Cloning and DNA sequence of the genes for two bacteriophage T5 tRNAs^{Ser}

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Bacteriophage T5 *Bgl*II/*Hind*III DNA fragment (803 basepairs), containing the genes for 2 tRNAs and 2 RNAs with unknown functions, was cloned in the plasmid pBR322. The analysis of DNA sequence indicates that tRNA genes code isoacceptor tRNAs^{Ser} (tRNA$_1^{Ser}$ and tRNA$_2^{Ser}$) with anticodons UGA and GGA, respectively. The main unusual structural feature of these tRNAs is the presence of extra non-basepaired nucleotides in the joinings of stem 'b' with stems 'a' and 'c'.

Bacteriophage T5 *tRNA gene* *RNA/DNA hybridization* *DNA sequencing* *Isoacceptor tRNA*

1. INTRODUCTION

In [1], coliphages T4 and T5 were found to induce the synthesis of phage-specific tRNAs. Bacteriophage T4 was later shown to encode 8 tRNAs and 2 low-M_r stable RNAs of an unknown function. The nucleotide sequence of these RNAs and their genes was determined [2], [3 and references cited therein] and it was shown that phage-specific tRNAs is a useful system for the study of different aspects of tRNA biosynthesis and function.

Unlike bacteriophage T4, the more intricately organized genome of bacteriophage T5 stable RNAs has been studied insufficiently. Using two independent methods:

(i) Hybridization of in vitro animoacylated tRNAs purified from phage-infected *E.coli* cells with DNAs of the different T5 deletion mutants [4,5];

(ii) Comparative analysis of stable RNAs produced by deletion mutants [5–7];

tRNAs and some other low-M_r RNAs have been mapped in the so-called deletion region of T5 DNA between 21.1% and 32.3% of its length. It was also shown that bacteriophage T5 codes tRNA species-specific for each of the 20 amino acids involved in protein synthesis including a number of isoacceptor tRNAs [5]. The existence of such a great number of stable RNA fractions as well as a distribution of their genes on the extensive region of genome (about 14 kilobasepairs) is the reason that neither the detailed location of these genes nor even their exact number is known as yet. The use of recombinant DNA techniques is one of the approaches to tackle this problem. In spite of the presence of recognition sites for a number of restriction endonucleases, only two DNA fragments from this region have been cloned: the *Eco*RI fragment (440 basepairs) and the *Hind*III fragment (1170 basepairs) [8–10]. Attempts to clone other fragments such as the *Eco*RI fragment (3250 basepairs) containing the genes for about half of all stable RNAs, failed. Here, we present the results on the cloning of part of the *Eco*RI fragment (3250 basepairs) in the form of *Hind*III/*Bgl*II subfragment (803 basepairs) in the plasmid pBR322 as well as its nucleotide sequence and the location of the genes for 2 tRNAs^{Ser}.

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2. MATERIALS AND METHODS

Methods used for cloning DNA fragments as well as the analysis of RNAs coded by the cloned fragments were as in [10]. The sequencing was performed as in [11].

3. RESULTS AND DISCUSSION

It was shown that the *Eco*RI fragment (3250 basepairs) has two recognition sites for restriction endonuclease *Bgl*II (fig.1). We attempted to clone *Bgl*II- and *Bgl*II/*Hin*dIII subfragments of the *Eco*RI fragment (3250 basepairs) in the plasmid pBR322 using the identity of the cohesive ends produced as a result of hydrolysis with endonucleases *Bam*HI and *Bgl*II. The analysis of recombinant plasmids selected by hybridization in situ with ^{32}P-labeled T5 4–5 S RNAs showed that the only fragment, we succeeded in cloning intact, was the *Bgl*II/*Hin*dIII fragment (803 basepairs). The recombinant plasmid which contained this fragment was designated as pBR322–T5$_{105}$. As to the other hybrid plasmids obtained in these experiments, considerable changes in their structure were observed.

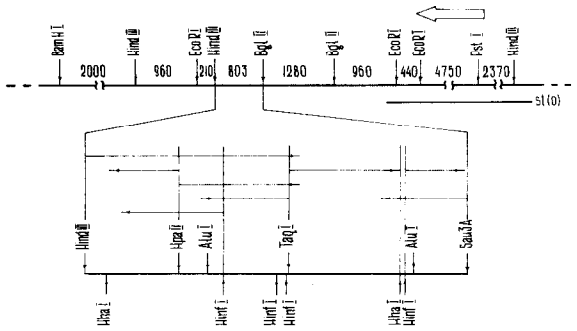

Fig.1. Restriction endonuclease map of the bacteriophage T5 deletion region showing cloned fragment used in the sequence determination. The strategy and extent of sequencing are indicated by arrows. The direction of transcription from the genes for stable RNAs is shown by the thickened arrow.

The hybridization of in vivo ^{32}P-labeled T5 4–5 S RNAs with DNA of the plasmid pBR322–T5$_{105}$, immobilized on nitrocellulose filters, and subsequent two-dimensional polyacrylamide gel electrophoresis of the RNAs eluted from the filters showed that the cloned fragment contained the genes (or parts of the genes) for the 4 stable RNAs: RNAI, RNA1, RNA4 and RNA7 (fig.2).

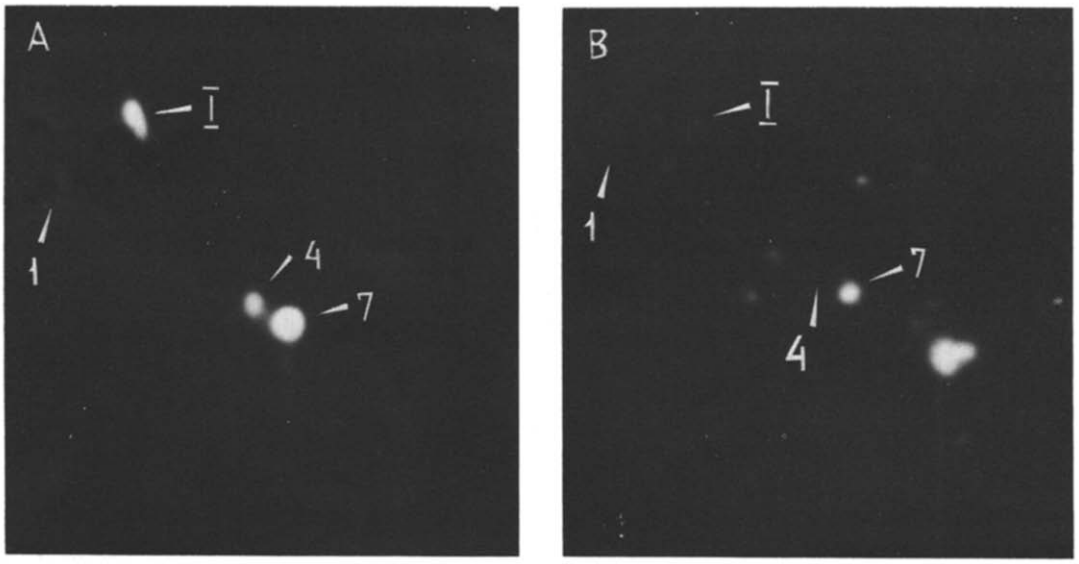

Fig.2. Two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled T5 RNAs hybridized with DNA of: (a) pBR322-T5$_{105}$; (b) T5^{+}; first dimension; 10% polyacrylamide/7 M urea/tris–borate, 10 V/cm, 15°C for 10 h; second dimension; 20% polyacrylamide/4 M urea/Tris–borate, 15 V/cm, 26°C, 15 h.

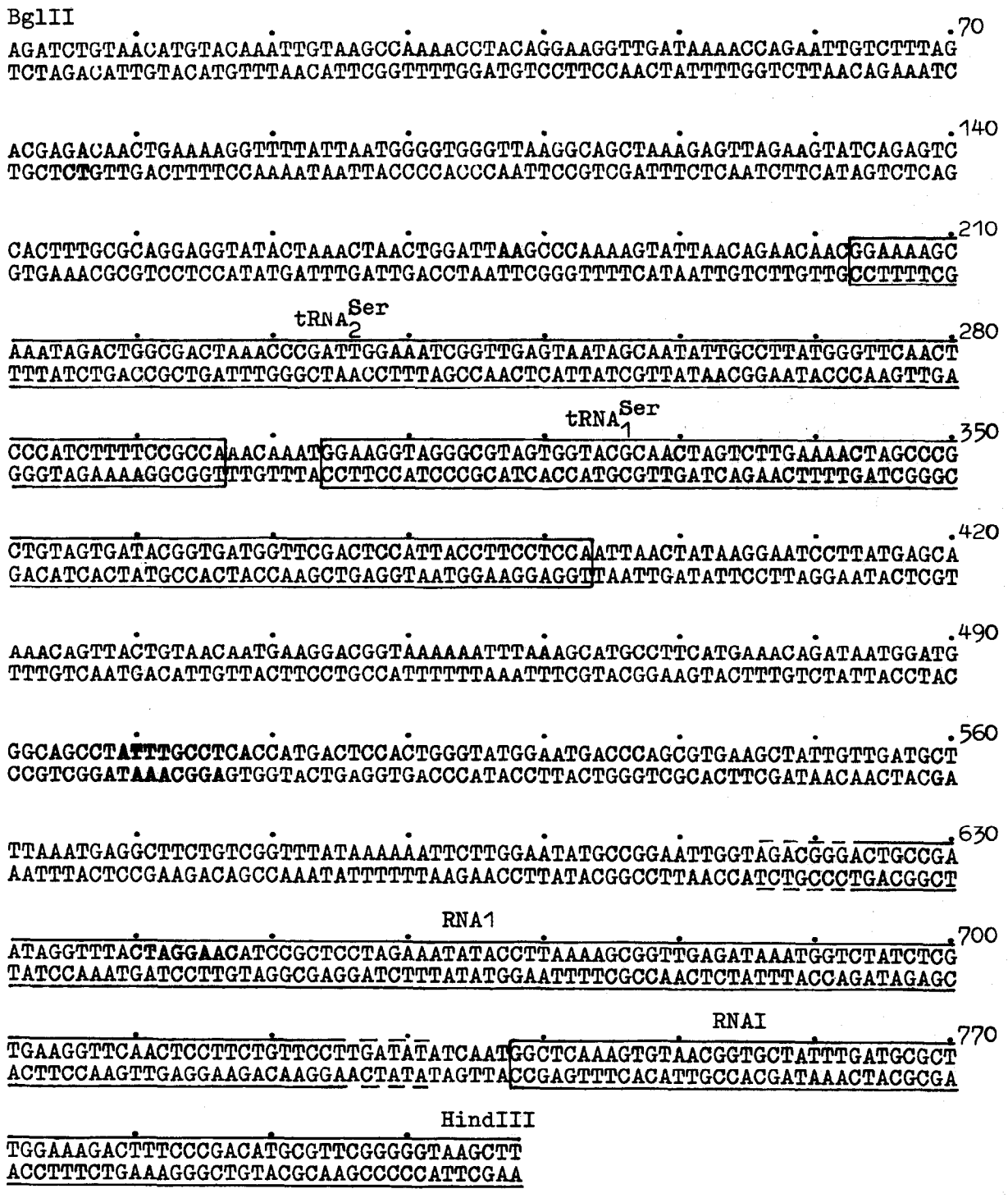

Fig.3. Nucleotide sequence of the *Hind*III/*Sau*3A fragment. Below the DNA sequence, the corresponding RNAs are represented with 5′- and 3′-terminal sequences as indicated.

With the aim of a more detailed delineation of these RNAs and precise localization of their genes, the DNA sequence of the *Hind*III/*Bgl*II fragment was determined as well as the fingerprint analysis of T1 and pancreatic RNase digests of these RNAs was performed. The information thus obtained appears to be sufficient for the unequivocal localization of the RNA genes on the DNA sequence (fig.3). Unfortunately, the purity of the obtained RNA1 preparations was insufficient for the identification and sequence analysis of the 3′- and 5′-terminal oligonucleotides. Because of this the precise boundaries of the gene for RNA1 are not pointed out. The arrangement only of the part of the gene for RNA1 corresponds to our data on the localization of another part of this gene on the *Eco*RI/*Hind*III fragment (210 basepairs) [10,12]. It should be noted that the functional meaning of the RNAI (107 bases) and RNA1 (about 120 bases) remains as yet unelucidated. Although RNA1 is known to contain the modified nucleotides T and ψ, these RNAs do not apparently represent tRNA precursors (according to the data on their increased stability in the experiments with the exchange of ^{32}P by the 'cold' phosphate).

The secondary structure of tRNA7 and tRNA4 can be represented in the classic cloverleaf pattern (fig.4). The sequence further shows that the anticodons of these RNAs are UGA and GGA what corresponds to serine codons UC^{A}_{G} and UCC, respectively. Both tRNAs have the most invariant nucleotides at the appropriate positions and similarly to another serine tRNAs they have a long extra-arm [13]. The main unique feature of $tRNA_1^{Ser}$ and $tRNA_2^{Ser}$ is the existence of extra-nucleotides in the region between stem 'b' and stems 'a' and 'c' what is accompanied by the absence of the canonical U at position 8.

The close proximity and common polarity of serine tRNAs is consistent with the possibility that

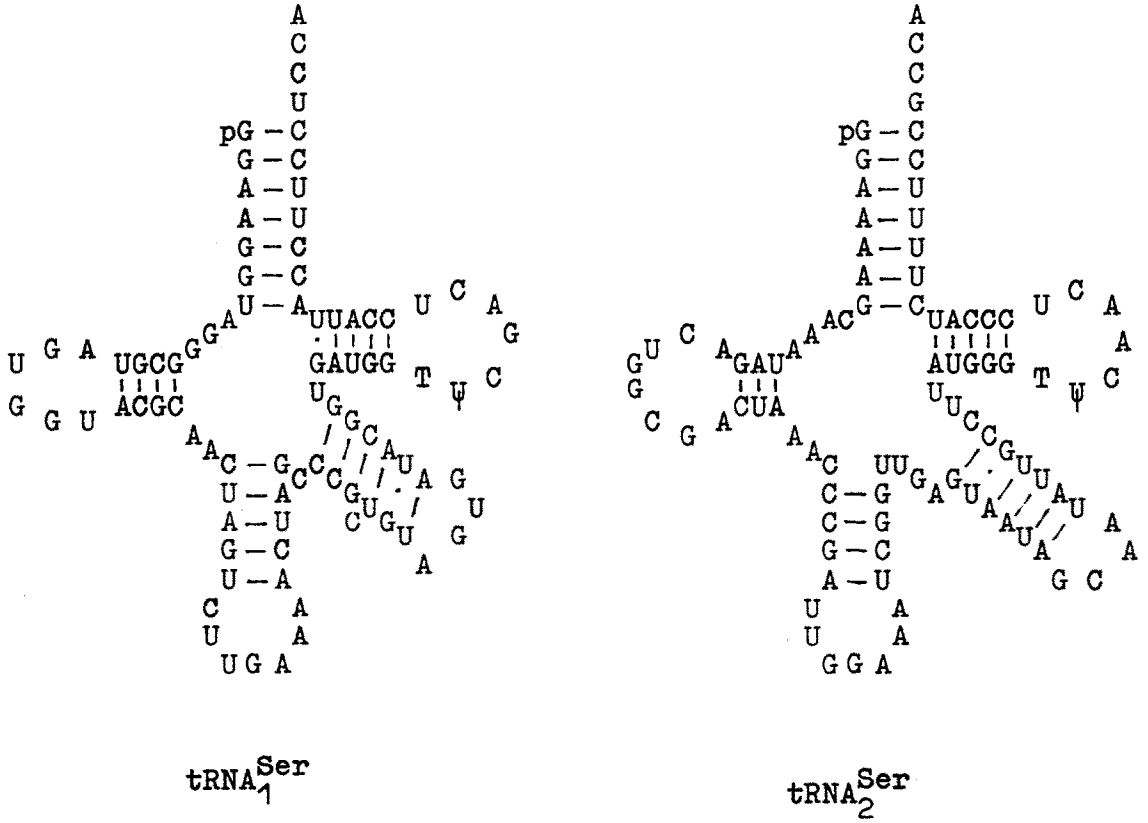

Fig.4. Cloverleaf structure of the bacteriophage T5 $tRNA_1^{Ser}$ (tRNA7) and $tRNA_2^{Ser}$ (tRNA4).

at any rate these tRNAs are transcribed in the form of the common precursor. However, in the region preceding tRNA genes the nucleotide sequence which should meet the requirements of the 'classic' promoter is absent. Thus, either promoter is situated outside the examined sequence or the promoters of stable RNAs have a non-typical structure.

In [14], two serine tRNAs were shown to be coded by the bacteriophage T5 deletion mutant *st*(o). In [5] one of two genes of $tRNAs^{Ser}$, found in [14], was arranged in the region of *st*(o) deletion. However, some difficulties were encountered in [5] with mapping of the $tRNA^{Ser}$ genes. Also, no explanation of the contradictions in their data on the synthesis of these tRNAs by the different bacteriophage T5 deletion mutants was presented in [5]. Thus, it is possible to assume that either the map in [5] is incorrect or bacteriophage T5 has the gene for another isoacceptor $tRNA^{Ser}$.

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