Regulation of the expression of bacteriophage T4 gene *30.9*

A. Driukas,
R. Nivinskas

Institute of Biochemistry,
Mokslininkų 12,
LT-2600 Vilnius, Lithuania

We searched for transcription regulation elements in the noncoding region immediately upstream gene *30.9*. Primer extension analysis of total RNA isolated from cells infected with T4 wild-type phage revealed several reverse transcriptase stops. Primer extension sequencing of RNA isolated from cells infected with T4 *33*$^-$, *55*$^-$, *45*$^-$, *motA*$^-$ mutant phage showed that the observed reverse transcriptase stops are not due to the phage T4 transcription regulation factor-dependent initiation of transcription. We constructed recombinant plasmids carrying the fusion gene *30.9*::*lacZ* with or without the noncoding region upstream and determined the β-galactosidase activities. The presence of noncoding region upstream the fusion gene *30.9*::*lacZ* resulted in a 14-fold increase of β-galactosidase activity. Our results indicate a possible existence of transcription regulation elements in the noncoding region upstream gene *30.9*.

Key words: bacteriophage T4, gene *30.9*, primer extension sequencing, gene fusion

INTRODUCTION

Bacteriophage T4 uses several stages of transcription. This temporal progression is achieved by modifying the host RNA polymerase and by using three different classes of T4 promoters: early, middle, and late. The phage T4 early promoters can be recognized by largely unmodified host RNA polymerase, except for partial ADP-ribosylation of the α subunit. Middle transcription requires two phage-encoded proteins: MotA transcription activator and AsiA coactivator. Transcription at the T4 late promoters requires the T4 σ protein gp55, a helper protein gp33 and activation by the sliding clamp of the T4 DNA replication machinery, gp45 [1].

All known genes expressed early in infection and part of late genes are transcribed counterclockwise, while in the clockwise direction of the genomic map only the late genes are transcribed. The late genes transcribed counterclockwise are generally included on early as well as on late transcripts. Gene *30.9* is located in the direction of early transcription and like many T4 genes could be transcribed from several promoters belonging to different temporal classes. Transcription of eight genes including *rIII* and *30.9 in vitro* has been shown to be initiated at P_E131.7 (upstream from gene *cd.2*) and terminated at t128.6 (downstream gene *30.9*) [2]. A middle mode *motA*-dependent transcript initiated from the gene *31* promoter P_M31 has been observed *in vivo* [3]. Late promoter consensus sequence immediately downstream termination codon of gene *31* experimentally was confirmed to be the active late promoter P_LrIII [4].

Intergenic space between genes *rIII* and *30.9* is 244 nt long (Fig. 1). Such a long noncoding region is quite unusual for the bacteriophage T4 genome

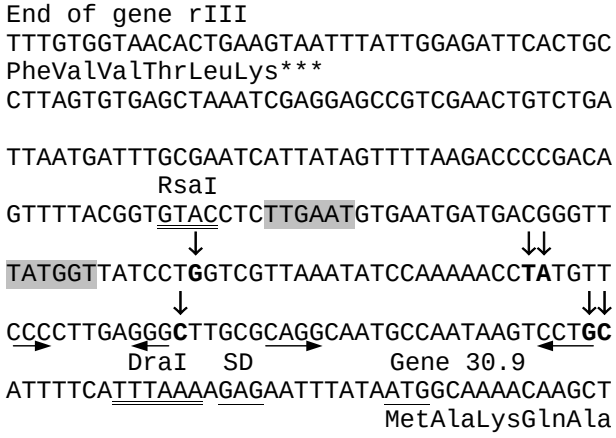

Fig. 1. Nucleotide sequence of the intergenic region between genes *rIII* and *30.9* of bacteriophage T4. The initiation codon and SD sequence for gene *30.9* are underlined. The sites of restriction endonucleases used for cloning are double-underlined. The termination codon of gene *rIII* is indicated by asterisks. Vertical arrows indicate reverse transcriptase stops observed by primer extension sequencing of RNA. Horizontal arrows indicate sequences predicted to form secondary mRNA structures. The putative *E. coli* promoter sequence is shaded

[5], though still not characterized. In this study we have used RNA sequencing to search for transcription regulation elements in the noncoding region immediately upstream gene *30.9*. Also we have examined the possibility of transcription initiation in the noncoding region upstream gene *30.9* without phage T4 infection background.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *Escherichia coli* strain DH5α was used for transformation and preparation of plasmid DNA and measurements of β-galactosidase activity. *E. coli* strain B^E (*su*$^-$) was obtained from Dr. L. W. Black. Bacteriophage T4D wild-type was supplied by Dr. W. B. Wood. Mutant phages T4 *motA*$^+$(*33amN134-55amBL292-45amE10*) and T4 *motA*$^-$ (*33amN134-55amBL292-45amE10-tsG1*) were obtained from Dr. N. Guild.

Plasmid constructions. The recombinant plasmids pBS30.9lac and pBD30.9lac carrying gene *30.9*::*lacZ* fusion were obtained as follows. Bacteriophage T4 genes *rIII* and *30.9* were PCR-amplified from phage with the aid of the synthetic primers Pr. 1, 5´-GCTCTGGGTCTTAAGCAGCC and Pr. 2, 5´-CCTCAACTTATGCTTTCTGC. The resulting DNA fragment was digested with *Bgl*II and *Eco*72I (cutting gene *30.9* at the position 127 nt) and inserted into *Bam*HI-*Sma*I sites of vector pT7-5 [6]. The obtained pTD5-1 was digested with *Rsa*I (cutting the noncoding region upstream gene *30.9* at the position 133 nt) and *Hind*III. The resulting DNA fragment was ligated into the *Sma*I-*Hind*III sites of plasmid pNM480 [7] containing gene *lacZ*. In the yielding plasmid pND480-1, the *30.9* frame was fused to the *lacZ* open reading frame. The plasmid pND480-1 was digested with *Dra*I (cutting the noncoding region upstream gene *30.9* at the position 15 nt) and *Bsu*15I. The resulting DNA fragment was subcloned into pNM480 *Sma*I-*Bsu*15I sites to give pND480-2. An *Eco*RI-*Eco*32I fragment from pND480-1 was inserted into the *Eco*RI-*Eco*32I sites of pBSR3lac30 (recombinant plasmid on the basis of the cloning vector pBSPL0 [8] carrying gene *rIII*::*lacZ* fusion). This resulted in the plasmid pBS30.9lac. The plasmid pBD30.9 has been obtained following the same procedure, but the *Eco*RI-*Eco*32I fragment was excised from plasmid pND480-2.

RNA methods. *E. coli* B^E (*su*$^-$) cells were grown at 37 °C (at 42 °C in the case of mutant phages) to a density of 2×10^8 cells/ml in LB medium. The cells were infected with bacteriophage T4D$^+$ or its mutants T4 *motA*$^+$ and T4 *motA*$^-$ at a m.o.i. of 5. RNA isolation, primer labeling and RNA sequencing were performed as described by McPheeters [9]. The assay was carried out with AMV reverse transcriptase (Promega), using the primer 5'-CCA GCGCGTTTAGAATCACC complementary to the region downstream the start codon of gene *30.9*.

Measurements of β-galactosidase activities. β-Galactosidase activities were determined as described by Miller [10]. The cultures were grown at 37 °C to an OD_{600} of 0.4–0.6 in LB medium and assayed.

RESULTS AND DISCUSSION

To search for transcription regulation elements in the noncoding region immediately upstream gene *30.9*, primer extension analysis of total RNA extracted from T4-infected cells was applied. The 5' ends of transcripts, as well as secondary mRNA structures and RNA endonuclease recognition sites are indicated by reverse transcriptase stops (dark bands at the same position in all four sequencing lanes). Primer extension sequencing of RNA isolated from wild-type T4D-infected cells revealed several strong reverse transcriptase stops at the positions CG and AU, 26–27 and 70–71 nt upstream the gene *30.9* (Fig. 2A). Also, a few faint stops at the positions C (54 nt) and G (93 nt) were observed. We analyzed sequences upstream the observed reverse transcriptase stops, but no sequences matching any of three phage T4 promoter consensus sequences were revealed. Instead of that, an *E. coli* promoter sequence upstream the stop at the position G (93 nt) was found. The sequences predicted to form secondary mRNA structures could be distinguished upstream the stops at the positions CG (26–27 nt) and G (54 nt). To test whether appearance of observed reverse transcriptase stops depended on phage T4 transcription regulation factors, primer extension sequencing of total RNA isolated from cells infected by either T4 *motA*$^+$ (*33*$^-$, *55*$^-$, *45*$^-$) or T4 *motA*$^-$ (*33*$^-$, *55*$^-$, *45*$^-$, *tsG1*) was performed. The products of genes *33*, *55* and *45* are required for late transcription [11] and the product of gene *motA* for middle transcription [12]. Only early transcripts independent of any phage transcription regulation factors are available in the case of infection with phage T4 *motA*$^-$. Figure 2B shows the results of primer extension sequencing of total RNA isolated from T4 *motA*$^+$ and T4 *motA*$^-$-infected cells. The reverse transcriptase stops observed in the sequencing of the wild-type T4 RNA also are visible in the case of phage T4 *motA*$^-$ RNA, indicating that they are not due to phage T4 transcription regulation factor-dependent initiation of transcription.

In order to test whether the noncoding region upstream gene *30.9* is transcriptionally active without the phage T4 infection background, the recombinant plasmids pBS30.9lac and pBD30.9lac carrying fusion genes *30.9*::*lacZ* were constructed (see

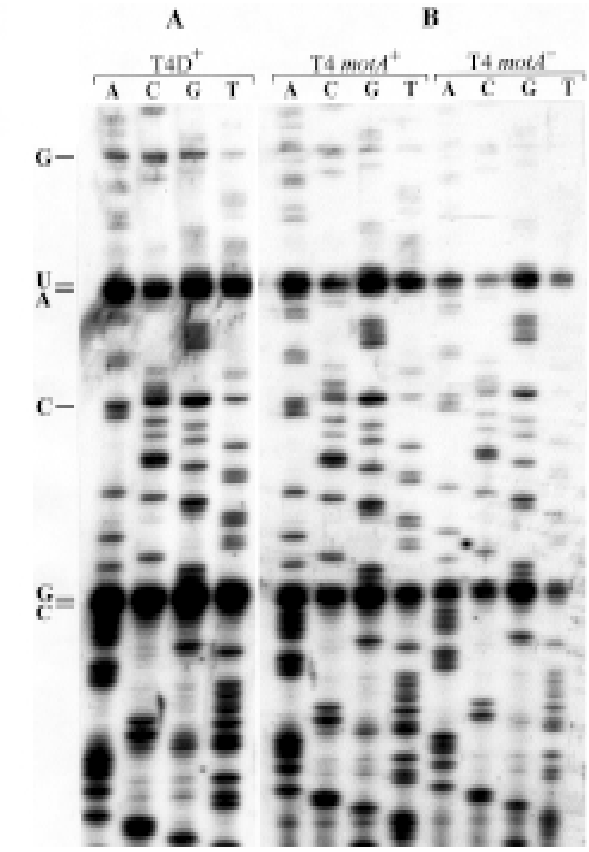

Fig. 2. Primer extension sequencing of the transcripts for gene *30.9*. Primer extension reactions were done on RNA isolated at 12 min post infection from cells infected with T4 wild-type (A) phage and on RNA isolated at 6 min post infection from cells infected with T4 *motA*$^+$ or T4 *motA*$^-$ (B) phages. The reverse transcriptase stops are noted. The sequencing lanes are labeled with the dideoxynucleotides used in the sequencing reactions

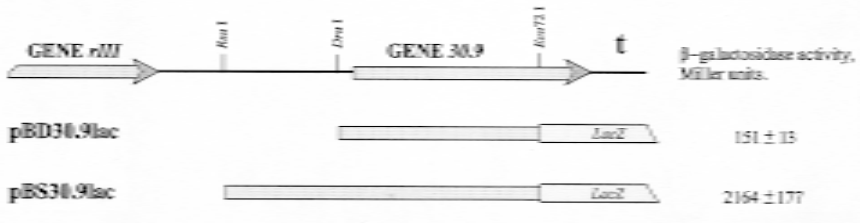

Fig. 3. The genomic region of bacteriophage T4 carrying gene *30.9*. The scheme of fusion plasmids pBS30.9lac and pBD30.9lac is shown below. The β-galactosidase activities (Miller units) produced by each fusion are on the right side of the scheme. Each value represents the average of at least six assays

Materials and Methods). Plasmid pBS30.9lac contains a 133 nt long noncoding region encompassing the observed reverse transcriptase stops upstream the fusion gene. In the case of plasmid pBS30.9lac only SD sequence is left upstream fusion gene (Figs. 1 and 3). Figure 3 shows the β-galactosidase activities produced by each fusion. The ratio between relative expression levels of the fusion gene *30.9*::*lacZ* in the case of pBS30.9lac and pBD30.9lac was approximately 14:1 (2164 units:151 units). The observed β-galactosidase activity in the case of pBD30.9lac could reflect the ground level of transcription, as no promoter sequence is located upstream the polylinker and the promoter for the *bla* gene directs transcription in the opposite direction, while the presence of a noncoding region upstream the fusion gene in the case of pBS30.9lac results in a 14-fold increase of β-galactosidase activity.

The obtained results support the presumption that transcription of gene *30.9* can be directed by transcription regulation elements located in the noncoding region upstream gene *30.9*. Also we showed that the putative transcriptional elements might be recognized by the native (unmodified) *E. coli* RNA polymerase. On the other hand, at least some of the observed reverse transcriptase stops indicate the mRNA secondary structures or degradation products. However, in order to identify and characterize the putative transcription regulation elements upstream gene *30.9,* further experiments are required.

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A. Driukas, R. Nivinskas

T4 BAKTERIOFAGO *30.9* GENO EKSPRESIJOS REGULIACIJA

S a n t r a u k a

Tarp T4 bakteriofago *rIII* ir *30.9* genų yra 244 bp ilgio tarpgeninė sritis. Siekiant ištirti, ar šioje srityje yra reguliacinės sekos, atsakingos už *30.9* geno ekspresiją, buvo taikytas pradmens prailginimo metodas. Analizuojant RNR, išskirtą iš ląstelių, infekuotų laukinio tipo T4 fagu, buvo identifikuotos kelios AMV atvirkštinės transkriptazės sustojimo vietos. Nustačius mutantinio T4 *33*$^-$, *55*$^-$, *45*$^-$, *motA*$^-$ fago RNR nukleotidų seką, buvo parodyta, kad stebėtos atvirkštinės transkriptazės sustojimo vietos nesusijusios su T4 fago transkripcijos veiksnių reguliuojama transkripcijos iniciacija. Siekiant įvertinti tarpgeninės srities poveikį *30.9* geno ekspresijai, sukonstruotos plazmidės, kuriose *E. coli* β-galaktozidazės *lacZ* genas sulietas pagal atvirą skaitymo rėmelį su *30.9* genu. β-Galaktozidazės aktyvumo matavimai parodė, kad tarpgeninės srities buvimas prieš *30.9*::*lacZ* rekombinantinį geną padidina šio geno ekspresiją keturiolika kartų. Gauti rezultatai patvirtina galimą transkripcijos reguliacijos sekų egzistavimą tarpgeninėje srityje prieš *30.9* geną.