

# The Mere Lack of rT Modification in Initiator tRNA Does Not Facilitate Formylation-Independent Initiation in *Escherichia coli*

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**Formylation of initiator methionyl-tRNA is essential for normal growth of eubacteria. However, under special conditions, it has been possible to initiate protein synthesis with unformylated initiator tRNA even in eubacteria. Earlier studies suggested that the lack of ribothymidine (rT) modification in initiator tRNA may facilitate initiation in the absence of formylation. In this report we show, by using *trmA* strains of *Escherichia coli* (defective for rT modification) and a sensitive in vivo initiation assay system, that the lack of rT modification in the initiators is not sufficient to effect formylation-independent initiation of protein synthesis.**

In stark contrast to its unformylated counterparts in archaea and eukarya, initiation of protein synthesis in eubacteria, mitochondria, and chloroplasts occurs with formylmethionyl (fMet)-tRNA (10). Therefore, this distinct mechanism of initiation with fMet-tRNA in eubacteria has been of interest for over three decades (1, 3, 5, 8, 12, 14, 18, 22, 23, 24, 25, 26). Formylation of the initiator tRNAs in eubacteria utilizes formyltetrahydrofolate (fTHF) as a cofactor, and this modification has been shown to be crucial in initiation of protein synthesis (10, 15). Nevertheless, under special conditions, it has been possible to initiate protein synthesis with unformylated initiator tRNA even in eubacteria. For instance, with the use of inhibitors of the folate biosynthesis pathway such as trimethoprim or aminopterin it was possible to deplete *Streptococcus faecalis* and *Bacillus subtilis* of fTHF. Growth under these conditions required supplementation of the medium with the end products of the other metabolic pathways that also utilize fTHF as a cofactor. Interestingly, these bacteria grew essentially normally even in the presence of the inhibitors (2, 18). Modified base analysis of the initiator tRNA from folate-deficient bacteria showed the lack of ribothymidine (rT) modification at position 54 in the highly conserved T $\psi$ C sequence (5). This observation led to a suggestion that the lack of rT modification could somehow lead to formylation-independent initiation in gram-positive bacteria (18). Subsequently, it was shown that at least in *B. subtilis*, the U<sup>54</sup>-methyltransferase uses fTHF as a cofactor (6, 7, 16). Thus, the lack of rT in tRNA could simply be a consequence of depletion of fTHF in these bacteria when grown in the presence of trimethoprim or aminopterin. Studies along these lines in *Escherichia coli* resulted in the isolation of a mutant, which was partially deficient in rT modification, and reciprocated with poor growth in the presence of the inhibitors of fTHF biosynthesis (3). Since in *E. coli* S-adenosylmethionine (rather than fTHF) is used as a cofactor by the U<sup>54</sup>-methyltransferase (19), the lack of rT modification could be implicated in formylation-independent initiation. This interpretation is consistent with the observation that in the

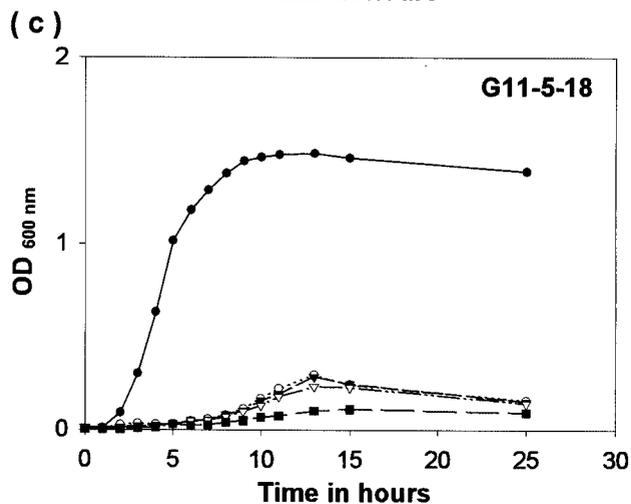
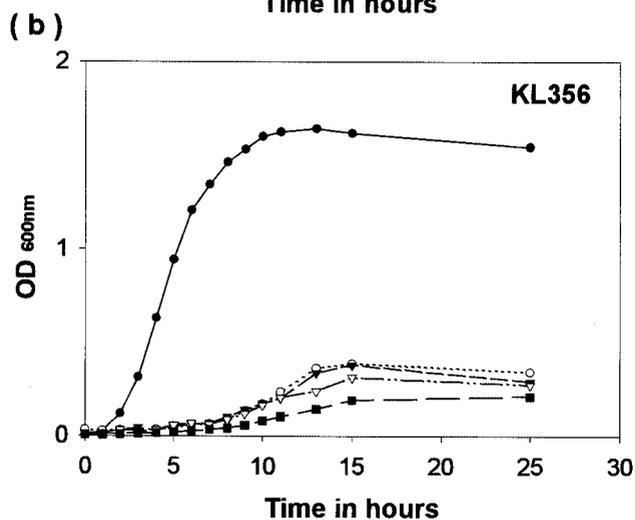
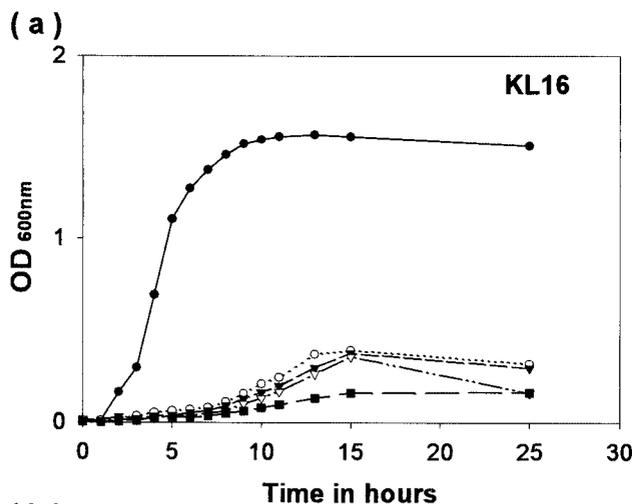
eukaryotic initiators, which are naturally used in their unformylated state, position 54 is not a U, and consequently these tRNAs lack rT at this position (22).

To study the role of the absence and/or deficiency of rT modification in the initiator tRNAs in formylation-independent initiation in eubacteria, we exploited the *trmA* strains of *E. coli* (defective for rT modification) by two approaches. First, we analyzed the growth phenotypes of these strains in the presence of the inhibitors of fTHF biosynthesis. In the second approach these strains were used to analyze initiation activities of the formylation-defective initiator tRNAs in a highly sensitive initiation assay system (27). The latter approach allowed us to circumvent the use of folate biosynthesis inhibitors in discerning the importance of the lack of the rT modification in initiator tRNA in initiation.

**Effect of folate biosynthesis inhibitors on growth of *E. coli trmA* strains.** Two of the *trmA* strains, KL356 (*trmA14*) and G11-5-18 (*trmA5*), defective in U<sup>54</sup> methyltransferase (4, 13) were obtained from *E. coli* Genetic Stock Center and grown in Luria-Bertani (LB) medium (supplemented with Gly, Ser, Met, adenosine, thymidine, and pantothenate [3]) in the absence or presence of various concentrations of the folate biosynthesis inhibitors (sulfathiazole and trimethoprim). As seen in Fig. 1, the wild-type (*trmA*<sup>+</sup>) or the *trmA* strains grew equally well in the absence of the drugs (panels a to c). Expectedly, the strain KL16 (*trmA*<sup>+</sup>) grew very slowly in the presence of the inhibitors (panel a). In contrast to the general expectation, even the *trmA* strains (KL356 and G11-5-18) grew very slowly in the presence of the folate biosynthesis inhibitors. In fact, the *trmA* strains were just as sensitive to the drugs as the wild-type strain, suggesting that the lack of rT modification in tRNA did not confer any growth advantage against the inhibitors. The minimal growth of all of the strains (to similar extent) at all concentrations of inhibitors suggests that it was most likely due to the pools of fTHF present in the cells prior to the addition of the inhibitors (Fig. 1a to c).

**Use of in vivo initiation assay to study initiation in *E. coli (trmA)* with formylation-defective initiator tRNAs.** The initiator tRNA mutant carrying a CUA anticodon change (U35A36 mutations) initiates protein synthesis from UAG as an initiation codon of the chloramphenicol acetyltransferase (CAT) reporter and confers chloramphenicol resistance (Cm<sup>r</sup>) to the

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●— No inhibitors  
 ○--- 50 mg each of trimethoprim and sulfathiazole  
 ▼--- 75 mg each of trimethoprim and sulfathiazole  
 △--- 100 mg each of trimethoprim and sulfathiazole  
 ■--- 200 mg each of trimethoprim and sulfathiazole

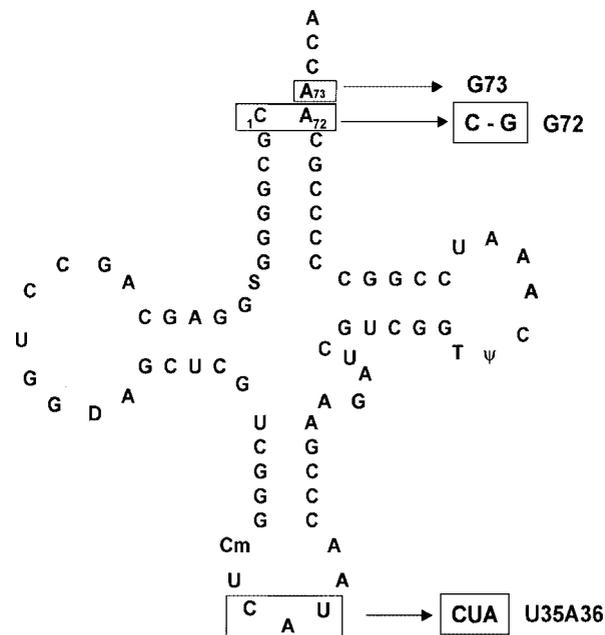


FIG. 2. Cloverleaf structure of the *E. coli* initiator  $tRNA_2^{Met}$ , indicating the sites of mutations (left panel), and the binary plasmid system used for the in vivo assay system (right panel). Plasmid pCAT<sub>am1</sub>metY<sub>CUA</sub> harbors the  $tRNA^{Met}$  (U53A36, G72/U53A36, or G72G73/U53A36) and the reporter CATam1 genes, whereas plasmid pACQS harbors the *E. coli* glutaminyl-tRNA synthetase gene.

host (27). Introduction of the G72, or G72G73 mutations, into the U53A36 background renders these tRNAs (G72/U53A36 and G72G73/U53A36 [Fig. 2, left panel]) inactive in initiation because of their defect at the step of formylation (11). While the G72/U53A36 tRNA is more than 475-fold worse than the U53A36 tRNA in formylation, the G72G73 tRNA is still worse, and its formylation cannot be detected in vitro. In addition, the G72 substitution creates a 1:72 bp, which may make the tRNA a substrate for peptidyl-tRNA hydrolase (9). Thus, in vivo, there is no accumulation of the formylated forms of these tRNAs (23, 26).

Both of the *trmA* *E. coli* strains KL356 (*trmA14*) and G11-5-18 (*trmA5*), defective in U<sup>54</sup>-methyltransferase, and the KL16 strain (*trmA*<sup>+</sup>) were transformed with the constructs harboring the formylation-defective tRNA mutants on the plasmids pCATam1metY<sub>CUA</sub>(G72) or pCATam1metY<sub>CUA</sub>(G72G73). As these tRNAs are aminoacylated by Gln, glutamine tRNA-synthetase (GlnRS) was overexpressed from a compatible plasmid (pACQS, Kan<sup>r</sup>) to help ensure their efficient aminoacylation (Fig. 2, right panel [27]). The initiation activity of these tRNAs was examined by their growth on chloramphenicol plates and by the detection of the reporter

FIG. 1. Growth of *E. coli* KL16 (*trmA*<sup>+</sup>), KL356 (*trmA14*), and G11-5-18 (*trmA5*) strains (a, b, and c, respectively) in LB medium (17) supplemented with Gly (45 mg/liter), Met (50 mg/liter), Ser (50 mg/liter), adenosine (28 mg/liter), thymidine (4 mg/liter), and calcium pantothenate (0.4 mg/liter), in the absence or presence of the folate biosynthesis inhibitors as shown (3). Amounts of trimethoprim and sulfathiazole are per liter.

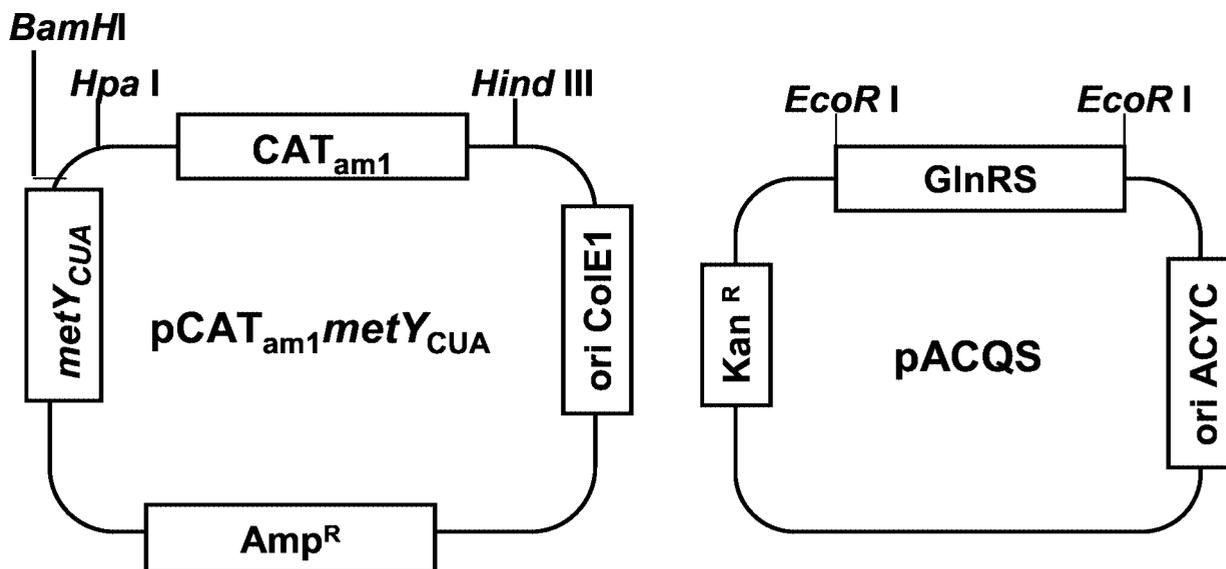
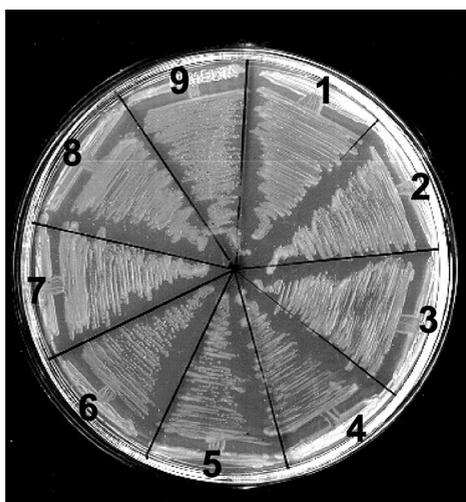


FIG. 2—Continued.

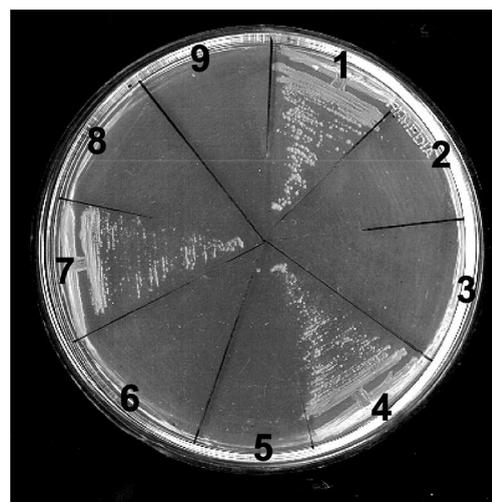
CAT protein in the cell extracts by enzyme assays and immunoblotting.

Expectedly, all of the three strains of *E. coli*, namely, KL16 (*trmA*<sup>+</sup>), KL356 (*trmA*), and G11-5-18 (*trmA*) harboring the formylation-proficient tRNA (U35A36) on the CATam1 reporter plasmid (ampicillin resistant [Amp<sup>r</sup>]) showed growth on plates containing ampicillin and kanamycin, as well as on plates containing ampicillin, kanamycin, and chloramphenicol (Fig. 3, sectors 1, 4, and 7). On the other hand, the transformants harboring the formylation-defective tRNAs (G72/

U35A36 and G72G73/U35A36) grew on the plates containing ampicillin and kanamycin but failed to grow on the plates containing ampicillin, kanamycin, and chloramphenicol (sectors 2, 5, and 8 and sectors 3, 6, and 9, respectively). Consistent with the growth phenotype on the chloramphenicol plates, the transformants harboring the formylation-defective tRNAs showed only the background level (~0.07 to 0.15%) CAT activity with respect to the U35A36 tRNA (Table 1) irrespective of the nature of the *trmA* allele. To ascertain further that the formylation-defective initiator tRNAs failed to initiate in



**Kan + Amp**



**Kan + Amp + Cm**

FIG. 3. *E. coli* transformants were streaked onto 2YT agar plates containing ampicillin and kanamycin (Kan + Amp) or ampicillin, kanamycin, and chloramphenicol (Kan + Amp + Cm) and incubated at 37°C for 18 h. The transformants of the strains KL16 (sectors 1 to 3), KL356 (sectors 4 to 6), and G11-5-18 (sectors 7 to 9) contained the following plasmids: sectors 1, 4, and 7, pCAT<sub>am1</sub>metY<sub>CUA</sub> and pACQS; sectors 2, 5, and 8, pCAT<sub>am1</sub>metY<sub>CUA</sub> (G72) and pACQS; and sectors 3, 6, and 9, pCAT<sub>am1</sub>metY<sub>CUA</sub> (G72G73) and pACQS. Antibiotic concentrations were as follows: ampicillin, 100 μg/ml; kanamycin, 25 μg/ml; and chloramphenicol, 30 μg/ml.

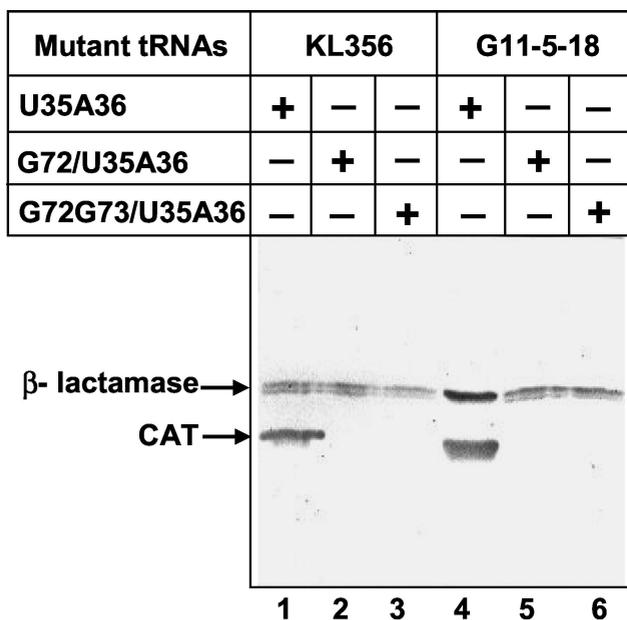


FIG. 4. The cell extracts (10  $\mu$ g of total protein) prepared from log-phase cultures were fractionated on a sodium dodecyl sulfate-polyacrylamide gel (12%) and electroblotted onto a polyvinylidene difluoride membrane. The membrane was probed with anti-CAT and anti- $\beta$ -lactamase rabbit antibodies, and the signals were detected with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G by using BCIP (5-bromo-4-chloro-3-indolylphosphate) and nitroblue tetrazolium (17).

the *trmA* strains of *E. coli*, we performed immunoblot analysis (Fig. 4). In agreement with the phenotypic and the CAT assays, the band corresponding to CAT was seen only in the transformants containing the formylation-proficient initiator (lanes 1 and 4) and not in the ones harboring formylation-defective tRNAs (lanes 2 and 3 and lanes 5 and 6). However, a band corresponding to  $\beta$ -lactamase ( $Amp^r$ ) was seen in all of the lanes confirming the presence of pCATam1*metY*<sub>CUA</sub> (G72; G72G73) plasmids. Importantly, this analysis ruled out overproduction of CAT in inactive form. Furthermore, the analysis of the total tRNA from these transformants by using acid urea gel Northern blots (26) showed that the mature tRNA corresponding to the G72/U35A36, as well as the G72G73/U35A36, tRNA was produced and aminoacylated in the cell (Fig. 5). As expected, the U35A36 tRNA was present predominantly in the formylated form (lanes 1 and 4), and the G72/U35A36 and G72G73/U35A36 tRNAs were detected in aminoacylated and

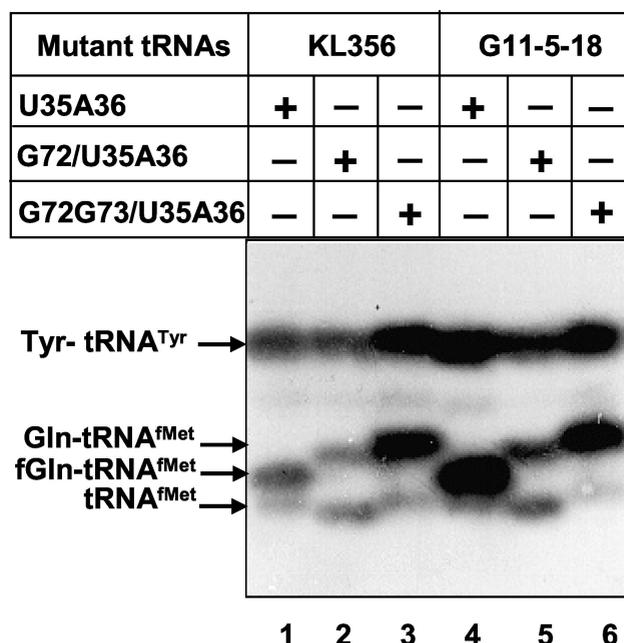


FIG. 5. Northern blot analysis. Total tRNA from various transformants (*trmA*) was isolated under acidic conditions, separated on acid urea gels, and electroblotted onto a Nytran membrane. The blots were hybridized to 5' <sup>32</sup>P-labeled oligonucleotides complementary to positions 29 to 47 of the tRNA<sub>2</sub><sup>fMet</sup> (U35A36) and to positions 2 to 44 of *E. coli* tRNA<sup>Tyr</sup> (26).

uncharged forms (lanes 2 and 5 and lanes 3 and 6, respectively). Our other studies showed that the CATam1 reporter gene in the G72/U35A36 or the G72G73/U35A36 tRNA encoding plasmids retains an intact reading frame with UAG as an initiation codon (24, 25). Furthermore, as the initiation factor 2 (IF2) or its equivalents in eukaryotes and archaea are important in the utilization of initiator tRNAs in initiation, in yet another control experiment, we measured initiation activity of the G72G73/U35A36 tRNA in these strains by CAT assays in the presence of IF2 overproduction. While there was a marginal increase in the initiation activity of this tRNA in all of the three strains, its activity in the *trmA* strains (KL356 and G11-5-18) did not increase beyond that of the *trmA*<sup>+</sup> control strain (KL16) (data not shown).

To ensure that the *trmA* strains were true to their genotypes, we carried out modified base analysis of the formylation-defective tRNAs (Fig. 6). The tRNAs isolated from the wild-type (*trmA*<sup>+</sup>) background contained rT modification (Fig. 6b and c,

FIG. 6. Modified base analysis. Fresh overnight cultures of the various transformants were subcultured (1% inoculum) in 5 ml of 2YT medium containing ampicillin (100  $\mu$ g/ml) and kanamycin (25  $\mu$ g/ml) and grown at 37°C with constant aeration for 4 h. The cells were collected by centrifugation, suspended in 1 ml of low-phosphate medium (20), supplemented with 500  $\mu$ Ci of [<sup>32</sup>P]orthophosphate, and incubated at 37°C for 1 h. Initiator tRNA was purified from total tRNA preparation by electrophoresis on a 15% preparative polyacrylamide gel under non-denaturing conditions. The <sup>32</sup>P-labeled tRNA (20,000 cpm) were treated with nuclease P1 (1  $\mu$ g) in 20  $\mu$ l of 50 mM ammonium acetate buffer (pH 5.3) at 37°C for 5 h, mixed with similarly digested yeast total RNA (5  $\mu$ g), and vacuum dried. Traces of ammonium acetate were eliminated by vacuum drying the contents twice more after dissolving them in 20  $\mu$ l of water each time. The contents were finally taken up in 2  $\mu$ l of water and applied onto cellulose thin-layer plates. Two-dimensional chromatography was performed by using isobutyric acid-water-ammonium hydroxide in a 66:33:1 (vol/vol/vol) ratio as the solvent for the first dimension and 0.1 M sodium phosphate (pH 7.2)-ammonium sulfate-*n*-propanol in a 100:60:2 (vol/wt/vol) ratio as the solvent for the second dimension exactly as described previously (21). The panels show a standard pattern (a) or a modified base analysis of U35A36 (b, d, and f) or G72G73/U35A36 (c, e, and g) tRNAs from KL16 (b and c), KL356 (d and e), and G11-5-18 (f and g) strains. The position of rT is indicated by arrows in panels b and c. rT is absent in panels d to e.

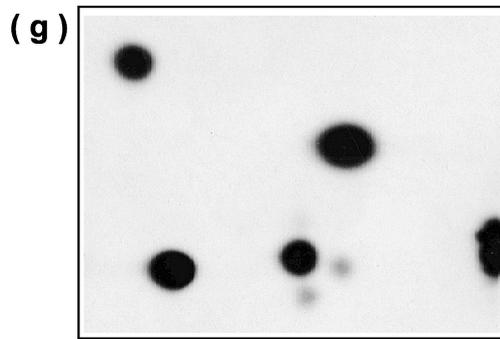
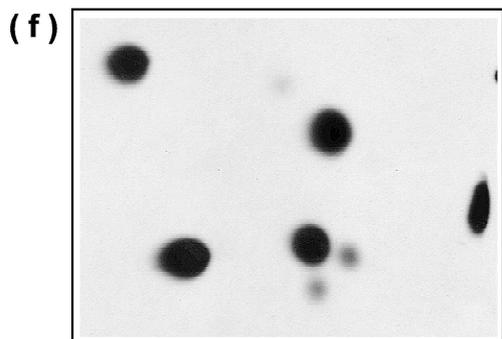
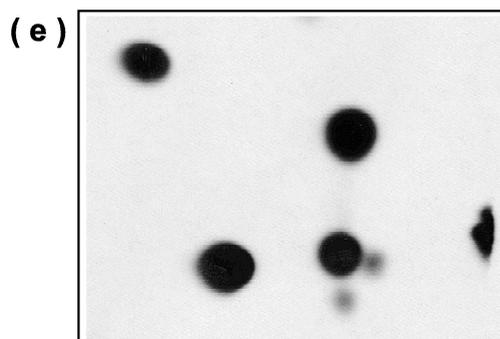
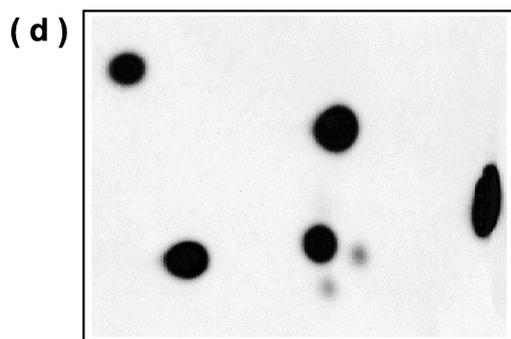
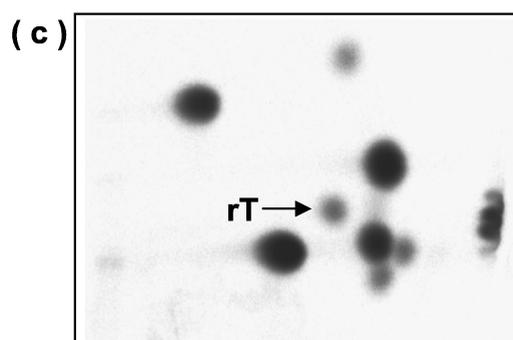
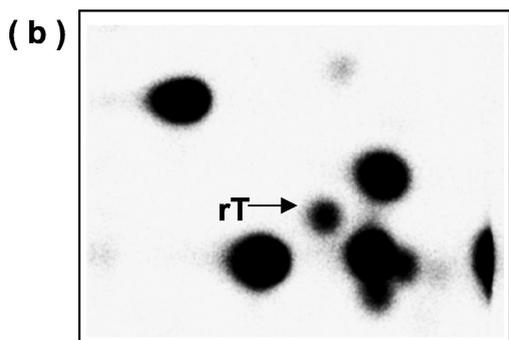
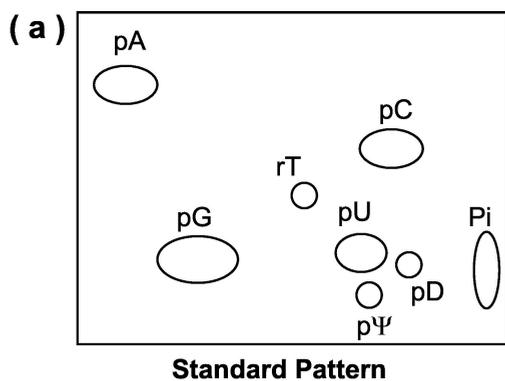


TABLE 1. Relative CAT activities in cell extracts of various transformants containing formylation-proficient (U35A36) or-defective (G72/U35A36 and G72G73/U35A36) tRNAs, along with the reporter CATam1 gene, in the strains of *E. coli* wild type (KL16) or mutants containing different *trmA* alleles (KL356 and G11-5-18)<sup>a</sup>

tRNA	Relative CAT activities (%) for strain:		
	KL16	KL356	G11-5-18
U35A36	100.00	71.00	80.57
G72/U35A36	0.15	0.08	0.11
G72G73/U35A36	0.09	0.14	0.14

<sup>a</sup> The CAT activities were normalized for any possible plasmid copy number variations among the various transformants with respect to  $\beta$ -lactamase levels (taken as the unit for the strain KL16). One-hundred percent activity in KL16 corresponded to 1,287 pmol of chloramphenicol converted to acetyl chloramphenicol per min per  $\mu$ g of total cellular proteins at 37°C

as shown by arrows), whereas those isolated from the *trmA* strains lacked this modification (Fig. 6d to g, compare with Fig. 6b and c).

Thus, our results clearly show that even though the formylation-defective tRNAs (G72/U35A36 and G72G73/U35A36) accumulated in the aminoacylated form and lacked rT modification, they failed to initiate protein synthesis. The inability of the *trmA* strains to accrue any growth advantage in the presence of folate biosynthesis inhibitors on the one hand, and the failure to support initiation with the formylation-defective initiator tRNAs on the other, shows that the absence of rT modification is not sufficient for formylation-independent initiation.

Recently, formylation-independent initiation was effected in *E. coli* (8, 14), *Pseudomonas aeruginosa* (14) and yeast mitochondria (12) by disruption of the formylase gene. Although the modification status of the initiator tRNA was not analyzed in any of these studies, the fact that the methyltransferase gene responsible for the conversion of U54 to T54 was not mutated suggested that the rT modification occurred in the initiator tRNA (12). Thus, taken together, these studies suggest that requirement of the lack of rT modification in the initiators is neither absolute nor sufficient for formylation-independent initiation of protein synthesis in *E. coli*. Our interpretation, however, does not rule out a possible role of the lack of rT modification in formylation-independent initiation in conjunction with mutations at some other location(s) in the *E. coli* chromosome. In fact, this may well explain the phenotype of the *E. coli* mutant that initiated independent of formylation and showed partial lack of rT modification (3).

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S.T. and T.K.D. contributed equally to this study.

#### REFERENCES

- Adams, J. M., and M. R. Capocchi. 1966. N-formyl-methionyl-s-tRNA as the initiator of protein synthesis. Proc. Natl. Acad. Sci. USA **55**:147–155.
- Arnold, H. 1977. Initiation of protein synthesis in *Bacillus subtilis* in the presence of trimethoprim or aminopterin. Biochim. Biophys. Acta **476**:76–87.
- Baumstark, B. R., L. L. Spemulli, U. L. RajBhandary, and G. M. Brown. 1977. Initiation of protein synthesis without formylation in a mutant of *Escherichia coli* that grows in the absence of tetrahydrofolate. J. Bacteriol. **129**:457–471.
- Bjork, G. R., and L. A. Isaksson. 1970. Isolation of mutants of *Escherichia coli* lacking 5-methyluracil in transfer ribonucleic acid or 1-methylguanine in ribosomal RNA. J. Mol. Biol. **51**:83–100.
- Delk, A. S., and J. C. Rabinowitz. 1974. Partial nucleotide sequence of a prokaryote initiator tRNA that functions in its non-formylated form. Nature **252**:106–109.
- Delk, A. S., and J. C. Rabinowitz. 1975. Biosynthesis of ribosylthymine in the transfer RNA of *Streptococcus faecalis*: a folate-dependent methylation not involving S-adenosylmethionine. Proc. Natl. Acad. Sci. USA **72**:528–530.
- Delk, A. S., J. M. Romeo, D. P. Nagle, Jr., and J. C. Rabinowitz. 1976. Biosynthesis of ribothymidine in the transfer RNA of *Streptococcus faecalis* and *Bacillus subtilis*. A methylation of RNA involving 5,10-methylenetetrahydrofolate. J. Biol. Chem. **251**:7649–7656.
- Guillon, J.-M., Y. Mechulam, J.-M. Schmitter, S. Blanquet, and G. Fayat. 1992. Disruption of the gene for Met-tRNA<sup>Met</sup> formyltransferase severely impairs growth of *Escherichia coli*. J. Bacteriol. **174**:4294–4301.
- Kosel, H., and U. L. RajBhandary. 1968. Studies on polynucleotides. LXXXVI. Enzymic hydrolysis of N-acylaminoacyl-transfer RNA. J. Mol. Biol. **35**:539–560.
- Kozak, M. 1983. Comparison of initiation of protein synthesis in prokaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
- Lee, C. P., B. L. Seong, and U. L. RajBhandary. 1991. Structural and sequence elements important for recognition of *Escherichia coli* formylmethionine tRNA by methionyl-tRNA transformylase are clustered in the acceptor stem. J. Biol. Chem. **266**:18012–18017.
- Li, Y., W. B. Holmes, D. R. Appling, and U. L. RajBhandary. 2000. Initiation of protein synthesis in *Saccharomyces cerevisiae* mitochondria without formylation of the initiator tRNA. J. Bacteriol. **182**:2886–2892.
- Marinus, M. G., N. R. Morris, D. Soll, and T. C. Kwong. 1975. Isolation and partial characterization of three *Escherichia coli* mutants with altered transfer ribonucleic acid methylases. J. Bacteriol. **122**:257–265.
- Newton, D. T., C. Creuzenet, and D. Mangroo. 1999. Formylation is not essential for initiation of protein synthesis in all eubacteria. J. Biol. Chem. **274**:22143–22146.
- RajBhandary, U. L. 1994. Initiator transfer RNAs. J. Bacteriol. **176**:547–552.
- Romeo, J. M., A. S. Delk, and J. C. Rabinowitz. 1974. The occurrence of a transmethylation reaction not involving S-adenosylmethionine in the formation of ribothymidine in *Bacillus subtilis* transfer-RNA. Biochem. Biophys. Res. Commun. **61**:1256–1261.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Samuel, C. E., and J. C. Rabinowitz. 1974. Initiation of protein synthesis by folate-sufficient and folate-deficient *Streptococcus faecalis* R. Biochemical and biophysical properties of methionine transfer ribonucleic acid. J. Biol. Chem. **249**:1198–1206.
- Santi, D. V., and L. W. Hardy. 1987. Catalytic mechanism and inhibition of tRNA (uracil-5-) methyltransferase: evidence for covalent catalysis. Biochemistry **26**:8599–8606.
- Seong, B. L., and U. L. RajBhandary. 1987. *Escherichia coli* formylmethionyl tRNA: mutations in GGGCCC 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.
- Silberklang, M., A. M. Gillum, and U. L. RajBhandary. 1979. Use of *in vitro* <sup>32</sup>P labeling in the sequence analysis of nonradioactive tRNAs. Methods Enzymol. **59**:58–109.
- Sprinzl, M., C. Horn, M. Brown, A. Ioudovitch, and S. Steinberg. 1998. Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res. **26**:148–153.
- Thanedar, S., N. V. Kumar, and U. Varshney. 2000. The fate of the initiator tRNAs is sensitive to the critical balance between interacting proteins. J. Biol. Chem. **275**:20361–20367.
- Thanedar, S. 2000. Use of formylation defective initiator tRNAs in the study of the significance of formylation in initiation of protein synthesis in *Escherichia coli*. Ph.D. thesis. Indian Institute of Science, Bangalore, India.
- Varshney, U., and U. L. RajBhandary. 1992. Role of methionine and formylation of initiator tRNA in initiation of protein synthesis in *Escherichia coli*. J. Bacteriol. **174**:7819–7826.
- Varshney, U., C. P. Lee, and U. L. RajBhandary. 1991. Direct analysis of aminoacylation levels of tRNAs *in vivo*. Application to studying recognition of *Escherichia coli* initiator tRNA mutants by glutaminyl-tRNA synthetase. J. Biol. Chem. **266**:24712–24718.
- Varshney, U., and U. L. RajBhandary. 1990. Initiation of protein synthesis from a termination codon. Proc. Natl. Acad. Sci. USA **87**:1586–1590.