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Novel *E. coli* mutants deficient in biosynthesis of 5-methylaminomethyl-2-thiouridine

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ABSTRACT

Novel E. coli mutants deficient in biosynthesis of 5-methylaminomethyl-2-thiouridine were isolated based on a phenotype of reduced readthrough at UAG codons. They define 2 new loci trmE and trmF, near 83' on the E. coli map. These mutants are different from strains carrying trmC mutations, which are known to confer a methylation deficiency in biosynthesis of 5-methylaminomethyl-2-thiouridine. tRNA from mutants carrying trmE or trmF mutations was shown to carry 2-thiouridine instead of 5-methylaminomethyl-2-thiouridine. This deficiency affects the triplet binding properties of the mutant tRNA. Our results suggest that the 5-methylaminomethyl group stabilizes the basepairing of this modified nucleotide with G, most likely through direct interaction with the ribosomal binding site(s).

INTRODUCTION

More than forty rare nucleotides have been found in transfer RNA of different organisms (1). They are uniquely associated with tRNA, but their biosynthesis and their role in tRNA function is not yet fully understood. Mutants deficient in tRNA modification provide a suitable substrate (undermodified tRNA) for further enzymological studies. They are also valuable tools in elucidating the role of modifications in tRNA function. However, even in E. coli only a handful of tRNA modification mutants have been isolated, by brute-force techniques (2,3), or as regulatory mutations (4,5).

We have previously shown (6) that a deficiency in 2-methylthio-N6-isopentenyl-adenosine in transfer RNA of E. coli drastically reduces readthrough at UGA codons, without seriously affecting normal protein synthesis. This finding suggests that readthrough at nonsense codons may be the phenotype of choice for detecting tRNA modification mutants.

Using a direct selection for reduced readthrough at UAG codons we have isolated and characterized 2 groups of novel E. coli mutants deficient in biosynthesis of 5-methylaminomethyl-2-thiouridine (mam5s2U). Transfer RNA from these mutants contain 2-thiouridine instead of mam5s2U. The role of the mam5 group in codon recognition was investigated in triplet binding experiments

Table 1. Bacterial Strains and Bacteriophages Used in this Work.

Designation	Pertinent genotype	Source/Reference
LL	E. coli B argR argF40 (UAG) lacZ(UAG)	This lab; see text
TP37	LL trmE	This work
RF30	LL trmF	This work
KL16	Hfr thi1 relA1 spotT1	This lab (6)
DEV 1	KL16 lacZ105 (UAG)	This lab (6)
DEV 14	KL16 lacZ659 (UAA)	This lab (6)
DEV 15	KL16 lacZ (UGA)	This lab (6)
DEV 16	DEV 1 val^r trmE	Transduction between DEV1 and TP37
DEV 17	DEV 1 val^r trmF	Transduction between DEV1 and RF30
DEV 18	DEV 14 bglR trmE	Transduction between DEV14 and DEV16 bglR
DEV 19	DEV 14 bglR trmF	Transduction between DEV14 and DEV17 bglR
DEV 20	DEV 15 bglR trmE	Transduction between DEV15 and DEV16 bglR
DEV 21	DEV 15 bglR trmF	Transduction between DEV15 and DEV 17 bglR
AN 382	uncB402	I.G. Young
GM 19	trmC	M.G. Marinus
W3110	trpB9579 tna	C. Yanofsky
pACYC184		A.C.Y. Chang (13)
T4	eL5a psu2	W.H. McClain; see text
λ tna		W.J. Brammar; see text
λ JF217		A. Torriani-Gorini; see text
λ KY7485		A. Torriani-Gorini; see text

using tRNAs charged with 14C lysine and ribosomes programmed with AAA and AAG. Our results suggest that the mam5 group stabilizes the interaction of the mam-5s2U-G wobble pair. A preliminary report of this work was presented at the Gorini Memorial Symposium on Prokaryotic Gene Expression, Lloyd Harbor, New York, June 1982.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophages

Bacterial strains and bacteriophages used in this work are listed in Table 1.

Media

Cells were routinely grown at 37^0C in tryptone broth or L-broth, as previously described (6). Minimal medium was that of Vogel and Bonner (7), supplemented as required. For tRNA and ribosome preparations cells were grown in Zubay broth (8). XG plates contained per liter: 10 g of Bacto-tryptone

(Difco), 5 g of NaCl and 15 g of Bacto-agar; 238 mg of isopropyl-β-D-glucopyranoside (IPTG) and 40 mg of 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (XG), dissolved together in 2 ml dimethylsulfoxide, were added after autoclaving.

Genetic Procedures

All standard genetic procedures were performed according to Miller (9). Transformations were performed as previously described (10). λ tna (11), a λ i^{21} derivative was prepared by mitomycin C induction of a lysogen (9) during early exponential growth in Zubay broth. All bglR derivatives were selected on minimal plates containing 0.3% salicin (Sigma) as a carbon source without mutagenesis (12). Plasmids containing a tna^{+} gene inserted at the HindIII site of pACYC184 (18) were selected in W3110 trpB9579 tna on minimal plates supplemented with 0.2% glycerol, 50 μg/ml 5-methyl-DL-tryptophan, 2 μg/ml indole (11) and 25 μg/ml chloramphenicol.

Strain Construction

In a genetic background containing the lacZ105 (UAG) mutation the trmE and trmF phenotypes are easily detected on XG plates, as a change from pale-blue colonies to white colonies. Construction of DEV 18,19,20, and 21 was a bit more involved. Maximal readthrough of lacZ659 (UAA) is already low, resulting in white colonies on XG. Further reduction in this readthrough level, can, however, be detected in β-galactosidase assays, provided special precautions are taken (6). DEV18 and DEV19 were identified in this way. Forty recombinants from a transduction between DEV14 and P1C1 grown on bglR derivatives of DEV16 and DEV17, selected for the ability to grow on salicin fell in 2 groups according to the β-galactosidase assays. About 80% of the recombinants showed reduced misreading levels, indicating the presence of trmE or trmF. Readthrough of the lacZ (UGA) mutation in DEV15 is high, resulting in deep blue colonies on XG plates. Among 40 recombinants obtained in a similar transduction with trmE and trmF donors, none showed an effect on readthrough levels. To prove that trmE and trmF had been introduced, but did not affect readthrough of UGA, P1 C1 was grown on 5 recombinants of each transduction and introduced back into DEV1. In 4 out of 5 cases, trmE and trmF were recovered, as evidenced by reduced readthrough of lacZ105 (UAG). These numbers are in good agreement with the mapping data (see below).

Direct Selection for Mutants with Reduced Misreading Levels and Reduced Efficiency of Suppression

This selection has been described previously (14). The main idea is as follows: Misreading of UAG codons can be monitored in 2 independent ways in strain LL (E. coli B, argR, argF40 (UAG), lacZ UAG)). Readthrough of lacZ(UAG) results in pale blue

colonies on XG plates. Readthrough of argF40 (UAG) results in an Arg-leaky phenotype, i.e. slow growth in the absence of arginine, provided the arginine pathway is derepressed (15). Mutants with reduced misreading levels were selected by plating LL cells with limiting amounts of bacteriophage T4 psu2$^+$ eL5a, a T4 phage containing a UAG mutation in the lysome gene (eL5a), which is suppressed by the T4 encoded UAA suppressor psu2$^+$, derived from $tRNA^{Gln}$ (16). The mixture was plated directly onto XG plates, where the 2 classes of survivors can be differentiated immediately: trivial T4 resistant mutants should still be pale blue on XG. However, rare survivors, which escape T4 killing by reducing the suppressor efficiency for psu2$^+$, are also expected to reduce misreading at UAG codons (15), resulting in white colonies on XG. Such candidates are further expected to show a concomitant change in their Arg-phenotype, from $Arg^{\pm}$ to Arg^-. For details, see Elseviers and Gorini (14).

DNA

Total E. coli DNA was isolated by the procedure of Muller and Crothers (17). Plasmid DNA was isolated by the procedure of Timmis et al. (18). λ tna was purified by a combination of published procedures. The crude lysate was centrifuged for 20' at 10,000 RPM in a GSA (Sorvall) rotor. The phage was then precipitated by the addition of 7% polyethylene glycol 6000 and 4% NaCl and allowed to settle overnight at 4^0C (19). λ tna was further purified by an equilibrium centrifugation in 41.5% (w/w) CsCl (KBI, technical grade) (20) and finally by spinning in a reverse block gradient (21) to remove bacterial DNA contamination. DNA was isolated from λ tna by phenol extraction and ethanol precipitation (20). Following HindIII digestion and electrophoresis on 1% agarose gels (10) a 3.7 kb fragment containing the tna$^+$ gene was identified (11). λ tna DNA and pACYC184 DNA were then mixed, digested with HindIII, and religated with T4 DNA ligase as previously described (10). Hybrid plasmids containing the tna$^+$ gene were selected as described above. Protein synthesis in plasmid containing minicells was monitored as described previously (10).

Ribosomes, Transfer RNA and Triplet Binding

Ribosomes. Cells were grown in Zubay broth as described (8), allowed to cool for 30' at 15^0C, harvested and frozen at -20^0C. They were resuspended at 1 ml/g of cells in a buffer containing 10 mM Tris-acetate pH 7.4, 10 mM Mg-acetate, 30 mM NH_4-acetate, 1 mM DTT, and 5 µg DNAse I (Sigma D1126) per g of cells. They were disrupted by alumina grinding or in the French Press at 6,000 psi. Cell debris was removed by two 30' spins in the SS34 (Sorvall) rotor at 16,000 RPM. Ribosomes were purified by a modification of Capecchi's procedure (22): SW41 Ti tubes containing 8.5 ml of 1 M sucrose in 50 mM Tris-acetate pH 7.8, 10 mM $MgCl_2$, 500 mM NH_4Cl, 1 mM EDTA and 0.5 mM DTT, were carefully overlayed with 4 ml of the S30 supernatant, obtained in the previous step, and

centrifuged overnight at 4^0C at 30,000 RPM in the SW41 Ti rotor. Ribosomes form a crystal clear pellet at the bottom. The pellet was rinsed several times with the same buffer without sucrose, and finally resuspended with the help of a glass rod in a small volume of a buffer containing 50 mM Tris-acetate pH 7.8, 10 mM Mg-acetate, 30 mM NH_4-acetate, 1 mM EDTA, 0.5 mM DTT, and 50% glycerol, and stored at -20^0C at a concentration of 0.5 to 1 A260/µl.

Transfer RNA. tRNA was prepared by the method of Zubay (23) from 1 or 2 liters of fresh overnight cultures, grown in Zubay broth. The yield was approximately 40 A260 of tRNA/g of cells. Aminoacyl tRNA synthetases, free of endogenous transfer RNA and RNAses were prepared from strain LL by following the procedure of Muench and Berg through the DEAE-cellulose step (24). tRNA was charged with lysine in a 200 µl reaction containing: 15-20 A260 units of tRNA, 30 µl of synthetases (approximately 0.6 mg protein), 1 µCi of L-(U-14C) lysine monohydrochloride (Amersham, specific activity 340 mCi/mmole), 1 mM ATP disodium salt, 4 mM reduced gluthathione, 10 mM $MgCl_2$ and 100 mM Na-cacodylate pH 7.0. Incubation was for 30' at 37^0C. Following incubation, the reaction was diluted with 1 ml sterile water and mixed with 1 ml of distilled phenol-chloroform-isoamylalcohol (50:48:2 v/v/v), previously equilibrated with water. The extraction was repeated twice. Residual phenol was removed from the aqueous phase by repeated extraction with anhydrous ether. Following the addition of 0.1 volume of 20% K-acetate pH 5.0 and 2 volumes of absolute ethanol, the tRNA was left to precipitate overnight at -20^0C, and recovered by centrifugation the next day. The ethanol precipitation was repeated twice. The final tRNA was resuspended in a small volume of sterile water and stored at -20^0C.

Triplet Binding experiments were performed according to Nirenberg and Leder (15) with slight modifications: each reaction contained in 30 µl; 3 A260 units of ribosomes, 10 pmoles of charged tRNA (based on TCA precipitable counts), 0.03 A260 units of codon, 50 mM Tris-acetate pH 7.2, 20 mM Mg-acetate and 50 mM NH_4-acetate. Incubation was for 20' at 24^0C and processing of samples was performed as described (25). Filters were dried under an infrared lamp and transferred to mini-scintillation vials, filled with 3.5 ml of Liquiscint (National Diagnostics). The vials were left at 4^0C overnight before vortexing and counting the next day.

Assays for Methyl Group Incorporation into tRNA. This assay was performed as described (2). The source of methylating enzymes was an S100 extract from a RNAse I^- derivative of DEV 1. The extract was dialyzed extensively against 40 mM Tris-acetate pH 7.8, 1 mM EDTA, 1 mM DTT, and 50% glycerol, and stored at -20^0C.

35S Labeling of tRNAs. Fresh overnight cultures were diluted 100-fold in 20 ml MOPS medium (26) with limiting K_2SO_4 (30 μM). When the A420 of the cultures reached 0.2, 0.6 mCi of $Na_2(35S)O_4$ was added and growth was continued for 1.5 generations. Labeled tRNA was purified by the Zubay procedure (23) in the presence of 1 A260 unit of carrier tRNA. The final isopropanol step in the purification was omitted. The resulting material contained approximately 10^6 cpm/A260 of tRNA. Digestion of tRNAs was performed according to Rogg et al. (27) using 1-2 10^5 cpm of labeled tRNA and 1 A260 unit of cold carrier tRNA. Samples evaporated to dryness in a SpeedVac concentrator (Savant) were then resuspended in 10 μl of sterile glass distilled water and spotted on 20 x 20 cm Cellulose F plates (Brinkmann). Chromatography tanks were lined with filter paper. The solvent system developed by Rogg et al., was used (27). Plates were developed for 6 hr or 15 cm in the first dimension in 1-butanol, isobutyric acid, concentrated NH_4OH, water (75:37.5:2.5:25, v/v/v/v), dried for 3 hrs in a ventilated hood and then run for 4 hrs in the second dimension, in saturated $(NH_4)_2SO_4$, 0.1 M Na-acetate pH 6.0, 2-propanol (79:19:2, v/v/v). Major nucleosides were detected by UV illumination; minor thiolated nucleosides by autoradiography, using Kodak XAR 5 film and Dupont Lightning Plus enhancing screens. Film was developed after 6 days of exposure at -70^0C.

RESULTS

Isolation of Mutants

Mutants with reduced UAG misreading and reduced efficiency of suppression for the bacteriophage T4 encoded suppressor $psu2^+$ were obtained as described in Materials and Methods. About 3000 white colonies were purified away from the phage and tested for concomitant change in the arginine phenotype, from $Arg^{\pm}$ to Arg^-; 160 such candidates were found and tested for residual β-galactosidase specific activity; 25 candidates with residual UAG misreading levels of less than 20% were studied further.

Genetic Mapping

On the basis of genetic mapping, the 25 candidates fall in at least 3 groups. Six out of 25 mutants define the new locus trmE (Mmemonic: tRNA modification), 13 out of 25 define the other new locus trmF. The remaining six mutants have not yet been mapped and may comprise one or more groups.

trmE and trmF were mapped near 83' on the E. coli map (28) by transduction experiments (see Fig. 1). It was further shown that trmE mutants were complemented by the specialized transducing phage λ tna (11). This places trmE in the immediate vicinity of tna. trmF maps between bglR and uncA but

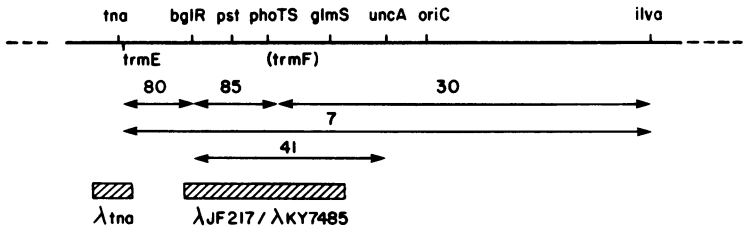

Figure 1. Genetic Mapping of trmE and trmF.
Numbers indicate cotransduction frequencies. Shaded blocks indicate chromosomal genes contained on the specialized transducing phages.

its precise location is less clear, because of high fluctuations in the transduction results. This is apparently a common problem with transductions in this area of the map, possible caused by the nearby loci dnaA and/or oriC (29). Furthermore, trmF mutants are not complemented by λ JF207 (30) and λ KY7485 (31) (see Fig. 1). It is possible that the trmF mutations obtained are dominant.

Effect on Misreading

trmE and trmF mutations were introduced into a set of E. coli K 12 strains containing respectively a lacZ UAA, lacZ UAG, and lacZ UGA mutation, all with maximal readthrough levels (6) by cotransduction with bglR, the ability to grow on salicin (12) (see Materials and Methods). The effect of trmE and trmF on misreading of the different nonsense codons is shown in Table 2. It is

Table 2. Readthrough at Nonsense Codons.

Genotype	Relative β-galactosidase specific activity (% of corresponding parent)		
	lacZ UAA	lacZ UAG	lacZ UGA
Wild type	100	100	100
	(0.015)[a]	(0.55)	(4.5)
trmE	30	6	100
trmF	20	7	100

Values shown are the average of at least 2 independent experiments. Experimental error was always within 10%. Special precautions for very low activities have been described (6).
a = Values in parentheses are β-galactosidase specific activities, expressed as % of KL16, a Lac^+ wild type strain.

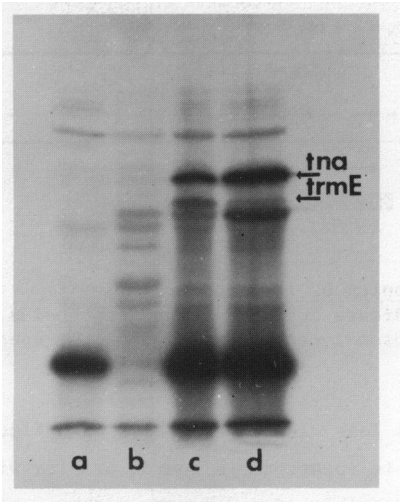

Figure 2. Expression of the trmE Gene Product in Minicells. Autoradiogram of SDS polyacrylamide gel following 35S methionine labeling of the minicell strain P678-54 containing: plasmid pACYC184 (lane a); no plasmid (lane b); plasmid pACYC184::*tna*$^+$ *trmE*$^+$ (lane c); plasmid pACYC184::*tna*$^+$ *trmE*$^-$ (lane d).

clear that UGA misreading is not affected. UAG misreading is drastically reduced, as selected; UAA misreading is also reduced but to a lesser extent. Neither mutation affects β-galactosidase specific activity in Lac$^+$ context. The observed effects are, therefore, specific for misreading.

Cloning of the trmE Gene

Complementation experiments with λ *tna* (see above) suggested that the wild type *trmE*$^+$ gene is present on λ *tna*. This phage was originally constructed *in vitro* by HindIII restriction-ligation (11). We transfered the chromosomal insert from λ *tna* to the plasmid pACYC184 by HindIII restriction ligation. The correct hybrid plasmid was selected by its ability to complement *tna*$^-$ (see Materials and Methods). In order to obtain a plasmid carrying a *trmE* mutation, we prepared a plasmid bank from total *E. coli* DNA, isolated from a *trmE* strain, digested with HindIII and religated to pACYC184, as prevously described (10). A hybrid plasmid containing the HindIII fragment carrying the *tna*$^+$ and *trmE*$^-$ genes was selected again by its ability to complement a *tna*$^-$ mutation (see Materials and Methods). The resulting candidates were checked on 1% agarose after HindIII digestion. A plasmid with a single insert, corresponding in size to the HindIII insert of λ *tna* (3.7 kb) was retained for further study. The plasmids obtained did not complement *trmE*$^-$, as expected.

Table 3. Triplet Binding.

Ribosomes	Wild type tRNA		trmE tRNA		trmF tRNA	
	AAA	AAG	AAA	AAG	AAA	AAG
wild type	100	52	51	4	53	6
trmE	101	54	98	9	73	12
trmF	89	24	37	1	43	4

100 corresponds to 2 pmoles bound under standard triplet binding conditions, i.e 3 OD washed ribosomes, 10 pmoles $tRNA^{Lys}$, 20 mM Mg^{++} (see Materials and Methods).

The trmE gene product was characterized by transforming the minicell strain P678-54 with the $trmE^+$ and the $trmE^-$ plasmid. Gene expression from the plasmids was monitored by 35S methionine labeling, followed by SDS polyacrylamide gel electrophoresis. The results are shown in Fig. 2. Both plasmids express the tna^+ gene product, a 52 K protein (32). In addition, the $trmE^+$ plasmid expresses a 48.5 K protein which is absent in the mutant plasmid. We conclude that the $trmE^+$ gene product is most likely this 48.5 K protein. In addition, it appears that the trmE mutation studied is itself a deletion or nonsense mutation.

Triplet Binding

Based on their phenotype, trmE and trmF mutations are expected to affect a component of the protein synthesis machinery, most likely the ribosome or something in tRNA, perhaps a deficiency in modification. To sort out these possibilities, we performed triplet binding experiments using crude tRNA charged with lysine and ribosomes programmed with AAA and AAG (see Materials and Methods). Neither mutant shows a deficiency in tRNA charging. The results of the triplet binding experiments are shown in Table 3. It is clear that the mutations affect tRNA and not ribosomes. In addition, a differential effect is observed with regard to binding to AAA or AAG programmed ribosomes: with AAA binding is reduced 2-fold; with AAG binding is reduced 10-fold. This effect is in good agreement with the effect of trmE and trmF mutations on readthrough of UAA and UAG, UAG being more drastically affected (see above).

Differences Between trmE, trmF, and trmC Mutations

Taken together our results suggest that trmE and trmF mutants have some alterations in the rare nucleoside 5-methylaminomethyl-2-thiouridine (mam5s2U). This modification is found in the wobble position of the anticodon in E. coli $tRNA_2^{Glu}$, $tRNA^{Lys}$ and possibly $tRNA_1^{Gln}$ (1). We therefore checked trmE and trmF tRNA for a possible deficiency in methylation, using as a control a

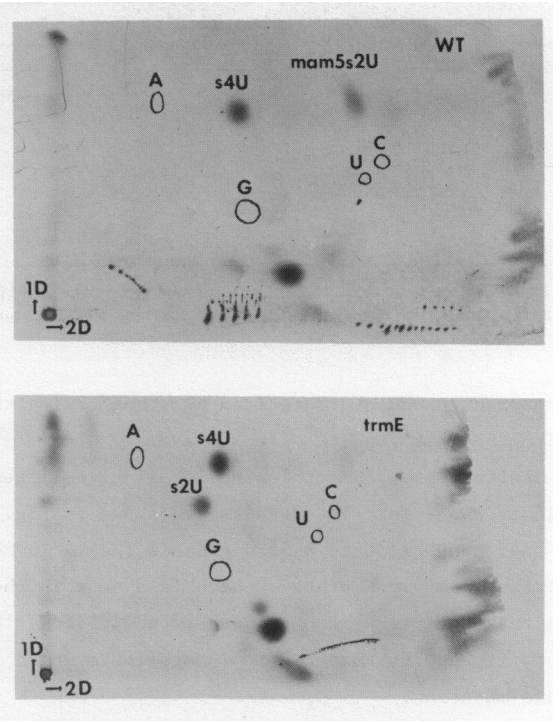

Figure 3. Thin Layer Chromatography of 35S Labeled tRNA Nucleotides. Only the bottom half of each autoradiogram is shown. Unmarked spots have not been positively identified.

known mutant deficient in methylation of mam5s2U, i.e. strain GM19, containing a trmC mutation. The methylation deficiency in GM19 was confirmed, but tRNA from trmE and trmF strains did not accept any S-adenosylmethionine counts in the post-labeling assay (see Materials and Methods; data not shown). TrmE and trmF mutants are different from trmC in at least 2 other respects: trmC mutations map near 55' on the E. coli map (2). We also found that tRNA from GM19 does not affect the binding properties of $tRNA^{Lys}$ to AAA and AAG programmed ribosomes (data not shown).

Thin Layer Chromatography

To further examine the deficiency in tRNA trmE and trmF strains, we isolated 35S labeled crude tRNA from parent and mutant strains and examined the thiolated nucleosides in 2 dimensional thin layer chromatography (see Materials and Methods). The results are shown in Fig. 3. Results for trmF (not shown) are identical to those obtained for trmE. Mam5s2U is clearly absent in the mutant tRNA. In addition, a new spot appears which was identified as 2-thiou-

ridine (s2U) on the basis of comigration with authentic standard, a generous gift of Dr. Vorbruggen, Schering AG, Berlin.

DISCUSSION

We have isolated 2 classes of E. coli mutants deficient in biosynthesis of 5-methylaminomethyl-2-thiouridine (mam5s2U). They define 2 different and novel genes for which we suggest the designation trmE and trmF. Their effect on tRNA function is nearly identical. We have also shown that the $trmE^+$ gene product is probably a 48.5 K protein. Our results also confirm our previous suggestion that in E. coli UAA and UAG codons are primarily misread by $tRNA^{Lys}$ $tRNA_2^{Glu}$, or, perhaps, $tRNA_1^{Gln}$ (6).

tRNA from the mutants contains s2U rather than mam5s2U, suggesting a deficiency in the biosynthesis of the mam5 group. Scraping of the thin layer chromatography spots suggests that at least 80% of the mam5s2U migrates as s2U in the mutants. We have also shown that trmE and trmF are different from trmC, a known mutation conferring a deficiency in methylation of mam5s2U, by at least 3 criteria: map position, in vitro methylation, and triplet binding of $tRNA^{Lys}$ to AAA and AAG. We suggest that tRNA from trmC strains probably contains am5s2U, but this point still remains to be proven.

The mam5s2U modification is usually described as a modification which restricts wobble (33). An unmodified uridine in the wobble position is expected to pair with A or G (34). In triplet binding experiments the modified base mam5s2U is reported to prefer A over G by a factor of 2 to 7 (35-38). A wobble pair U.G requries hydrogen bonding between O(2) of uridine and NH(1) of guanine (34). When S(2) replaces O(2) in 2-thiouridine, the corresponding hydrogen bond is expected to be weakened (39). It was also shown (40) that there is no detectable interaction between G and s2U in triplet binding experiments using synthetic codons containing s2U instead of U.

The fact that this preference of A over G is increased 5 fold in mutants lacking the mam5 group suggests that the role of this side chain is to stabilize the mam5s2U.G wobble pair. This makes good physiological sense, because both $tRNA^{Lys}$ and $tRNA_2^{Glu}$ of E. coli are believed to be unique species which must be able to recognize both of their respective codons, AAA and AAG for $tRNA^{Lys}$, and GAA and GAG for $tRNA_2^{Glu}$. However, if the mam5 group is there to counteract the restricted wobble imposed by the s2U group, one wonders why this uridine is not left unmodified to begin with. A possible explanation is as follows: the mam5s2U modification is specific for tRNAs with 5' (mam5s2U)UX 3' anticodons. In view of the poor stacking of A and U residues (41) this

sequence may require modification in order to stabilize the 3' stacked conformation of the anticodon (42). The 2-thiouridine modification could accomplish this by its strong stacking interaction with the N(1) position of the neighboring uridine (43).

How does the mam5 group exert its stabilizing effect on the mam5s2U.G interaction? In their study on the stability of tRNA.tRNA complexes with complementary anticodons Grosjean et al. (44) examined mam5s2U pairing with A,C, U, and G. The interaction with G is by far the most unstable, suggesting that the mam5 group does not act directly at the level of codon anticodon interaction. It would appear, therefore, that the mam5 group interacts with a site in the binding domain of the ribosomal A and/or P site. Weakened codon-anticodon interactions on the ribosome have also been reported for 2 other tRNA modification mutants: miaA deficient in 2-methylthio-N6-isopentenyladenosine (6,45), and hisT, deficient in pseudouridylate synthetase I (46). Taken together these results suggest that several tRNA modifications have evolved to stabilize inherently weak codon-anticodon interactions. Our results strongly suggest that they do so by direct interaction with the ribosome. This fits very well with the extended anticodon concept proposed by Yarus (47). This model is further supported by our observation that an antagonistic effect exists between miaA mutations and certain Sm-resistant rpsL mutations, which alter protein S12 of the 30S ribosomal subunit (6). We have observed a similar effect in trmE rpsL and trmF rpsL double mutants and the antagonism is also known to exist in the case of hisT mutants (48).

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