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supG and *supL* in *Escherichia coli* code for mutant lysine tRNAs$^+$

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ABSTRACT

We have determined the nucleotide sequences of lysine tRNAs isolated from strains containing one or the other of two Escherichia coli ochre suppressors, supG and supL. Each strain, besides producing wild-type lysine tRNA, has a mutant lysine tRNA species that apparently can read the polypeptide chain termination codons UAA and UAG. The mutant tRNAs from supG and supL strains are identical. In each case the suppressor tRNA has an A36 for U36 nucleotide substitution. Furthermore, the hypermodified nucleoside at position 37 has been changed from t^6A to ms^2i^6A.

INTRODUCTION

The ochre suppressors supG and $su^+\beta$ are known to cause the insertion of a basic amino acid, presumably lysine, during polypeptide synthesis (1,2, 3). More recently, $su^+\beta$ was reported to be an anticodon mutant of lysine tRNA (4). The wild-type gene for $su^+\beta$ was designated lysT, and supG was placed very close to lysT on the Escherichia coli genetic map (5). Ozeki et al. (4) suggested that the ochre suppressor supL (6) is allelic to $su^+\beta$ and demonstrated that there are at least two genes for lysine tRNA at 16.5 min on the E. coli map (5). Recently, we reported results that suggest that supG and supL may be allelic and we provided evidence that there are only two genes for lysine tRNA in the E. coli chromosome (7). We suggested that ochre suppressors of the supG, supL or $su^+\beta$ type can arise from one or the other copy of lysT. In this paper, we describe the isolation and nucleotide sequence analysis of lysine tRNAs from strains containing either supG or supL.

MATERIALS AND METHODS

The bacterial strains examined were all E. coli K12. supG (1) was obtained from T. Matney and supL (6) from the E. coli Genetic Stock Center. Each suppressor was introduced, by phage-mediated transduction, into strain FTP1769, whose genotype is argE(Am) glyV55 Δ(tonB-trpAB)/F'trpA(UAA243).

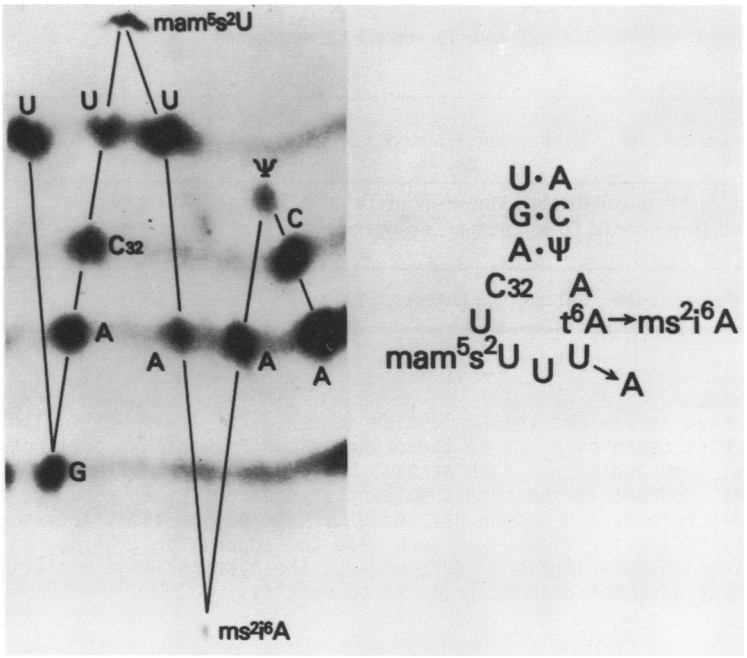

Figure 1. Chromatogram and diagram of the anticodon region of an ochre suppressor lysine tRNA from E. coli K12. Left, analysis of the 5' terminus (10) of fragments from a PEI-cellulose print of a gel ladder (not shown) obtained after hydrolysis, 5'-^{32}P-labeling with polynucleotide kinase and gel electrophoresis of the supL mutant lysine tRNA. After RNase T_2 treatment in situ, the released 5'-terminal nucleoside diphosphates were contact-transferred to a PEI-cellulose sheet for chromatographic analysis in 0.55 M ammonium sulfate. The sequence is displayed from 5' to 3', left to right, and contains the anticodon loop, nucleotides 32 to 38. Right, the anticodon region of the cloverleaf structure of wild-type lysine tRNA. The arrows indicate the two changes observed in the mutant lysine tRNA obtained from both supG- and supL-containing strains. mam^{5}s^{2}U = 5-methylaminomethyl-2-thiouridine; t^{6}A = N-[(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine; ψ = pseudouridine; ms^{2}i^{6}A = 2-methylthio-N^6-isopentenyladenosine. Under certain conditions, some of the wild-type lysine tRNA molecules may contain a derivative of t^{6}A at position 37 (9).

For nucleotide sequence analysis, each tRNA was isolated by electrophoresis in a two-dimensional and tertiary polyacrylamide gel system previously described (8,9). The procedure for RNA sequence analysis was the direct readout method of Gupta and Randerath (10).

RESULTS

Having determined the nucleotide sequence of wild-type lysine tRNA from E. coli K12 (9), we analyzed the lysine tRNAs from supG and supL ochre sup-

pressor strains. Sequence analyses were performed in both the ammonium sulfate and the ammonium formate chromatographic systems suggested by Gupta and Randerath (10). In the present study, mutational changes in the tRNA primary structure were determined by direct comparison of the entire sequence of the wild-type and mutant tRNAs. However, only the portion of the mutant tRNA that is different from normal lysine tRNA will be shown and discussed. The mutational changes in the tRNAs encoded by supG and supL were found to be identical.

The anticodon loop sequence, nucleotides 32 to 38, of wild-type lysine tRNA is CUmam^{5}s^{2}UUUt^{6}AA (anticodon underlined). As shown in Figure 1, each ochre suppressor tRNA is altered in two ways. First, the uridine in the 3' position of the anticodon (nucleotide 36) has been replaced by adenosine. Second, the posttranscriptionally modified nucleoside adjacent to the 3' end of the anticodon (position 37) has been changed from t^{6}A (see the comment in the figure legend) to ms^{2}i^{6}A. There appears to be no unmodified adenosine at that position. The identity of ms^{2}i^{6}A was deduced from its characteristic migratory behavior in the two chromatographic systems mentioned above.

DISCUSSION

The nucleotide sequence analyses reported in this paper demonstrate that the ochre suppressor genes supG and supL code for mutant lysine tRNAs. The mutant products of supG and supL were found to be identical. In each case the suppressor tRNA differed from wild-type lysine tRNA by an A for U36 nucleotide substitution in the anticodon. Furthermore, although the modification of U34 that is found in the wild-type tRNA remained unchanged, the t^{6}A at position 37 was replaced by ms^{2}i^{6}A (Fig. 1, right).

Recently, the DNA sequence of a tRNA operon containing two lysine tRNA genes was analyzed, and it was determined that the ochre suppressor su$^+\beta$ is an anticodon mutant of one of the two genes (4; H. Inokuchi, M. Yoshimura and H. Ozeki, in preparation). The disposition, however, of modified nucleosides in the suppressor tRNA, particularly the A37 modification, was not determined. But in general, the sequence data are consistent with our recent suggestion that ochre suppressors of the supG, supL or su$^+\beta$ type can arise from either copy of lysT on the E. coli chromosome (7). The same appears to be true of misacylated mutant lysine tRNAs that function as suppressors of missense mutations (7,9).

In studies with missense suppressors derived from the glycine tRNAs, all of which have an unmodified A37, it has been found that nucleotide changes of

C36 to U or A were sufficient to cause at least partial modification of A37 to t^6A or ms^2i^6A respectively (8,11,12; Prather, Mims and Murgola, unpublished). Nevertheless, the present case of conversion of wild-type lysine tRNA to an ochre suppressor represents the first example of the change of one of those modifications to the other. This observation is relevant to discussions of the recognition of tRNAs by different tRNA modifying enzymes.

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