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An Anticodon Sequence Mutant of *Escherichia coli* Initiator tRNA: Possible Importance of a Newly Acquired Base Modification next to the Anticodon on Its Activity in Initiation†

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Initiator tRNAs from eubacteria and chloroplasts lack a base modification next to the anticodon. This is in contrast to virtually all other tRNAs from these sources. We show that a mutant *Escherichia coli* initiator tRNA which has an anticodon sequence change from CAU to CUA now has a 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine (ms^2i^6A) modification, produced by posttranscriptional modification of A, next to the anticodon. This newly acquired base modification may be important for the function of the mutant tRNA in initiation. In a *miaA* mutant strain of *E. coli* defective in biosynthesis of ms^2i^6A, the mutant initiator tRNA is 10- to 12-fold less active in initiation. The mutant tRNA is aminoacylated and formylated normally in the *miaA* strain. Thus, the absence of the base modification affects the activity of the mutant tRNA at a step subsequent to its formylation.

Virtually all tRNAs sequenced to date contain a base modification at position 37 next to the anticodon (32). This base modification can be quite simple, requiring the activity of a single enzyme, or quite elaborate, requiring the activities of as many as four or five different enzymes (1, 21). The nature of the base modification depends upon several factors: the parental base in which the modification occurs, the base preceding it in the anticodon sequence, the presence or absence of determinants in the tRNA for the base-modifying enzyme(s), and the organism from which the tRNA is isolated (1, 21).

Eubacterial and chloroplast initiator tRNAs are unusual in that they all contain an unmodified A at position 37 (7, 32). For *Escherichia coli* initiator tRNA, it has been suggested that the absence of this base modification is important for its unique ability to initiate protein synthesis with such codons as AUG, GUG, and UUG, which differ in the first position (7). The demonstration that yeast cytoplasmic initiator tRNA which has a base modification next to the anticodon can use AUG and GUG for initiation of protein synthesis in an *E. coli* in vitro system would seem to argue against this possibility (27). However, in vivo, the same yeast initiator tRNA is unable to read GUG and UUG as initiation codons in a wild-type strain (6, 30). Therefore, it is still possible that the absence of a base modification next to the anticodon allows the *E. coli* initiator tRNA to translate GUG and UUG as initiation codons in *E. coli.*

In our studies on structure-function relationships of *E. coli* initiator tRNA, we have used a CAU→CUA anticodon sequence mutant (U35A36 mutant) which can initiate protein synthesis in *E. coli* from UAG instead of AUG (38). Because most *E. coli* tRNAs with A36 in the anticodon contain a 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine (ms^2i^6A) modification at position 37 (1, 20, 21, 33), we have examined whether the U35A36 mutant tRNA now carries such a modification. Mass spectrometric and modified base composition analyses of the tRNA show that the U35A36 mutant tRNA contains ms^2i^6A, a conclusion supported by accurate molecular mass measurement of the intact tRNA. The U35A36 mutant tRNA isolated from a *miaA* strain (8, 39) deficient in biosynthesis of ms^2i^6A lacks ms^2i^6A. Also, the U35A36 mutant tRNA isolated from the *miaA* strain migrates faster during polyacrylamide gel electrophoresis than tRNA isolated from a $miaA^+$ isogenic strain.

Our results indicate that the ms^2i^6A modification may be important for the activity of the U35A36 mutant tRNA in initiation. We have examined whether the reduced activity of the U35A36 mutant tRNA in initiation in the *miaA* strain can be rescued by overproduction of IF3. We show that overproduction of IF3 has no effect.

MATERIALS AND METHODS

Strains and plasmids. *E. coli* CC104 *ara* Δ(*lac pro*) *XIII*(F′ *proB*$^+$) and TX2590 *ara* Δ(*lac pro*) *XIII*(F′ *proB*$^+$) *miaA*::*omega* Km^r were obtained from Malcolm E. Winkler, University of Texas, Houston Medical School (34). The plasmids pRSV *CATam1.2.5*, harboring the genes for the mutant chloramphenicol acetyltransferase (CAT) and the U35A36 mutant tRNA (38), pAC, containing the IF3 gene under the control of the *lac* promoter (17), and pACD, containing the gene for GlnRS or GlnRS and IF2 under the control of the *lac* promoter, were described before. The pACYC184 plasmid was obtained from New England Biolabs.

Construct for overproduction of IF3. The IF3 gene (3) under the control of the *lac* promoter in plasmid pACIF3 (17) was excised with *Bgl*II and *Pst*I. The cohesive ends were blunted with T4 DNA polymerase in the presence of deoxynucleoside triphosphates (dNTPs). The *Bgl*II-*Pst*I fragment was ligated to the large *Ava*I-*Pvu*II fragment of pACYC184. The *Ava*I site of the pACYC184 fragment was filled in with T4 DNA polymerase in the presence of dNTPs.

Growth of cells. Transformants of the CC104 and TX2590 strains of *E. coli* were grown overnight at 37°C in Luria-Bertani medium supplemented with the appropriate antibiotics. An aliquot of the overnight culture was diluted 20-fold, and that of cells overproducing IF2 was diluted 10-fold (17), into 3 ml of fresh medium containing antibiotics as necessary and grown for 3 h. The TX2590 strain was routinely grown in the presence of 25 μg of kanamycin per ml. When required, ampicillin, tetracycline, and IPTG (isopropyl-β-D-thiogalactopyranoside) were added to final concentrations of 100 μg/ml, 7.5 μg/ml, and 1 mM, respectively.

Preparation of cell extracts. Cells from 1.2 ml of culture were pelleted by centrifugation for 10 min at 4°C with a Beckman microcentrifuge and lysed as described previously (37). The cell lysate was centrifuged for 10 min at 4°C, and an aliquot of the supernatant was mixed with 1 volume of CAT storage buffer (20 mM Tris-HCl [pH 8.0], 200 mM NaCl, 10 mM β-mercaptoethanol, and 70% [wt/vol] glycerol) and stored at −20°C.

Enzyme assays. The activities of CAT and β-lactamase in cell extracts were

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† We dedicate this paper to Helga and Walter Kersten, University of Erlangen on the occasion of their retirement from the University.

assayed as described previously (37). The relative CAT activities are defined as the ratios of the specific activities of CAT to those of β-lactamase in the same extract.

Isolation and Northern (RNA) blot analysis of total tRNA. Total tRNA was isolated from *E. coli* by phenol extraction under acidic conditions (36). An aliquot containing total RNA at an optical density at 260 nm of 0.0025 was subjected to electrophoresis on a 6.5% polyacrylamide gel containing 8 M urea at pH 5.0 and 4°C. The tRNAs were transferred to Nytran membrane, and the initiator tRNAs were detected with a probe complementary to nucleotides 40 to 56 of *E. coli* initiator tRNA. The endogenous tyrosine tRNA was detected with a probe complementary to nucleotides 10 to 25 of the tRNA. Uncharged tRNA markers were prepared by incubating an aliquot of the isolated tRNA with an equal volume of 100 mM Tris-HCl, pH 9.0, for 1 h at 37°C.

Large-scale isolation and purification of mutant initiator tRNA. *E. coli* CC104 (*miaA*$^+$) and TX2590 (*miaA*) transformed with the pRSV *CATam.1.2.5* plasmid carrying the U35A36 mutant tRNA gene were used for isolation of the mutant tRNA. Total tRNA was isolated from 1 liter of cells by phenol extraction. The yields of tRNA from the *miaA*$^+$ and *miaA* strains were 908 and 558 A_{260} units, respectively.

Aliquots of total tRNA (165 A_{260} units) were electrophoresed on a 15% polyacrylamide gel (20 by 40 by 0.15 cm) (29) at 450 V until the xylene cyanol dye reached the bottom of the gel. The tRNAs were visualized by UV shadowing, and the band corresponding to the mutant initiator tRNA was excised. The tRNA was eluted from the gel slice with 5 ml of 20 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), and 0.2 M LiCl overnight at 37°C. The eluate was filtered through a Millipore-GV filter unit (pore size, 0.22 μm), and the tRNA was precipitated at 4°C overnight following the addition of 0.5 ml of 3 M sodium acetate, pH 6.0, and 2.5 volumes of ethanol. The yield of pure tRNA was about 10 to 15% of the total tRNA applied.

HPLC analysis of U35A36 mutant tRNA. tRNA was completely hydrolyzed to nucleosides with a mixture of nuclease P1, snake venom phosphodiesterase, and bacterial alkaline phosphatase as described earlier (5). The enzymatic digest was analyzed for the presence of ms^{2}i^{6}A by reversed-phase high-pressure liquid chromatography (HPLC) with a protocol designed for nucleoside analysis, as described previously (23).

Determination of tRNA molecular mass by electrospray ionization mass spectrometry. tRNA from *E. coli miaA* and *miaA*$^+$ strains was precipitated as described previously (16) and then dissolved in isopropanol (30 μl), H_2O (12 μl), and 100 μM CDTA (*trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid) in isopropanol (3 μl) and 0.1% triethylamine (3 μl). The solution was continuously infused at 2 μl/min into the electrospray ion source of an API III+ quadrupole mass spectrometer (PE/Sciex, Norwalk, Conn.). Mass measurements were made under conditions previously reported (16) with the analyzer Q1 for mass analysis and with Q2 and Q3 operating in radiofrequency-only mode. Molecular mass calculations were made with the HyperMass software supplied with the Sciex data system. The molecular mass spectra (see Fig. 3) were derived (9) with maximum entropy (MaxEnt) deconvolution software (VG Biotech, Cheshire, United Kingdom).

General methods. These were all as described elsewhere (17).

RESULTS

Virtually all *E. coli* tRNAs which have A residues at positions 36, 37, and 38 in the anticodon loop and a 5-bp anticodon stem contain the ms^{2}i^{6}A modification at position 37 (1, 20, 21, 33). The U35A36 mutant of *E. coli* initiator tRNA which initiates protein synthesis from UAG instead of AUG now has A36 in addition to A37 and A38 (Fig. 1). Therefore, we investigated (i) whether the mutant tRNA contains ms^{2}i^{6}A and (ii) whether the ms^{2}i^{6}A plays a role in its activity in initiation.

Presence of ms^{2}i^{6}A in the U35A36 mutant initiator tRNA. We have previously used Northern blot analyses to separate the uncharged-, aminoacyl-, and formylaminoacyl-forms of mutant initiator tRNAs fractionated on polyacrylamide gels containing 8 M urea at pH 5.0 and 4°C (36). The first indication that the U35A36 mutant initiator tRNA might contain a base modification came from Northern blot analyses of the mutant tRNA when it was highly overproduced in *E. coli* by overproduction of IF2 (17). Under these conditions, the mutant tRNA yielded two bands corresponding to the formylaminoacyl-tRNA instead of the single band obtained normally (17). The most likely reason for this result is that tRNA in the two bands differed by a single base modification and that this base modification is ms^{2}i^{6}A. Therefore, we used Northern blot analyses of tRNA isolated from a *miaA*$^+$ strain and an isogenic *miaA* strain deficient in ms^{2}i^{6}A modification to examine whether the U35A36 mutant tRNA isolated from these two strains would migrate differently on polyacrylamide gels. Results in Fig. 2 show that the formylaminoacyl-tRNA band corresponding to the U35A36 mutant isolated from the *miaA* strain (Fig. 2, band B′, lane 8) migrates faster than that isolated from the *miaA*$^+$ strain (band B, lane 7). The endogenous Tyr-tRNA, which contains the ms^{2}i^{6}A modification (12), also behaved similarly to the U35A36 mutant tRNA (compare band D′ in lane 8 with band D in lane 7). In contrast, the electrophoretic mobilities of the wild-type initiator tRNA (band B) isolated from the *miaA* (lane 4) and *miaA*$^+$ (lane 3) strains were essentially the same.

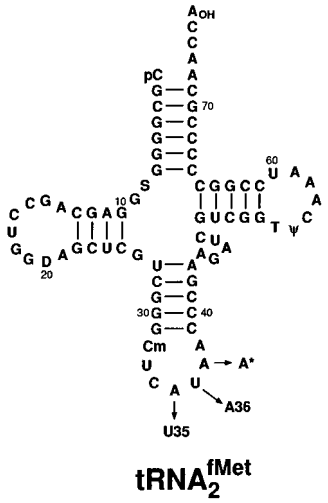

FIG. 1. Sequence of *E. coli* tRNA$_2^{fMet}$ in cloverleaf form. Arrows indicate the sequence changes (U35 and A36) and the site of the ms^{2}i^{6}A (A*) found in the mutant tRNA in a *miaA*$^+$ strain.

Treatment of the tRNAs with mild alkali to hydrolyze the ester linkage between the amino acid or formyl amino acid and the tRNA resulted, as expected, in an increase in mobility for all of the tRNAs (compare lanes 1, 2, 5, and 6 with lanes 3, 4, 7, and 8, respectively; for details, see the legend to Fig. 2). However, the difference in mobilities noted above for the corresponding tRNAs isolated from *miaA*$^+$ and *miaA* strains is retained. These results indicate very strongly that the U35A36

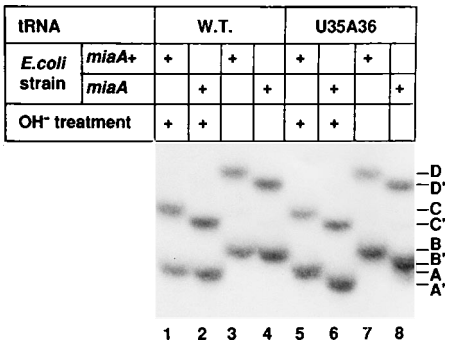

FIG. 2. Northern blot analysis of tRNA electrophoresed on a 6.5% polyacrylamide gel containing 8 M urea at pH 5.0 and 4°C. tRNA was isolated from *miaA*$^+$ (lanes 1, 3, 5, and 7) and *miaA* (lanes 2, 4, 6, and 8) strains of *E. coli* transformed with plasmids carrying the wild-type (W.T.) initiator tRNA gene (lanes 1 to 4) or the U35A36 mutant initiator tRNA gene (lanes 5 to 8). The blot was probed with a mixture of 5′-^{32}P-labeled oligonucleotides complementary to nucleotides 40 to 56 of *E. coli* initiator tRNA and nucleotides 10 to 25 of *E. coli* tyrosine tRNA. The letters at the right refer to tRNA bands.

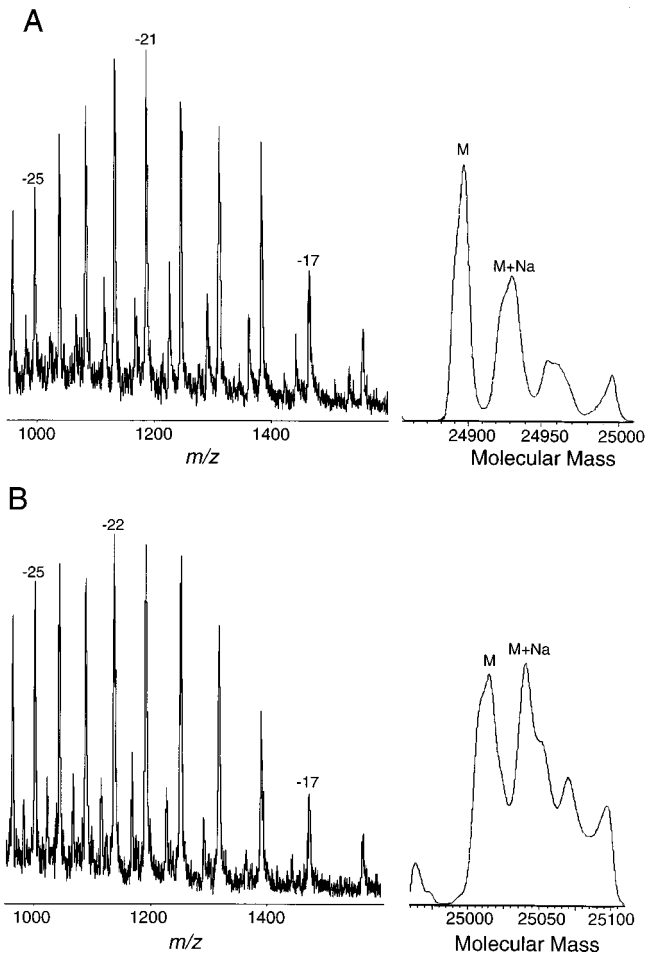

FIG. 3. Electrospray ionization mass spectra of the U35A36 mutant initiator tRNAs isolated from *miaA* (A) and *miaA*$^+$ (B) strains of *E. coli*. The measured mass-to-charge ratios (*m*/*z*) of ions containing from 16 to 26 net negative charges (Table 1) representing tRNA molecular mass (left) are used to calculate the molecular masses (M) of the neutral tRNAs: *miaA* tRNA, 24,898.2 Da; *miaA*$^+$ tRNA, 25,012.8 Da. The minor ion series corresponds to a background impurity with an M_r of 24,532 in both mass spectra. Shown on the right are mass spectra of the neutral tRNA species, reconstructed from the ion series shown. Isotope peaks at higher masses represent adduct ions resulting from the replacement of H^+ by increasing numbers of cations, principally Na^+. Molecular mass is measured in daltons.

TABLE 1. Measured *m*/*z* values from mass spectra for the U35A36 mutant initiator tRNAs isolated from *miaA* and *miaA*$^+$ strains[a]

Net charge (*z*)	*m*/*z*	
	miaA	*miaA*$^+$
−16	1,554.9	1,562.3
−17	1,463.4	1,469.8
−18	1,382.1	1,389.0
−19	1,309.4	1,315.3
−20	1,243.8	1,249.5
−21	1,184.6	1,189.9
−22	1,130.8	1,136.0
−23	1,081.5	1,086.6
−24	1,036.5	1,041.1
−25	995.1	999.6
−26	956.8	961.3

[a] See Fig. 3 for the mass spectra. The derived molecular masses for tRNAs from *miaA* and *miaA*$^+$ strains are 24,898.2 and 25,012.8 Da, respectively.

mutant tRNA carries an ms^2i^6A modification. Furthermore, tRNA carrying the base modification can be separated electrophoretically from the one lacking it.

Definitive evidence that the U35A36 mutant tRNA contains ms^2i^6A was obtained by mass spectrometric and modified base composition analyses of purified tRNAs isolated from *miaA*$^+$ and *miaA* strains (purification of the mutant tRNA is described in Materials and Methods). Electrospray ionization mass spectrometry was used for the accurate measurement of the molecular masses of both tRNAs (16). The molecular masses of the U35A36 mutant tRNA from the *miaA*$^+$ and *miaA* strains were 25,012.8 Da and 24,898.2 Da, respectively (Fig. 3; Table 1). The estimated error for these measurements is ≤2 Da (16). The difference in the masses of the tRNAs of 114.6 Da corresponds closely to the expected difference in the molecular masses of ms^2i^6A and A of 114.2 Da. Moreover, HPLC analysis of the modified base composition of the U35A36 mutant tRNA isolated from the *miaA*$^+$ strain showed a peak with the same retention time as that of authentic ms^2i^6A, 44.2 min, and an experimentally indistinguishable 254 nm/280 nm HPLC peak height ratio from UV detection of 0.84, compared with 0.80 for authentic ms^2i^6A. The corresponding peak was absent in the same mutant tRNA from the *miaA* strain (data not shown). The analysis also showed that the U35A36 mutant tRNA from the two strains contained the other expected modified nucleosides, pseudouridine, 2′-*O*-methylcytidine, and thymine riboside, in a 1:1:1 ratio as well as 4-thiouridine (data not shown).

Importance of ms^2i^6A in function of the U35A36 mutant tRNA in initiation. The ms^2i^6A modification affects the function of the U35A36 tRNA in initiation in vivo. The initiator activity of the mutant tRNA in the *miaA*$^+$ and *miaA* strains was determined by measuring the level of CAT expression from a mutant CAT gene containing UAG as the initiation codon (38). The specific activities of CAT were normalized to β-lactamase specific activities in the same extract to correct for fluctuations in CAT activity arising from changes in plasmid copy number. The normalized CAT specific activities were then expressed as a percentage of the normalized CAT specific activity in the extract of the *miaA*$^+$ transformant harboring the U35A36 gene, which was set at 100% (values are averages of assays of two or three separate extracts). The CAT, the β-lactamase, and the tRNA genes are all on the same pRSV plasmid (37). The CAT activity in the *miaA* strain expressing the U35A36 mutant tRNA is only about 8% of that in the *miaA*$^+$ strain. In contrast, the CAT activities in extracts of the *miaA*$^+$ and *miaA* strains carrying the wild-type CAT reporter gene and the wild-type initiator tRNA gene were essentially identical (133.5 and 135.1%, respectively).

The results of Northern blot analyses (Fig. 2, lanes 7 and 8) show that the U35A36 mutant tRNA is fully formylated in both the *miaA*$^+$ and the *miaA* strains (bands B and B′, respectively). Furthermore, relative to the endogenous Tyr-tRNA (bands D and D′, respectively), there is no significant difference in the levels of the U35A36 mutant tRNA in the two strains. Thus, the reduced activity in initiation of the U35A36 mutant tRNA lacking the ms^2i^6 modification is due to a defect at a step subsequent to the formylation of the tRNA.

Effect of overproduction of IF2 and IF3 on activity of the U35A36 mutant tRNA in initiation in *miaA*$^+$ and *miaA* strains. The initiation factors IF2 and IF3 facilitate the binding of fMet-tRNA to the P site on the ribosome (11, 26). We have, therefore, investigated the effect of overproduction of IF2 and IF3 on activities of the U35A36 mutant tRNA in the *miaA*$^+$

TABLE 2. Effect of overproduction of GlnRS, GlnRS and IF2, and IF3 on the initiator activity of the U35A36 mutant tRNA in *miaA*$^+$ and *miaA* transformants

Strain	Relative CAT activity (%)[a]			
	pACD vector alone	GlnRS	GlnRS and IF2	IF3
miaA$^+$	100	92	1,170	138
miaA	10	13	209	16

[a] Relative CAT activities in extracts of *miaA*$^+$ and *miaA* transformants harboring the pRSV plasmid carrying the *CATam1.2.5* and the U35A36 tRNA genes. These transformants also contained the pACD plasmid without (vector alone) or with the genes for GlnRS, GlnRS and IF2, and IF3. The values reported are averages of assays of three separate extracts.

and *miaA* strains of *E. coli* (Table 2). Overproduction of Gl nRS alone had essentially no effect. Overproduction of GlnRS and IF2 increased the level of CAT expression from 100 to 1,170% in the *miaA*$^+$ strain. This effect of IF2 on the activity of the U35A36 mutant tRNA is reported elsewhere (17). In the *miaA* strain also, overproduction of GlnRS and IF2 increased the CAT activity from 10 to 209%. However, the activity of the U35A36 mutant tRNA in the *miaA* strain is still only about 18% of that in the *miaA*$^+$ strain. In contrast to the effect of overproduction of GlnRS and IF2, overproduction of IF3 had only small effects on the activity of the U35A36 mutant tRNA in the *miaA*$^+$ and *miaA* strains. Control experiments using immunoblot analyses of cell extracts showed that IF3 was highly overproduced in these cells, as was described elsewhere for the IF3 gene cloned into a different vector (17).

DISCUSSION

We have shown by mass spectrometric and modified base composition analyses that A37 next to the anticodon of *E. coli* initiator tRNA is modified to ms^{2}i^{6}A when the anticodon CAU is changed to CUA. Interestingly, although all 10 eubacterial and 3 chloroplast initiator tRNAs sequenced to date (32) lack a base modification next to the anticodon, the U35A36 mutant *E. coli* initiator tRNA with the base modification is quite active in initiation (see Results). This result suggests that the absence of a base modification next to the anticodon in the wild-type tRNA is not among the determinants used by components of the protein synthetic machinery to distinguish between initiator tRNA and elongator tRNAs (26).

The newly acquired ms^{2}i^6 modification of A next to the anticodon may be important for the activity of the mutant tRNA in initiation. The U35A36 mutant tRNA is essentially quantitatively formylated in the *miaA* strain. Therefore, the reduced activity of the U35A36 tRNA in initiation in the *miaA* strain is due to an effect at a step subsequent to formylation, possibly the step of binding to the ribosomal P site. The second codon on the CAT gene used as a reporter specifies aspartic acid. tRNA^{Asp} does not have ms^{2}i^{6}A (32). Therefore, binding of Asp-tRNA to the ribosomal A site should not be affected in the *miaA* strain. It is also unlikely that the lack of the ms^{2}i^6 modification in the U35A36 mutant initiator tRNA affects the binding of Asp-tRNA to the A site by influencing tRNA-tRNA interactions between the tRNA on the P site and the tRNA on the A site. Nucleotide 37 on the P site tRNA is distal to residues thought to be involved in tRNA-tRNA interactions on the ribosome (31).

Why should modification of A37 in the U35A36 mutant tRNA be important for initiation when the wild-type tRNA lacks such a modification? A likely reason is that the mutant initiator tRNA reads the codon UAG instead of AUG. The presence of the ms^{2}i^6 modification at A37 may be necessary to stabilize base pairing between U in the UAG codon and A36 in the tRNA anticodon (21). Suppressor tRNAs lacking the ms^{2}i^6 modification in *E. coli* (2, 10, 22) or the i^6 modification in *Saccharomyces cerevisiae* (15), which also reads codons beginning with U such as UAA, UAG, or UGA, are weaker suppressors, possibly because of the loss of stabilization afforded by the stacking of ms^{2}i^{6}A or i^{6}A on top of the U:A base pair (35). The interesting aspect of our results is that while the efficiency of suppression of nonsense codons depends upon competition between suppressor tRNAs and release factors at the ribosomal A site (20) and is a measure of the activity of the tRNA at the A site, the initiation activity that we are studying is a measure of the activity of the tRNA at the ribosomal P site.

If the reduced activity of the U35A36 mutant initiator tRNA in the *miaA* strain is due to a weakened codon-anticodon interaction, it is interesting that overproduction of IF3 does not compensate for this (Table 2). It is thought that IF3, bound to the 30S ribosomal subunit, uses the anticodon stem and loop to discriminate initiator tRNA from other tRNAs and to ensure correct codon-anticodon interactions at the P site (13). It is possible that initiation from UAG does not involve IF3, although this is unlikely, given the fact that the U35A36 mutant initiator tRNA and the UAG codon have all the features identified to be important for IF3 recognition (13). The other possibility is that IF3 levels and concentrations in vivo, relative to those of free ribosomal 30S subunits, are already saturating (14). Consequently, further increases in IF3 do not have any effect.

Our finding that the U35A36 mutant initiator tRNA contains ms^{2}i^{6}A is consistent with the suggested requirements for the presence of this base modification at position 37 of *E. coli* tRNAs (33). In previous work, missense and UGA suppressor mutants of *E. coli* glycine tRNA, which have A36 instead of C36, and ochre suppressor mutants of lysine tRNA, which have A36 instead of U36, were shown to have acquired an ms^{2}i^6 modification of A37 (4, 24). The current work shows that a mutant derived from an initiator tRNA can also be recognized by the enzymes responsible for biosynthesis of ms^{2}i^{6}A (1).

The U35A36 mutant initiator tRNA and the tyrosine tRNA lacking the ms^{2}i^6 modification can be separated clearly from the corresponding tRNAs containing it by polyacrylamide gel electrophoresis (Fig. 2). This finding could be useful for routine analysis of the effect of chromosomal mutations or the effect of mutations in a tRNA gene on the presence or absence of certain base modifications in a tRNA. In studies of the effect of mutations on aminoacyl-tRNA synthetase recognition of tRNAs (reviewed in references 18 and 28), the efficiency of suppression of nonsense codons is often used as a measure of the aminoacylation of the tRNA in vivo. However, in vivo suppression efficiency is determined not only by the concentration of the aminoacylated form of the suppressor tRNA but also by the ribosomal activity of the suppressor tRNA (19, 25, 36). Since the presence or absence of certain base modifications (for example, ms^{2}i^{6}A) can affect the ribosomal activity of a tRNA, it is important to know whether a mutation that was introduced affects the base modification of the tRNA, particularly in and around the anticodon.

Finally, the work described in this article provides an excellent example of the application of electrospray ionization mass spectrometry in the analysis of such macromolecules as tRNAs (16). The measured molecular mass of 24,898.2 Da for the U35A36 mutant initiator tRNA isolated from the *miaA* strain is remarkably close to the value of 24,896 Da expected on the basis of the tRNA sequence. The observed difference of 114.6

Da in the molecular masses of the U35A36 mutant tRNAs isolated from the *miaA*$^+$ and *miaA* strains is also strikingly close to the expected difference of 114.2 Da. This closeness is all the more remarkable considering that this difference was calculated on the basis of two very large experimentally measured values, 24,898.2 and 25,012.8 Da.

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REFERENCES

1. **Björk, G. R.** 1995. Biosynthesis and function of modified nucleosides, p. 165–205. *In* D. Söll and U. L. RajBhandary (ed.), tRNA: structure, biosynthesis, and function. American Society for Microbiology, Washington, D.C.
2. **Bouadloun, F., T. Srichaiya, L. A. Isaksson, and G. R. Björk.** 1986. Influence of modification next to the anticodon in tRNA on codon context sensitivity of translational suppression and accuracy. J. Bacteriol. **166:**1022–1027.
3. **Brombach, M., and C. L. Pon.** 1987. The unusual translational initiation codon AUU limits the expression of the *infC* (initiation factor IF3) gene of *Escherichia coli*. Mol. Gen. Genet. **208:**94–100.
4. **Carbon, J., and E. W. Fleck.** 1979. Genetic alteration of structure and function in glycine transfer RNA of *Escherichia coli*: mechanism of suppression of the tryptophan synthetase A78 mutation. J. Mol. Biol. **85:**371–391.
5. **Crain, P. F.** 1990. Preparation and enzymatic hydrolysis of DNA and RNA for mass spectrometry. Methods Enzymol. **193:**782–790.
6. **Donahue, T. F., A. M. Cigan, E. K. Pabich, and B. C. Valavicius.** 1988. Mutations at a Zn(II) finger motif in the yeast e1F-2β gene alter ribosomal start-site selection during the scanning process. Cell **54:**621–632.
7. **Dube, S. K., K. A. Marcker, B. F. C. Clark, and S. Cory.** 1968. Nucleotide sequence of N-formyl-methionyl-transfer RNA. Nature (London) **218:**232–233.
8. **Eisenberg, S. P., M. Yarus, and L. Soll.** 1979. The effect of an *Escherichia coli* regulatory mutation on transfer RNA structure. J. Mol. Biol. **135:**111–126.
9. **Ferrige, A. G., M. J. Seddon, B. N. Green, S. A. Jarvis, and J. Skilling.** 1992. Disentangling electrospray spectra with maximum entropy. Rapid Commun. Mass Spectrom. **6:**707–711.
10. **Gefter, M. L., and R. L. Russell.** 1969. Role of modifications in tyrosine transfer RNA: a modified base affecting ribosome binding. J. Mol. Biol. **39:**145–157.
11. **Gualerzi, C., and C. L. Pon.** 1990. Initiation of mRNA translation in prokaryotes. Biochemistry **29:**5881–5889.
12. **Harada, F., H. J. Gross, F. Kimura, S. H. Chang, S. Nishimura, and U. L. RajBhandary.** 1968. 2-methylthio-N^6-(Δ^2-isopentenyl) adenosine: a component of *E. coli* tyrosine transfer RNA. Biochem. Biophys. Res. Commun. **33:**299–306.
13. **Hartz, D., J. Binkley, T. Hollingsworth, and L. Gold.** 1990. Domains of initiator tRNA and initiation codon crucial for initiator tRNA selection by *Escherichia coli* IF3. Genes Dev. **4:**1790–1800.
14. **Hershey, J. W. B.** 1987. Protein synthesis, p. 613–647. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
15. **Laten, H., J. Gorman, and R. M. Bock.** 1978. Isopentenyladenosine deficient tRNA from an antisuppressor mutant of *Saccharomyces cerevisiae*. Nucleic Acids Res. **5:**4329–4342.
16. **Limbach, P. A., P. F. Crain, and J. A. McCloskey.** 1995. Molecular mass measurement of intact ribonucleic acids via electrospray ionization quadrupole mass spectrometry. J. Am. Soc. Mass Spectrom. **6:**27–39.
17. **Mangroo, D., and U. L. RajBhandary.** Mutants of *Escherichia coli* initiator tRNA defective in initiation: effect of overproduction of methionyl-tRNA transformylase and the initiation factors IF2 and IF3. J. Biol. Chem., in press.
18. **McClain, W. H.** 1993. Rules that govern tRNA identity in protein synthesis. J. Mol. Biol. **234:**257–280.
19. **McClain, W. H., J. Schneider, and K. Gabriel.** 1994. Distinctive acceptor-end structure and other determinants of *Escherichia coli* tRNA^{Pro} identity. Nucleic Acids Res. **22:**522–529.
20. **Murgola, E. J.** 1995. Translational suppression: when two wrongs DO make a right, p. 491–509. *In* D. Söll and U. L. RajBhandary (ed.), tRNA: structure, biosynthesis, and function. American Society for Microbiology, Washington, D.C.
21. **Nishimura, S.** 1979. Modified nucleosides in tRNA, p. 59–79. *In* P. R. Schimmel, D. Söll, and J. N. Abelson (ed.), Transfer RNA: structure, properties, and recognition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Petrullo, L. A., P. J. Gallagher, and D. Elseviers.** 1983. The role of 2-methylthio-N^6-isopentenyladenosine in readthrough and suppression of nonsense codons in *Escherichia coli*. Mol. Gen. Genet. **190:**289–294.
23. **Pomerantz, S. C., and J. A. McCloskey.** 1990. Analysis of RNA hydrolyzates by liquid chromatography-mass spectrometry. Methods Enzymol. **193:**796–824.
24. **Prather, N. E., E. J. Murgola, and B. H. Mims.** 1983. *supG* and *supL* in *Escherichia coli* code for mutant lysine tRNAs. Nucleic Acids Res. **11:**8283–8286.
25. **Raftery, L. A., and M. Yarus.** 1987. Systematic alterations in the anticodon arm make tRNA^{Glu}-Su$_{oc}$ a more efficient suppressor. EMBO J. **6:**1499–1506.
26. **RajBhandary, U. L.** 1994. Initiator transfer RNAs. J. Bacteriol. **176:**547–552.
27. **RajBhandary, U. L., and A. Kumar.** 1970. A formylatable methionine transfer RNA of yeast: comparison of coding properties and sequences around the anticodon with *E. coli* formylmethionine transfer RNA. J. Mol. Biol. **50:**707–711.
28. **Saks, M. E., J. R. Sampson, and J. Abelson.** 1994. The transfer RNA identity problem: a search for rules. Science **263:**191–197.
29. **Seong, B. L., and U. L. RajBhandary.** 1987. *Escherichia coli* formylmethionine tRNA: mutations in G-G-G:C-C-C sequence conserved in anticodon stem of initiator tRNAs affect initiation of protein synthesis and conformation of anticodon loop. Proc. Natl. Acad. Sci. USA **84:**334–338.
30. **Sherman, F., and J.-W. Stewart.** 1982. Mutations altering initiation of yeast iso-1-cytochrome C: contrasts between the eukaryotic and procaryotic initiation process, p. 301–333. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Smith, D., and M. Yarus.** 1989. tRNA-tRNA interactions within cellular ribosomes. Proc. Natl. Acad. Sci. USA **86:**4397–4401.
32. **Sprinzl, M., N. Dank, S. Nock, and A. Schön.** 1991. Compilation of tRNA and tRNA gene sequences. Nucleic Acids Res. **19:**2127–2171.
33. **Tsang, T. H., M. Buck, and B. N. Ames.** 1983. Sequence specificity of tRNA-modifying enzymes: an analysis of 258 tRNA sequences. Biochim. Biophys. Acta **741:**180–196.
34. **Tsui, H.-C. T., H.-C. E. Leung, and M. E. Winkler.** 1994. Characterization of broadly pleiotropic phenotypes caused by a *hfq* insertion mutation in *E. coli* K12. Mol. Microbiol. **13:**35–49.
35. **Vacher, J., H. Grosjean, C. Houssier, and R. H. Buckingham.** 1984. The effect of point mutations affecting *Escherichia coli* tryptophan tRNA on anticodon-anticodon interactions and on UGA suppression. J. Mol. Biol. **177:**329–342.
36. **Varshney, U., C.-P. Lee, and U. L. RajBhandary.** 1991. Direct analysis of aminoacylation levels of tRNAs *in vivo*. J. Biol. Chem. **266:**24712–24718.
37. **Varshney, U., C.-P. Lee, B. L. Seong, and U. L. RajBhandary.** 1991. Mutants of initiator tRNA that function both as initiators and elongators. J. Biol. Chem. **266:**18018–18024.
38. **Varshney, U., and U. L. RajBhandary.** 1990. Initiation of protein synthesis using a termination codon. Proc. Natl. Acad. Sci. USA **87:**1586–1590.
39. **Yanofsky, C., and L. Soll.** 1977. Mutations affecting tRNA^{Trp} and its charging and their effect on regulation of transcription termination at the attenuator of the tryptophan operon. J. Mol. Biol. **113:**663–677.