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# Nucleotide sequence of initiator tRNA from *Mycobacterium smegmatis*

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ABSTRACT

The nucleotide sequence of initiator tRNA from Mycobacterium smegmatis was determined to be pCGCGGGGUGGAGCAGCUCGGDAGCUCGCUGGGCUCAUAACCCAGAGm$^7$GUCG CAGGU$\psi$CGm$^1$AAUCCUGUCCCCGCUACCA$_{OH}$. The nucleotide sequence of Mycobacterium initiator tRNA was found to be the same as that of Streptomyces initiator tRNA, except that $G_{46}$ and $A_{57}$ were replaced by m$^7G_{46}$ and $G_{57}$, respectively. The striking feature of Mycobacterium initiator tRNA is the absence of ribothymidine at residue 54, and the presence of 1-methyladenosine at residue 58 which makes the sequence of this tRNA similar to that of eukaryotic initiator tRNA.

## INTRODUCTION

We previously reported that initiator tRNA of Streptomyces griceus which belongs to Actinomycota posseses unmodified uridine at residue 54 instead of ribothymidine (1). In addition, the fifth base from the 3'-terminus of Streptomyces initiator tRNA is $U_{72}$, while $A_{72}$ is located in the same position as other prokaryote initiator tRNAs sequenced so far (2). Moreover, Streptomyces initiator tRNA contains 1-methyladenosine (m$^1$A) at residue 58 in the T$\psi$C-loop, which has been found in cytoplasmic initiator tRNAs from eukaryotes (1).

These structural characteristics of Streptomyces initiator tRNA indicate that Streptomyces is phylogenetically quite distinct from other prokaryotes. It would be interesting to know whether these structural features found in Streptomyces initiator tRNA are common to organisms belonging to Actinomycota.

This paper reports the nucleotide sequence of initiator tRNA from Mycobacterium smegmatis, which belongs to Actinomycota, and discusses the common unique structural features of Actinomycota initiator tRNAs which differ from those of other prokaryotic initiator tRNAs.

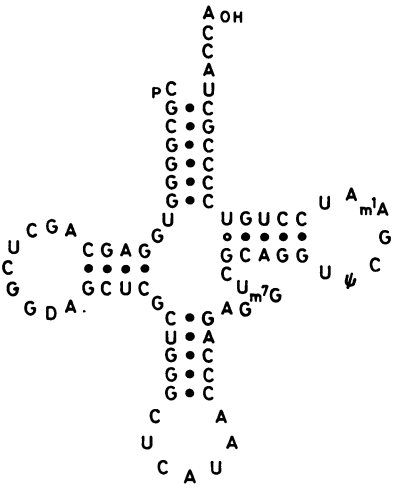

Fig. 1. Nucleotide sequence of initiator tRNA from Mycobacterium smegmatis arranged in a clover-leaf model.

## MATERIALS AND METHODS

Mycobacterium smegmatis $SN_2$ was cultured in Youmans and Karlson's minimal medium containing 0.1% Tween-80 at 37°C (3). Unfractionated tRNA from Mycobacterium smegmatis was prepared by procedures described previously (3). DEAE-Sephadex A-50 and RPC-5 column chromatographies were used successively for the purification of Mycobacterium initiator tRNA (4,5). Three different gel electrophoreses using 10, 20 and 15% polyacrylamide gels were

Table 1. Structural characteristics found in initiator tRNAs

| Position of nucleotide residue | Prokaryotes | | | | Eukaryotes |
|---|---|---|---|---|---|
| | Mycoplasma | Eubacteria | Streptomyces | Mycobacterium | |
| 1 | C | C | C | C | A |
| 20 | D | D | D | D | A |
| 33 | U | U | U | U | U,C |
| 37 | A | A | A | A | $t^6A$ |
| 54 | U | T | U | U | A |
| 57 | G | A | A | G | G |
| 58 | A | A | $m^1A$ | $m^1A$ | $m^1A$ |
| 60 | U | U | U | U | A |
| 72 | A | A | U | U | U |

Abbreviations used were:

D; dihydrouridine, T; ribothymidine, $m^1A$; 1-methyladenosine, $t^6A$; N-[9-β-D-ribofuranosyl-purin-6-yl)carbamoyl]-L-threonine.

performed for the final purification of initiator tRNA as described previously (6,7). For the assay of the methionine accepting ability of initiator tRNA, a crude Escherichia coli aminoacyl tRNA synthetase mixture was used. The materials and procedures used for sequence analysis of tRNA by post-labeling techniques were the same as described previously (6-8).

## RESULTS AND DISCUSSION

The nucleotide sequence of initiator tRNA from Mycobacterium smegmatis was determined by combined use of several post-labeling procedures as described previously (8). The total nucleotide sequence obtained from the sequencing procedures is arranged in a cloverleaf form in Fig. 1.

It is interesting to note that Mycobacterium initiator tRNA lacks ribothymidine. This result coincides with the previous data that unfractionated total Mycobacterium tRNA does not contain ribothymidine (3). As other striking features, Mycobacterium initiator tRNA posseses $U_{54}$, $G_{57}$, $m^1A_{58}$ and $U_{72}$. In general, prokaryote initiator tRNAs, except Mycoplasma and Streptomyces initiator tRNAs, have $T_{54}$, $A_{57}$, $A_{58}$ and $A_{72}$ as shown in Table I.

Initiator tRNA of Mycoplasma, which belongs to Mycoplasmomycota and has the smallest chromosomal DNA among the self-growing organisms, does not contain ribothymidine at residue 54, however other structural features of the tRNA are the same as those of eubacteria initiator tRNAs (9). On the contrary, initiator tRNA of Streptomyces, which belongs to Actinomycota, has the same structural feature as that of Mycobacterium initiator tRNA. The overall sequence homology between Mycobacterium initiator tRNA and Streptomyces initiator tRNA is 98%. These sequencing data of Mycobacterium and Streptomyces initiator tRNAs clearly indicate that the presence of $U_{54}$, $m^1A_{58}$ and $U_{72}$ in place of $T_{54}$, $A_{58}$ and $A_{72}$ is specific to Actinomycota initiator tRNAs. In addition, these sequence characteristics show that Streptomyces and Mycobacterium are phylogenetically quite distinct from other prokaryotes.

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