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Nucleotide sequence of *Streptomyces griseus* initiator tRNA

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ABSTRACT

The primary structure of initiator tRNA from *Streptomyces griseus* was determined by post-labeling procedures. The nucleotide sequence is pC-G-C-G-G-G-G-U-G-G-A-G-C-A-G-C-U-C-G-G-D-A-G-C-U-C-G-C-U-G-G-G-C-U-C-A-U-A-A-C-C-C-A-G-A-G-G-U-C-G-C-A-G-G-U-ψ-C-A-m^{1}A-A-U-C-C-U-G-U-C-C-C-C-G-C-U-A-C-C-A_{OH}. The unique feature of the sequence of this tRNA is that residue 54 is occupied by unmodified U, while ribothymidine is located in that position in most initiator tRNAs from eubacteria.

INTRODUCTION

Prokaryotic initiator tRNAs so far sequenced have several common structural characteristics which differ from those of eukaryotic initiator tRNAs; namely, lack of base-pairing between the 5'-terminal base and the fifth base from the 3'-terminus, presence of the TψC-sequence in the TψC-loop, and uridine residues at the positions 20, 33 and 60 (1).

It was previously shown that unfractionated total *Mycobacterium* tRNA does not contain ribothymidine (2). *Mycobacterium* belongs to Actinomycetes and is clearly distinguished from *Bacillus* in Eubacteriomycetes. Thus it would be interesting to know the sequence of initiator tRNA from Actinomycetes and to identify the nucleotide residue which replaces ribothymidine in TψC-loop. In this paper, we report the nucleotide sequence of initiator tRNA of *Streptomyces griseus* which belongs to Actinomycetes, and discuss its structural characteristics compared with those of other eubacteria initiator tRNAs.

MATERIALS AND METHODS

Streptomyces griseus was cultured in 3% tryptic soy broth. Unfractionated tRNA was prepared by the procedure described by Zubay (3). Initiator tRNA was purified by successive application of DEAE-Sephadex A-50, BD-cellulose and RPC-5 column chromatographies (4-6). For the final purerification of the initiator tRNA, two-dimensional polyacrylamide gel electro-

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phoresis was performed (7,8). For detection of initiator tRNA species during purification, a crude *Escherichia coli* aminoacyl tRNA synthetase mixture was used to charge methionine. The materials and procedures used for sequence analysis of tRNA by post-labeling techniques were the same as described previously (7,8).

RESULTS AND DISCUSSION

Most of the nucleotide sequence of *Streptomyces griseus* initiator tRNA was determined by the partial formamide degradation method (7,8). The assignment of the 5'-terminal nucleotide of the tRNA was done by nuclease P1 digestion of 5'-terminal labeled tRNA, followed by two-dimensional cellulose thin-layer chromatography (9). The six nucleotide residues from the 3'-terminus of the tRNA was determined by the method reported by Peattie (10). Modified nucleotides present in *Streptomyces griseus* initiator tRNA were independently identified by the post-labeling procedure using unfractionated tRNA (11), rather than formamide degradation fragments, in addition to identification made during the course of the sequencing procedure. A combination of the resulting data made it possible to obtain the total nucleotide sequence of *Streptomyces griseus* initiator tRNA, as arranged in the clover-leaf form as shown in Fig. 1.

Streptomyces initiator tRNA contained only three modified nucleosides, D_{20}, ψ_{55} and m^1A_{57}; this is the lowest modified nucleoside content of the natural tRNAs so far sequenced (1). The 5'-terminal base and the fifth base

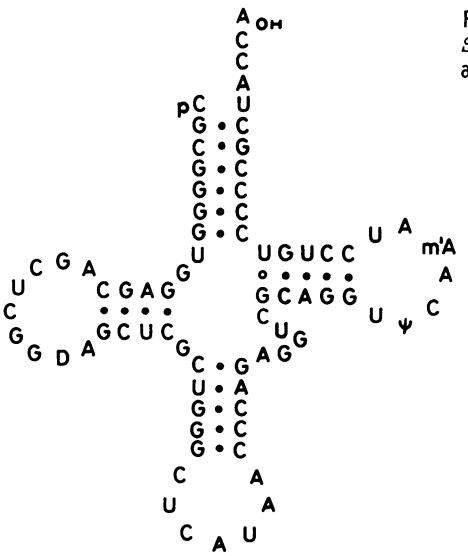

Fig. 1. Nucleotide sequence of *Streptomyces griseus* initiator tRNA arranged in a clover-leaf model.

from the 3'-terminus was unpaired like other eubacteria initiator tRNAs. However, the fifth base from the 3'-terminus of *Streptomyces* initiator tRNA was found to be U instead of A, as clearly indicated in the sequencing ladder obtained by Peattie's method using 3'-end labeled tRNA. Uridine at the same position is found in all eukaryotic initiator tRNAs, base-pairing with the adenylate residue at the 5'-terminus. Prokaryotic initiator tRNAs so far sequenced have distinctive characteristics as compared with eukaryotic initiator tRNAs, by having C_1, D_{20}, A_{37}, T_{54}, A_{57} and U_{60} in place of A_1, A_{20}, t^6A_{37}, A_{54}, G_{57} and A_{60} (Table 1). *Streptomyces* initiator tRNA is clearly distinguished from other initiator tRNAs since it possesses U_{54}, m^1A_{58} and U_{72} instead of T_{54}, A_{58} and A_{72}. These alterations make the sequence of *Streptomyces* initiator tRNA similar to that of eukaryotic initiator tRNA, indicating that *Streptomyces* is phylogenetically quite distinct from other prokaryotes.

The nucleotide sequence of *Mycobacterium smegmatis* initiator tRNA has been recently obtained (B.R. Vani, Y. Kuchino and S. Nishimura, unpublished results). It was found that *Mycobacterium smegmatis* initiator tRNA has the same structural feature do that of *Streptomyces griseus* initiator tRNA. Both

Table I. Structural characteristics of initiator tRNAs found in specific nucleotide residues.

Position of nucleotide residue	Prokaryotes		Eukaryotes	
	Eubacteria	*Streptomyces griseus*	Unicellular	Multicellular
1	C	C	A	A
20	D	D	A	A
33	U	U	U	C
37	A	A	t^6A	t^6A
54	T	U	A	A
57	A	A	G	G
58	A	m^1A	m^1A	m^1A
60	U	U	A	A
72	A	U	U	U

Abbreviations used were:

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

Streptomyces and *Mycobacterium* belong to the same phylum, Actinomycota. Thus it is likely that the presence of U_{54} and U_{72} in place of T_{54} and A_{72} is specific to organisms which belong to Actinomycota.

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