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The nucleotide sequence of *Bacillus subtilis* tRNA genes

Yuko Yamada*, Misao Ohki+ and Hisayuki Ishikura*

*Laboratory of Chemistry, Jichi Medical School, Minamikawachi-machi, Tochigi-ken 329-04, Japan, and +Biology Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, Japan

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ABSTRACT

Clones carring *Bacillus subtilis* tRNA genes were isolated from a λ816 library. A recombinant phage λ816-BS83 which was hybridized effectively with unfractionated tRNA probes contained a 3-kb fragment. By a Southern's blot analysis, it was found that tRNA genes were located in Eco RI-Hinc II region of this fragment. Sequence determination revealed the presence of a cluster of four tRNA genes in this region. The gene organization was as follows: $tDNA^{Lys}$-9bp-$tDNA^{Glu}$-81bp-$tDNA^{Asp}$-30bp-$tDNA^{Phe}$. The RNA sequences expected from $tDNA^{Lys}$ and $tDNA^{Phe}$ were identical with the reported RNA sequences. Two tRNA genes, $tDNA^{Lys}$ and $tDNA^{Asp}$ encoded the CCA sequence of 3'-terminal region, but the other two, $tDNA^{Glu}$ and $tDNA^{Phe}$ did not. A promoter-like sequence which corresponds to the σ^{55}-recognition site was found in a region about 100bp upstream from $tDNA^{Lys}$.

INTRODUCTION

Bacillus subtilis changes in the percentage of isoaccepting species of $tRNA^{Lys}$ and $tRNA^{Val}$ in different growth stages. It is very interesting to know the mechanism of tRNA gene expression in different stages. Two clusters of tRNA genes have been reported to exist in the spacer regions of rRNA genes. One of two clusters encoded $tRNA^{Ile}$ and $tRNA^{Ala}$ genes (1 and Yoshikawa, H.: personal communication) and the other encoded $tRNA^{Asn}$, $tRNA^{Thr}$, $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{Pro}$, and $tRNA^{Ala}$ genes (2). All tRNA genes described above were oriented in the same direction as rRNA genes. The expression of those tRNA genes seems to be related with that of rRNA genes.

Here, we report the structure of a cluster of tRNA genes. This cluster consisted of four tRNA genes; $tDNA^{Lys}$, $tDNA^{Glu}$, $tDNA^{Asp}$, and $tDNA^{Phe}$, and contained no rRNA genes. Two of four tRNA genes ($tDNA^{Lys}$ and $tDNA^{Asp}$) encoded the CCA sequence but the other two genes ($tDNA^{Glu}$ and $tDNA^{Phe}$) did not. A promoter-like sequence which corresponds to the σ^{55}-recognition site was found in a locus about 100bp upstream from $tDNA^{Lys}$. A stem and loop structure followed by poly T which was considered as a

terminator is located at a region about 10bp downstream from $tDNA^{Phe}$. The cloned fragment hybridized to four unidentified tRNA species isolated from two-dimensional polyacrylamide gels. Since it is highly unlikely that a cluster of four tRNA pseudogenes exists in this DNA fragment, we assume that these tRNA genes are functionally expressed.

MATERIALS AND METHODS

Restriction endonucleases Eco RI, Alu I, Hinf I, Mbo II, Taq I and Hae III were purchased from New England BioLabs. Endonuclease Sau 96 I and large fragment *E. coli* DNA polymerase I were from Bethesda Research Laboratory, Inc. Hinc II, Pvu II, and T_4-polynucleotide kinase were obtained from Takara Shuzo Co., Ltd. T_4-polynucleotide kinase was also purchased from Boehringer Mannheim Biochemicals. Alkaline phosphatase of bovine intestine was from Sigma Chemical Co., Ltd. [γ-^{32}P]ATP (specific activity, 5000Ci/mmol) and [α-^{32}P]ATP (specific activity, 2000-3000Ci/mmol) were obtained from Amersham.

A gene bank of *Bacillus subtilis* W168 was constructed using λ816 vector phage (λp*lac*5 *srI*λ3° *imm*^{21}ts *srI*λ4° *nin*5 *srI*λ5°) being gift from Dr. K Murray. *B. subtilis* W168 DNA and λ816 DNA were digested with Eco RI, separately. After ligation and *in vitro* packaging, recombinant phages were plated with an indicator bacteria, *E. coli* K802 (*hsr met gal*K *lac*Y *su*II) being gift from Dr. F. R. Blattner. The 4500 colorless plaques were selected on plates containing 4-bromo-5-chloro-3-indolyl-β-D-galactoside. Each 30 plaques were pooled and 150 batches stored. The probe for tRNA gene was prepared by kination of crude tRNA extracted from *B. subtilis* log-phase cell with [γ-^{32}P]ATP and T_4-polynucleotide kinase. The recombinant phages carring tRNA genes were screened by hybridization of [^{32}P]tRNA probe with phage DNA obtained from 5 ml liquid lysates and fixed on nitrocellulose membrane filters. Phages purified from a hybridization-positive batch were multiplied and again subjected to hybridization test. λ816-BS83, one of transducing phage carring tRNA genes was obtained in this screening and contained a 3-kb insert. This fragment was subcloned into pBR322 for further analysis. Transformation of *E. coli* HB101 with a recombinant DNA was carried out essentially as described by Mandel and Higa (3). A strain harboring the target plasmid was selected and grown on a large scale as described (4). The presence of tRNA gene in this fragment was confirmed according to the Southern hybridization method (5) after digestion of the fragment with appropriate endonucleases.

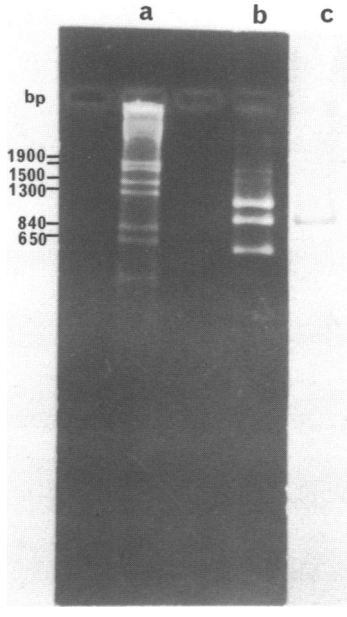

Fig. 1 Southern hybridization analysis.
The Eco RI fragment (3-kb) digested with Hinc II (lane b) and λDNA treated with Eco RI and Hind III as marker DNA (lane a) were electrophoresed on 2% agarose gel. Electrophoresis was perfomed at 15 mA for 15 hrs with 90 mM Tris-90 mM boric acid-4 mM EDTA as buffer. The bands of DNA in the gel were stained with ethidium bromide (1 μg/ml). The bands in the gel were transfered to nitrocellulose membrane filter and hybridized with [^{32}P]tRNA probe (lane c) as described (5).

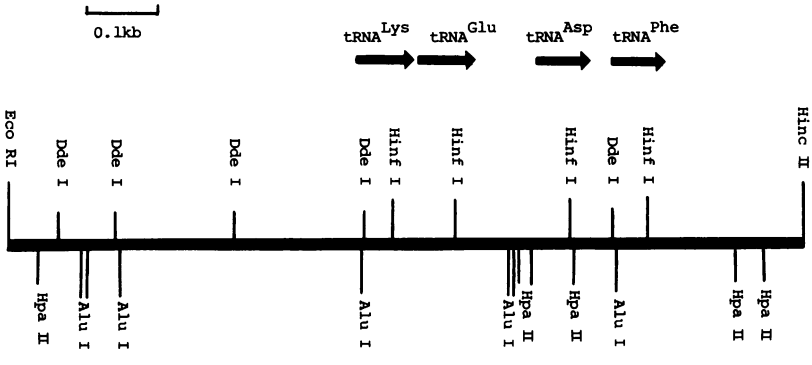

Fig. 2 Restriction map of the Eco RI-Hinc II fragment.
tRNA genes are shown above in alignment with the map.
Transcription proceeds from *left* to *right*.

DNA sequencing was performed essentially as described by Maxam and Gilbert (6).

RESULTS

A recombinant phage λ816-BS83 contained a 3-kb Eco RI insert which

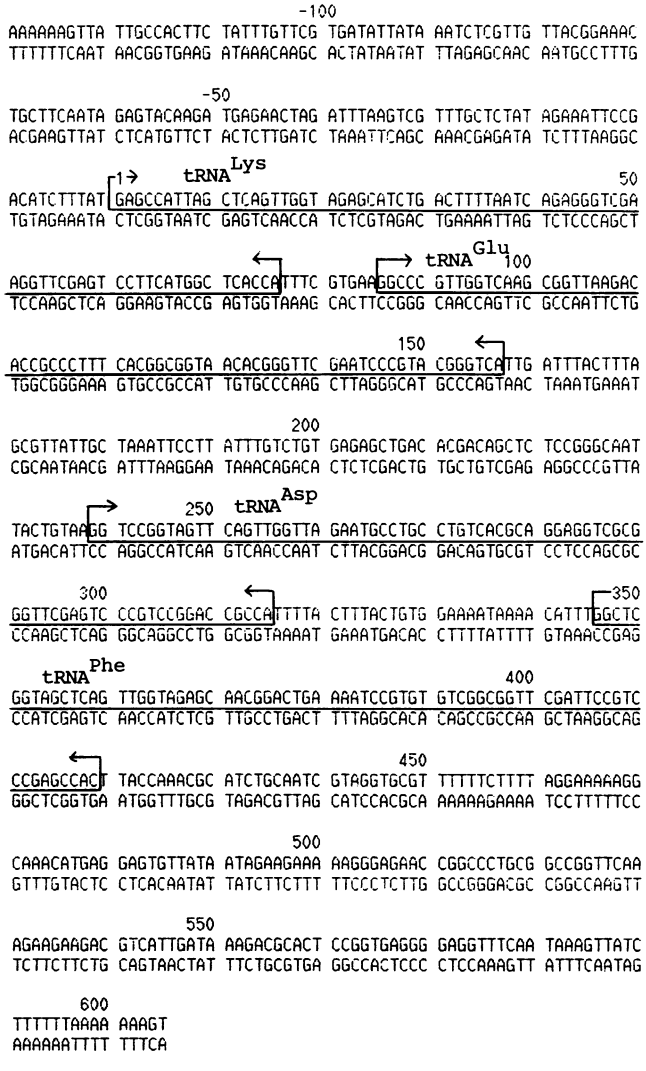

Fig. 3 Nucleotide sequence of the Eco RI-Hinc II fragment. Nucleotide sequence was numbered from the first nucleotide G of $tDNA^{Lys}$. Underlined and boxed sequences indicate the coding sequences corresponding to processed tRNAs.

hybridized with [^{32}P]probe tRNA by the Southern blotting method. The 3-kb fragment gave 3 bands with Hinc II. Among these bands, a 1-kb fragment hybridized with [^{32}P]probe (Fig. 1). This Eco RI-Hinc II fragment was therefore sequenced.

The restriction map of the Eco RI-Hinc II fragment was deduced mainly by the standard methods of single and double digestions and confirmed from nucleotide sequence (Fig. 2). DNA sequence was determined for fragments 5'-end- or 3'-end-labeled at the following sites: Eco RI, Hinc II, Hinf I, Alu I, Hpa II, and Dde I. The tRNA genes were searched in both strands for GTTC sequence and for the presence of 5bp-stem and 7b-loop structure corresponding to anticodon region and GTΨC region.

In this sequence, four tRNA genes were found in the same direction. The gene organization is as follows: tDNA^{Lys}-spacer(9bp)-tDNA^{Glu}-spacer(81bp)-tDNA^{Asp}-spacer(30bp)-tDNA^{Phe} (Fig. 3). The RNA sequence expected from tDNA^{Lys} was identical with tRNA$_1^{Lys}$ we had already determined (7). The tRNA$_1^{Lys}$ is a major lysine-isoaccepting tRNA in late-log phase cells. The RNA sequence expected from tDNA^{Phe} was identical with tRNA^{Phe} reported by Arnold and Keith (8) except for the absence of terminal CA sequence of 3'-region. The nucleotide sequences of tRNA^{Glu} and tRNA^{Asp} from *B. subtilis* are undetermined. When compared with those of *E. coli* (9),

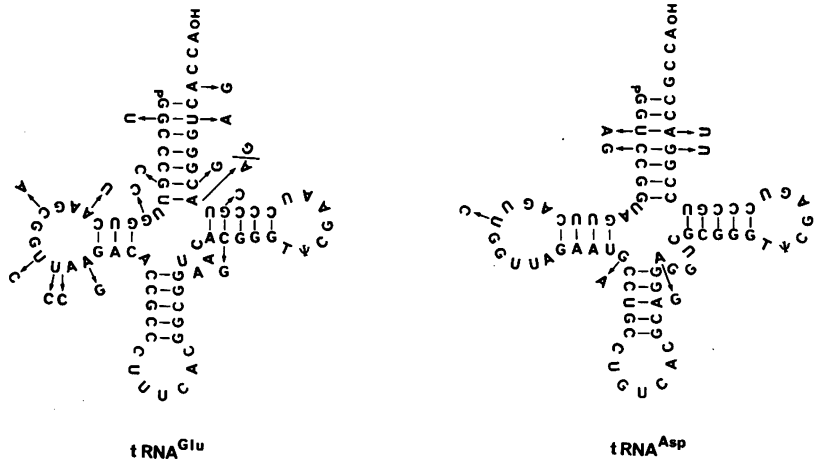

Fig. 4 Comparisons tRNA^{Glu} and tRNA^{Asp} with those of *E. coli*. The primary structures are deduced from DNA sequences and shown in the normal tRNA cloverleaf secondary structure. Nucleotides which differ from *E. coli* tRNAs are indicated by arrows.

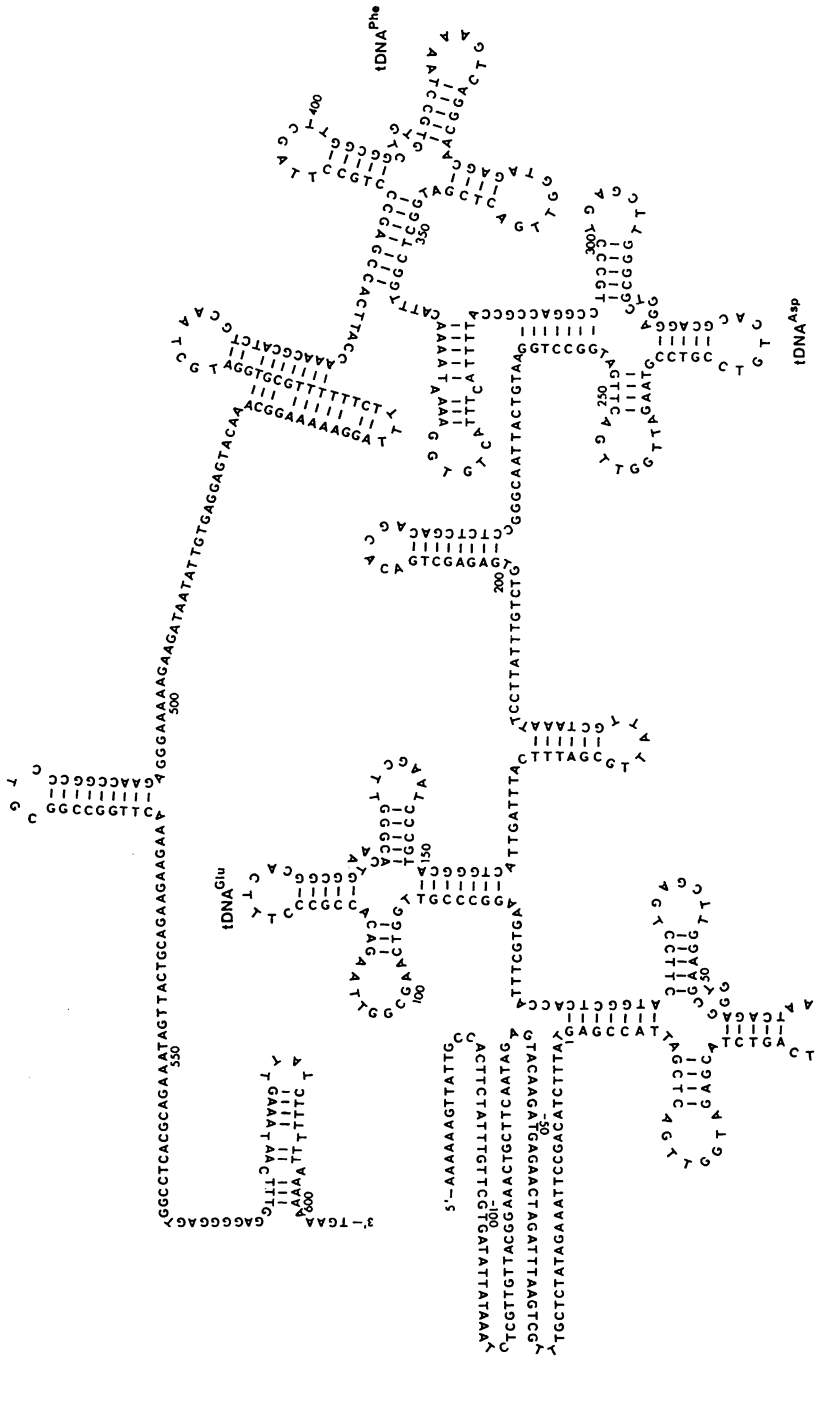

Fig. 5 Possible secondary structure of the single stranded DNA carring four tRNA genes.

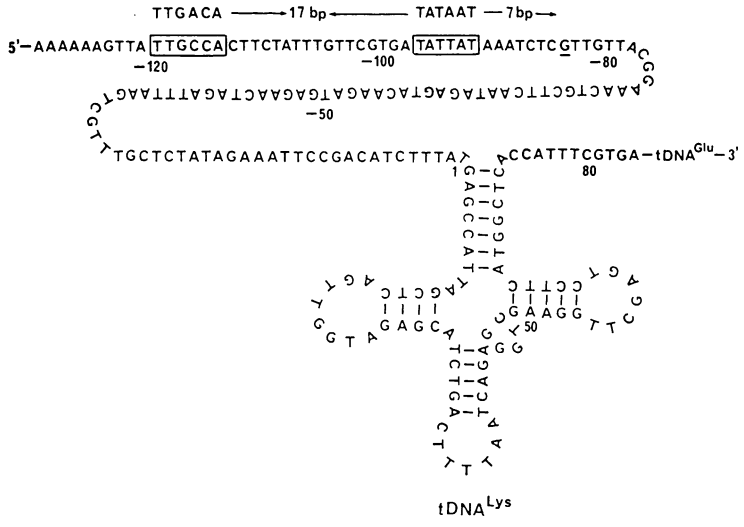

Fig. 6 Nucleotide sequence at a region upstream of $tDNA^{Lys}$ and putative promoter structure of $tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Asp}$, and $tRNA^{Phe}$ genes.
The boxes show the sequences of -35 region and -10 region.
The sequences described above the boxes are consensus sequences for σ^{55}-factor. The underline shows a putative starting point of transcription.

the RNA sequences expected from $tDNA^{Glu}$ and $tDNA^{Asp}$ showed 15 and 7 substitutions, respectively (Fig. 4). As shown in Fig. 5, all four genes can be represented in the tRNA cloverleaf structure. Two tRNA genes, $tDNA^{Lys}$ and $tDNA^{Asp}$, encoded the CCA sequence, but the other two, $tDNA^{Glu}$ and $tDNA^{Phe}$, did not.

Several initiation factors have been defined in *B. subtilis* (10). The σ^{55}-factor, an initiation factor in the vegetative cell, recognizes the same sequence as *E. coli* σ factor. Two promoter-like sequences for σ^{55}-factor were found; one in a region about 100bp upstream from $tDNA^{Lys}$ and the other in a region between $tDNA^{Glu}$ and $tDNA^{Asp}$. The first promoter set showed homologies of 5 out of 6 base pairs to the consensus sequence of σ^{55}-recognition both in the "-10" (-97→-92) and the "-35" (-120→-115) regions. In this promoter set, 17 base pairs were present between "-10" and "-35" regions. This region seems to be a functional promoter (Fig. 6). Though in the second set also 17bp were present between the "-10" (181→186) and the "-35" (158→163), they showed homologies of only 4 out of 6 base pairs both in the "-10" and "-35" regions. The second promoter set will be no

more than a weak promoter, even if it works as a promoter. None of sites for the other σ-factors were found. The region of an oligo-T sequence (450→455) was presumed as a terminator (Fig. 5).

DISCUSSION

The cloned DNA which we have sequenced, contains a cluster of four tRNA genes. That these tRNA genes are functionally expressed is confirmed by the fact that the fragment hybridized with four tRNA species obtained from spots separated by two dimensional polyacrylamide gel electrophoresis (11). The cluster of four tRNA genes have a σ^{55}-promoter site at the region upstream of $tDNA^{Lys}$ and a terminator site at the position downstream of $tDNA^{Phe}$. The secondary structure of its terminator can be described in a very unique form, which might exert some attenuator effect. The nucleotide sequence corresponding to rRNA was not detected in this Eco RI-Hinc II fragment. We beleive that this tRNA gene-cluster is not a spacer gene of rRNA genes. These tRNA genes are probably transcribed in the vegetative cell stage. All four tRNA genes determined here were tailed with a stretch of oligo-T at the ends of their structural genes. This stretch might be an influential signal for the processing of tRNA gene-transcript or it might be mere fortuity.

Our results showed that two tRNA genes, $tDNA^{Lys}$ and $tDNA^{Asp}$, encoded the CCA sequence of 3'-terminal region, but the other two, $tDNA^{Glu}$ and $tDNA^{Phe}$ did not. Wawrousek and Hansen have observed that $tRNA^{Asn}$ and $tRNA^{Thr}$ genes from *B. subtilis* did not encoded the CCA sequence (2). We conclude it to be a general phenomenon that in *B. subtilis* some tRNA genes encode the CCA sequence and the others do not. This raises a new problem of tRNA-processing in *B. subtilis*. The indispensability of the tRNA-nucleotidyltransferase which had been detected in *B. subtilis* (12) will have to be examined. All the tRNA genes from *E. coli* encode the CCA sequence, whereas none of eukaryotic tRNA genes do. Thus, *B. subtilis* tRNA genes are evolutionally characterized as intermediates between *E. coli* and eukaryotic tRNA genes. In this sense, it is worthy noting that one of two spacer tRNA genes from a blue-green alga, *Anacystis nidulans*, encoded the CCA sequence but the other did not (Tomizuka, H. and Sugiura, M.: personal communication).

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