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NOTES

Escherichia coli B Lacks One of the Two Initiator tRNA Species Present in *E. coli* K-12

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We show that the *metY* locus which specifies $tRNA_2^{fMet}$ in *Escherichia coli* K-12 specifies $tRNA_1^{fMet}$ in *E. coli* B. This conclusion is based on results of Southern blot analysis of *E. coli* B and K-12 DNAs and on polymerase chain reaction amplification, cloning, and sequencing of an ~200-bp region of DNA corresponding to the *metY* loci of *E. coli* B and *E. coli* K-12. We also show that the *metY* locus of *E. coli* B is transcriptionally active. *E. coli* strains transformed with the multicopy plasmid vector pUC19 carrying the *metY* locus of *E. coli* B overproduce $tRNA_1^{fMet}$ in *E. coli* B and *E. coli* K-12 in contrast to strains transformed with pUC19 carrying the corresponding locus from *E. coli* K-12, which overproduce $tRNA_2^{fMet}$.

Of the two classes of methionine tRNAs present in all organisms, the initiator, $tRNA^{fMet}$, is used exclusively for initiation of protein synthesis, whereas the elongator is used for inserting methionine into internal peptidic linkages. In eubacteria, mitochondria, and chloroplasts, the initiator is used as formylmethionyl-tRNA (fMet-tRNA) (14). Two isoacceptor species of $tRNA^{fMet}$ are present in *Escherichia coli* K-12. These differ in a single nucleotide at position 46 (Fig. 1). $tRNA_1^{fMet}$, the major species, has m^7G, whereas $tRNA_2^{fMet}$, the minor species, has A (7, 8). $tRNA_1^{fMet}$ is specified by the *metZ* locus, which consists of two tandem $tRNA_1^{fMet}$ genes (13), whereas $tRNA_2^{fMet}$ is specified by the *metY* locus, which is part of an operon in which the $tRNA_2^{fMet}$ gene is the promoter-proximal gene for the major promoter of the *nusA-infB* operon (10). There are indications that the genes for the two $tRNA^{fMet}$ species are regulated differently (17). Transcription from the *metZ* promoter is sensitive to ppGpp, whereas that from the *metY* promoter is not.

Why *E. coli* K-12 contains two isoacceptor $tRNA^{fMet}$ species is unknown. Besides its role in translational initiation, *E. coli* $tRNA^{fMet}$ may have other functions in the cell. The fMet-tRNA is known to bind to *E. coli* RNA polymerase, and this binding affects promoter selectivity of the RNA polymerase (5, 19, 21). Interestingly, the affinity of *E. coli* RNA polymerase for fMet-tRNA is higher than that of the initiation factor IF-2. Thus, there is the possibility that one or both of the fMet-tRNA species regulates transcriptional activity of RNA polymerase towards certain genes. In addition, several lines of evidence indicate that $tRNA_1^{fMet}$ has a more stable structure than $tRNA_2^{fMet}$. These include differences in (i) rates of photo-cross-linking of the modified base S^4U8 to C13, (ii) thermal stability of the dihydrouridine stem, and (iii) nuclear magnetic resonance spectra of the two $tRNA^{fMet}$ species (3, 4, 6). This difference in stability, which is accentuated under conditions of low ionic strength and in the absence of magnesium, may be a consequence of the loss of a tertiary base pair interaction in $tRNA_2^{fMet}$ between the G of the C13-G22 base pair in the D stem and A46 in the variable loop. The two $tRNA^{fMet}$ species also differ in Michaelis constants for aminoacylation (4), the K_m for $tRNA_2^{fMet}$ (5 μM) being higher than that for $tRNA_1^{fMet}$ (1.3 μM).

These differences in the structures and properties of the two $tRNA^{fMet}$ species in *E. coli* K-12 raise the possibilities that one or both of these tRNA species have unique functions in the cell and that both tRNAs are necessary for cell growth and viability of *E. coli*. The results described in this paper indicate that these possibilities are unlikely. We show that *E. coli* B lacks the $tRNA_2^{fMet}$ species and that the *metY* locus of *E. coli* B specifies $tRNA_1^{fMet}$ instead of $tRNA_2^{fMet}$ in *E. coli* K-12.

Northern (RNA) blot analysis of *E. coli* B and K-12 tRNAs. Some indication that *E. coli* B may lack the $tRNA_2^{fMet}$ species came from comparison of chromatographic patterns of *E. coli* B and K-12 tRNAs on RPC-3 columns and the finding that a peak corresponding to $tRNA_2^{fMet}$ in *E. coli* K-12 was missing in *E. coli* B tRNA (personal communication from A. D. Kelmers cited in reference 6). However, this has not been studied further. With the recent finding that the $tRNA^{fMet}$ species could be readily separated by polyacrylamide gel electrophoresis (25), we carried out Northern blot analysis of *E. coli* B and K-12 tRNAs by using a $tRNA^{fMet}$-specific probe (23). This experiment, which allows detection of extremely low levels of $tRNA^{fMet}$, showed that while *E. coli* K-12 tRNA yielded two hybridization bands corresponding to $tRNA_1^{fMet}$ and $tRNA_2^{fMet}$, *E. coli* B tRNA yielded only one band corresponding to $tRNA_1^{fMet}$ (14a).

There are several possible explanations for the absence of $tRNA_2^{fMet}$ in *E. coli* B: (i) the *metY* locus does not specify a tRNA, (ii) the *metY* locus specifies a tRNA but the tRNA gene is transcribed poorly or not at all or $tRNA_2^{fMet}$ is unstable in *E. coli* B, and (iii) the *metY* locus of *E. coli* B specifies $tRNA_1^{fMet}$ instead of $tRNA_2^{fMet}$. We show below that the last of these possibilities is the correct one.

Southern blot analysis of genomic digests of *E. coli* B and K-12 DNAs. Figure 2 shows the results of a Southern blot

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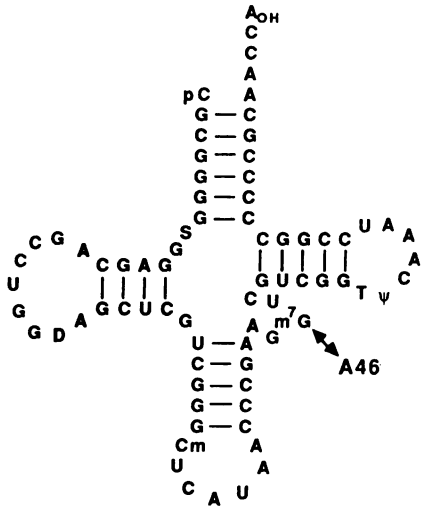

FIG. 1. Cloverleaf structure of *E. coli* $tRNA^{fMet}$. Arrows indicate the single difference between $tRNA_1^{fMet}$ (m^7G46) and $tRNA_2^{fMet}$ (A46) of *E. coli* K-12.

analysis of DNA from *E. coli* B105 and JM103 (an *E. coli* K-12 strain) digested with *Pst*I, *Sau*3A, or a mixture of *Pst*I and *Sau*3A (26). The probe was an ~450-bp DNA fragment which includes the *metY* locus encoding the $tRNA_2^{fMet}$ gene (Fig. 3). The bands that hybridize strongly to the DNA probe correspond to fragments derived from the *metY* locus. An identical-sized hybridization band (~1.9 kb) in *Pst*I digests (lanes 5 and 6) of both *E. coli* B and *E. coli* K-12 DNAs suggests that a tRNA gene related in sequence to $tRNA_2^{fMet}$ of *E. coli* K-12 is present also at the *metY* locus of *E. coli* B. This band also hybridizes to a labeled $tRNA^{fMet}$ probe.

The bands that hybridize weakly in the blot described above correspond most likely to fragments derived from the

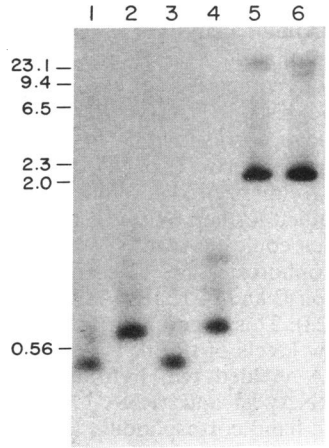

FIG. 2. Southern blot analysis of restriction digests of *E. coli* B105 r_B^- m_B^- Gal^- $RecA^+$ (lanes 2, 4, and 6; obtained from G. W. Walker, Massachusetts Institute of Technology) and *E. coli* K-12 (strain JM103; lanes 1, 3, and 5) DNAs using as probe an ~450-bp fragment containing the *metY* locus and the region bounded by the *Pst*I and *Taq*I sites (Fig. 3). The DNAs were cut with *Pst*I (lanes 5 and 6), *Sau*3A (lanes 3 and 4), or *Pst*I and *Sau*3A (lanes 1 and 2) and electrophoresed on a 1.2% agarose gel. Numbers on the left indicate locations of DNA size markers (λ DNA digested with *Hin*dIII).

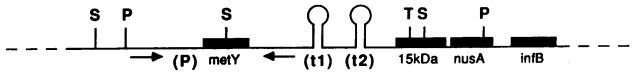

FIG. 3. Organization of the *nusA-infB* operon including the *metY* locus which specifies $tRNA_2^{fMet}$ in *E. coli* K-12. (P), major promoter for the operon; (t1) and (t2), rho-independent transcription termination signals; P, S, and T (*Pst*I, *Sau*3A, and *Taq*I, respectively), locations of restriction sites relevant to this work. Arrows represent the approximate locations of the primers used for PCR amplification of genomic DNA.

metZ locus. This is supported by the fact that in a Southern blot of *Taq*I-*Pst*I digests of *E. coli* B and *E. coli* K-12 DNAs, two identical-sized fragments, ~450 bp corresponding to the *metY* locus and ~1.2 kbp corresponding to the *metZ* locus, were found to hybridize to the probe (data not shown).

The single nucleotide difference at positions 46 in $tRNA_1^{fMet}$ and $tRNA_2^{fMet}$ generates a *Sau*3A site (GATC) in the $tRNA_2^{fMet}$ gene that is absent in the $tRNA_1^{fMet}$ gene. Results of Southern blot analysis (Fig. 2) of *Sau*3A (lanes 3 and 4) or *Pst*I-*Sau*3A double digests (lanes 1 and 2) of *E. coli* B and K-12 DNAs show that *E. coli* K-12 DNA has this *Sau*3A site at the *metY* locus, whereas *E. coli* B DNA does not. The fragment that hybridizes to the DNA probe in digests of *E. coli* B DNA is longer than that in digests of *E. coli* K-12 DNA (compare lane 4 with lane 3 and lane 2 with lane 1). A second fragment of *E. coli* K-12 DNA which is also expected to hybridize to the probe is probably too small (~140 bp) to be retained in the agarose gel used. The sizes of the fragments that hybridize to the probe are as expected on the basis of the known sequence of this region of DNA (10) and distribution of *Sau*3A and *Pst*I restriction sites (22). Thus, while the *metY* locus of *E. coli* B encodes a tRNA which is related to $tRNA^{fMet}$, it does not encode $tRNA_2^{fMet}$.

PCR amplification, cloning, and sequence analysis of the *metY* locus of *E. coli* B and K-12. Two oligonucleotides, each 22 long, flanking the *metY* locus of *E. coli* K-12 (Fig. 3, arrows), were used to amplify this region of *E. coli* B and K-12 DNAs (23) by using polymerase chain reaction (PCR). A predominant PCR product ~220 bp long was obtained in each case. The PCR products were sequenced directly (24). We also cloned and sequenced the cloned DNA. The sequences of the PCR products from *E. coli* B and K-12 were identical (data not shown) except for one change: the A corresponding to A46 of $tRNA_2^{fMet}$ in *E. coli* K-12 (Fig. 4a) was replaced by a G corresponding to m^7G46 of $tRNA_1^{fMet}$ in *E. coli* B (Fig. 4b). Thus, the $tRNA_2^{fMet}$ gene in the *metY* locus of *E. coli* K-12 is replaced by a $tRNA_1^{fMet}$ gene in *E. coli* B.

***metY* locus of *E. coli* B is transcriptionally active.** Figure 5 shows the polyacrylamide gel electrophoresis pattern of tRNAs isolated from *E. coli* B (lane 3), K-12 (lane 2), and K-12 transformed with plasmids carrying PCR-amplified DNAs corresponding to the *metY* loci of *E. coli* B (lane 4) and K-12 (lane 1). Comparison of lane 3 with lane 2 shows that the band corresponding to $tRNA_2^{fMet}$ of *E. coli* K-12 is absent in *E. coli* B. This confirms the conclusion given above that *E. coli* B does not have the $tRNA_2^{fMet}$ species. Similarly, comparison of lane 4 with lane 2 shows that the *metY* locus specifying $tRNA_1^{fMet}$ in *E. coli* B overproduces $tRNA_1^{fMet}$. In contrast, the *metY* locus from *E. coli* K-12 overproduces $tRNA_2^{fMet}$ (lane 1). Aminoacylation assays on isolated tRNAs indicate an approximately 16-fold increase in total methionine acceptance over the combined initiator and elongator methionine tRNAs in untransformed cells. Thus,

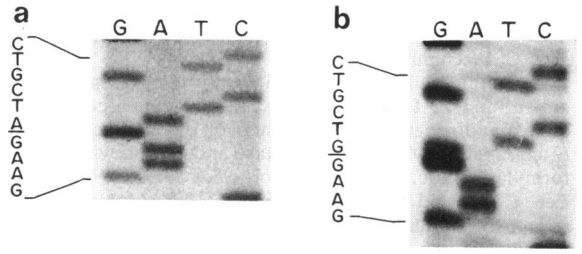

FIG. 4. Comparative sequence analysis of $tRNA^{fMet}$ genes in the *metY* locus of *E. coli* B (b) and K-12 (a). Underlined nucleotides indicate the only difference in sequence of an ~220-bp region (remainder of sequence not shown). Genomic DNAs were isolated from *E. coli* K-12 (strain JM103, $RecA^+$) and *E. coli* B (strain B105, r_B^- m_B^- Gal^- $RecA^+$), and the *metY* regions of the DNAs were amplified by PCR. The incubation mixture for PCR (100 μl) contained the genomic DNA (1 μg), the two deoxyoligonucleotide primers (5'-TACGTCCGTCTCGGTACACCAA-3' and 5'-CAAAT CCCACTACGAAGGCCGA-3'; each 22 long; 1 μM each), the four deoxynucleoside triphosphates (200 μM each), and *Taq*I DNA polymerase (5 U). Following 30 cycles of PCR with a Perkin-Elmer Cetus Thermal Cycler, the amplified DNA was recovered. Electrophoresis on a 1.5% agarose gel yielded a single band ~220 bp long in each case. The amplified DNAs were phosphorylated at their 5' ends with polynucleotide kinase and cloned into the *Sma*I site of pUC19 (New England BioLabs). The cloned DNA was sequenced in its entirety on both strands by the dideoxy chain termination method with Sequenase enzyme (U.S. Biochemicals).

the *metY* locus in *E. coli* B is transcriptionally active in *E. coli* K-12. In other experiments, we have shown that plasmids carrying the *metY* locus of *E. coli* B or K-12 also overproduce $tRNA_1^{fMet}$ or $tRNA_2^{fMet}$, respectively, in *E. coli* B. These results are consistent with the finding given above (Fig. 4) that in the ~220 bp of genomic DNAs of *E. coli* B and K-12, which includes the *metY* promoter, the only difference in sequence is a single nucleotide within the tRNA coding sequence.

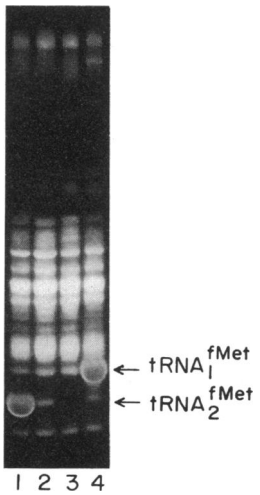

FIG. 5. Electrophoresis on a 15% nondenaturing polyacrylamide gel (25) of tRNA isolated from *E. coli* B105 (lane 3); TG1, an *E. coli* K-12 $RecA^+$ strain (lane 2); and TG1 transformed with pUC19 carrying the *metY* locus of *E. coli* K-12 (lane 1) or *E. coli* B (lane 4). Each lane contained ~25 μg of tRNA. tRNAs were detected by staining with ethidium bromide.

Our finding that *E. coli* B lacks the $tRNA_2^{fMet}$ species suggests that this species is not essential for viability or growth of *E. coli* cells. As such, it supports the recent result of Kenri and coworkers (11) that disruption of the $tRNA_2^{fMet}$ gene in *E. coli* K-12 has no effect on its growth rate. These workers have also shown that mutants of *E. coli* K-12 with disruptions in the $tRNA_2^{fMet}$ gene can initiate protein synthesis from both AUG and GUG codons. Taken together, our results suggest that $tRNA_2^{fMet}$ is dispensable in *E. coli* and that it does not fulfill a role that cannot be fulfilled by $tRNA_1^{fMet}$.

A similar disruption of the $tRNA_1^{fMet}$ gene in *E. coli* K-12 results in a strain that grows slightly slower at 37 and 42°C and significantly slower at 30°C. These results suggest that $tRNA_1^{fMet}$ is also not essential for viability of *E. coli* cells (12). Although $tRNA_2^{fMet}$ levels have not been measured in these cells, the slow-growth phenotype of these cells in which the tandem $tRNA_1^{fMet}$ genes have been disrupted is more likely a consequence of reduced levels of $tRNA^{fMet}$ than of a special role for $tRNA_1^{fMet}$ that cannot be fulfilled by $tRNA_2^{fMet}$. Since $tRNA_1^{fMet}$ represents ~75 to 80% of total $tRNA^{fMet}$ in *E. coli* K-12, it is expected that disruption of both of the $tRNA_1^{fMet}$ genes would reduce levels of total $tRNA^{fMet}$ in the cell. In the yeast *Saccharomyces cerevisiae*, disruption of three of the four initiator tRNA genes (1) leads to a twofold reduction in levels of initiator tRNA (9); these cells grow extremely slowly. An alternative possibility is that a feedback mechanism regulates levels of $tRNA^{fMet}$, but there is no evidence for such a mechanism.

It is interesting to note that the PCR-amplified DNAs which were cloned into *E. coli* K-12 lack the rho-independent tandem transcription termination signals (Fig. 3) associated with the *metY* locus and yet overproduce the tRNAs (Fig. 5). Thus, transcription termination at these sites may not be necessary for processing of the tRNA transcripts. It is possible that the main role of these signals is for regulated expression of the genes downstream in the operon (2, 10, 18). Other workers (20) have commented on a possible role of these termination signals in NusA-mediated autoregulation of the *nusA-infB* operon via transcriptional attenuation.

Finally, we showed previously that $tRNA_1^{fMet}$ and $tRNA_2^{fMet}$ of *E. coli* K-12, which differ by a single nucleotide substitution, could be separated clearly from each other by polyacrylamide gel electrophoresis under nondenaturing conditions (25). Furthermore, because $tRNA_2^{fMet}$ was among the fastest migrating tRNAs under these conditions, a single step of gel electrophoresis could be used to separate wild-type and mutant $tRNA_2^{fMet}$ species from all other tRNAs. Our finding that *E. coli* B lacks $tRNA_2^{fMet}$ species entirely has now allowed us to clone and express many of the mutant $tRNA_2^{fMet}$ genes in *E. coli* B instead of K-12 and thereby obtain the mutant tRNAs completely free of contamination by any wild-type $tRNA_2^{fMet}$. This in turn has greatly facilitated work on structure-function relationships of initiator tRNAs (15, 16, 27).

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