

In vitro suppressor activities were assayed with the RNA from f2 bacteriophage carrying the sus 4A amber mutant in the coat protein gene [4]. The probability of translating the amber codon as a function of tRNA concentration is shown in fig. 3. 12 tRNA is about 50% as effective as su^+ tRNA but 15 tRNA, although effective at low concentrations, fails to stimulate at higher tRNA concentrations.

These results show that mutant 12 has a lower affinity for the activating enzyme, but can bind well to ribosomes in the presence of the transfer factor. Its lower efficiency of suppression might be due to slower rate of activation. Mutant 15 is normally activated, binds well in all assays but suppresses poorly and anomalously. This suggests that this tRNA is defective in some step on the ribosome subsequent to binding.

Other mutants have been isolated and are being studied. They may offer the opportunity of analysing the relation of the structure of the tRNA to its function.

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