Studies on the expression of genes *rIII* and *30.9* of bacteriophage T4 *in vivo*

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We have constructed two bacteriophage T4 mutants carrying genes *rIII* and *30.9* fused with the 5′ end of the *lacZ* β-galactosidase (βGal) gene of *Escherichia coli*. The obtained mutant phages were tested for the kinetics of βGal expression during T4 infection in *E. coli* cells. We have determined that βGal activity becomes detectable 5–6 min following infection and reaches the plateau about 35 min post infection at 30 °C in the case of both phages. This indicates that bacteriophage T4 genes *rIII* and *30.9* are expressed early as well as late in infection under the control of three different T4 promoters.

Key words: bacteriophage T4, genes *rIII* and *30.9*, gene fusions, expression pattern

INTRODUCTION

The temporal control of differential gene expression of bacteriophage T4 ensures the optimal supply of individual gene products when they are needed in the life cycle. Genes whose products are required for the transition from host to phage metabolism and for DNA replication are expressed prereplicatively. Other genes are expressed exclusively or predominantly after the onset of DNA replication. These genes code for structural components of the viral particle, enzymes that package vegetative DNA into capsids and proteins that destroy the cell wall of the host bacteria to implement phage release [1].

Bacteriophage T4 genes are temporally regulated mostly at the level of transcription. The progression in time is achieved by modifying the host RNA polymerase and by using three different classes of T4 promoters: early, middle and late. 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 late genes are transcribed. Most phage T4 genes located in the counterclockwise direction can be transcribed from several different promoters so that many late transcripts overlap with those initiated from early or middle promoters. Posttranscriptional control mechanisms prevent translation of several late genes from RNA cotranscribed with early genes, so few or no protein products of these late genes are made early. In the cases of genes *e*, *49*, *soc* and I-*Tev*I an RNA stem-loop blocks translation of the early transcripts [2].

Bacteriophage T4 genes *rIII* and *30.9* are located in the direction of early transcription. Three promoters transcription from which was confirmed experimentally are located upstream genes *rIII* and *30.9* without intervening terminators (Fig.1). Transcription of eight genes including *rIII* and *30.9* is directed from the early promoter P_E131.7 upstream gene *cd.2* and terminated at t128.6 (downstream gene *30.9*) [3]. A middle mode *motA*-dependent transcript is initiated from gene *31* promoter P_M31 [4]. Finally, the late promoter P_LrIII upstream gene *rIII* defines late transcription of genes *rIII* and *30.9* [5]. Bacteriophage T4 gene *rIII* is a “rapid lysis” gene, mutations in which result in the absence of lysis inhibition. Gene *rIII* mutants have been widely used in early phage studies, though the biochemical function of the gene product has not yet been identified. Gene *30.9* is a nonessential bacteriophage T4 gene with unknown function.

The aim of this work is to determine expression patterns of phage T4 genes *rIII* and *30.9 in vivo*.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *Escherichia coli* strain DH5α was used for the transformation, preparation of plasmid DNA and measurements of β-galactosidase activity. *E. coli* strain B^E (*su*$^-$) was obtained from Dr. L. W. Black. Bacteriophage T4 wild-type was supplied by Dr. W. B. Wood.

Plasmid constructions. The recombinant plasmid pBSrIIIlac30 carrying gene *rIII*::*lacZ* fusion was obtained as follows. The recombinant plasmid pRA5-

2 with T4 genes *31.2-30.9* constructed previously on the basis of the plasmid vector pT7-5 [6] was treated with *Rsa*I. The resulting 0.83 kb DNA fragment containing gene *rIII* was recloned into the *Sma*I site of pT7-5. The *Eco*RI-*Hind*III DNA fragment containing gene *rIII* was excised from the yielding plasmid pTR3-1 and subcloned into *Eco*RI-*Hind*III sites of the vector pBSPL0$^+$ [7] resulting in the plasmid pBSR3. At the same time the plasmid pNM480 [8] had been cleaved with *Bam*HI and *Hind*III, treated with DNA polymerase I Klenow fragment and ligated, eliminating *Bam*HI-*Hind*III restriction sites in the pNM480 polylinker. The resulting plasmid was digested with *Eco*RI-*Dra*I, and the obtained DNA fragment containing *lacZ* gene was cloned into the pT7-5. The *Sma*I-*Hind*III DNA fragment from the resulting plasmid pT75lacZ was inserted into *Bpu*1102I(fill-in)-*Hind*III sites of the plasmid pBSrIII. In the yielding plasmid pBSrIIIlacZ the *rIII* frame was fused to the *lacZ* open reading frame. The *Ade*I-*Eco*RI(fill-in) DNA fragment carrying the 3′ end of gene *lacZ*, the 3′ end of gene *30.1* and the 5′ end of gene *30* was excised from the recombinant plasmid pRT02-1 [9] and ligated into the *Ade*I-*Hind*III(fill-in) sites of the pBSrIIIlacZ. This resulted in the plasmid pBSrIIIlac30.

For construction of the recombinant plasmid pBS30.9lac30, a 0.69 kb *Bgl*II-*Eco*72I fragment containing gene *rIII* and the 5′ end of gene *30.9* was isolated from the plasmid pRA5-2 and subcloned into *Bam*HI-*Sma*I sites of vector pT7-5. The obtained plasmid pTD5-1 was digested with *Rsa*I and *Hind*III. The resulting 0.26 kb DNA fragment was ligated into the *Sma*I-*Hind*III sites of plasmid pNM480. In yielding plasmid pND480-1 the *30.9* frame was fused to the *lacZ* open reading frame. An *Eco*RI-*Eco*32I fragment from the plasmid pND480-1 was inserted into the *Eco*RI-*Eco*32I sites of pBSrIIIlac30. This resulted in the plasmid pBS30.9lac30.

The obtained gene fusions were transferred to the T4 genome. T4 mutant phages forming blue plaques were selected on *E. coli* strain DH5α plating with X-gal.

Measurements of β-galactosidase activities. β-Galactosidase activities were determined as described by Miller [10].

RESULTS AND DISCUSSION

Analysis of bacteriophage T4 prereplicative proteins revealed that proteins performing related functions generally show similar patterns of synthesis. This gives some hope that understanding the levels and kinetics of production of not yet identified proteins will aid in determining their functions [11].

To study the expression patterns of phage T4 genes *rIII* and *30.9 in vivo*, the generated *rIII*::*lacZ* and *30.9*::*lacZ* fusions from the recombinant plasmids (see Materials and Methods) via homologous recombination were introduced into the phage genome (Fig. 1). The obtained mutant phages were designated as T4 *rIII*::*lacZ* and T4 *30.9*::*lacZ*.

Mutant phages T4 *rIII*::*lacZ* and T4 *30.9*::*lacZ* were used for infection of *E. coli* DH5α strain cells. βGal activities were assayed at different times after infection. We have determined that βGal activity in *E. coli* cells becomes detectable about 5–6 min following infection and reaches the plateau about 35 min post infection at 30 °C (Fig. 2) in the case of both phages. Given the fact that during T4 infection transcription rate at 30 °C is 18 nt/s [12], it takes about 2.3 and 0.3 minutes for RNA polymerase to reach gene *rIII* from the promoters P_E131.7 (2.5 kb upstream gene *rIII*) and P_M31 (0.37 kb upstream gene *rIII*), respectively. Taking into account that transcription from the early promoter P_E131.7 starts immediately after infection and transcription from the middle promoter P_M31 starts 1.5 min later, transcription of the gene *rIII* directed from the both promoters should begin about 1.8–2.3 min post infection. Since the translation is usually coupled to transcription, the product the of 3.3 kb long gene fusion can appear about 5 min after infection. As the gene *30.9* is located 0.42 kb further downstream of gene *rIII*, βGal activity becomes detectable with a slight delay in the case of the gene fusion *30.9*::*lacZ*. On the other hand, the βGal activity reaching the plateau only about 35 min post infection shows expression of genes *rIII* and *30.9* at the late stage of infection from the promoter P_LrIII. Though

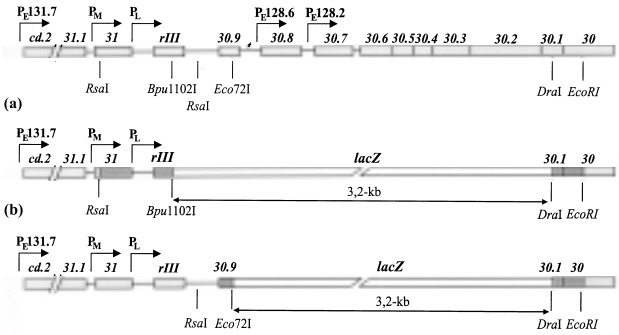

Fig. 1. Construction of T4 mutants carrying gene fusions *rIII*::*lacZ* (*a*) and *30.9*::*lacZ* (*b*). The schematic outline of T4 genomic region between early promoter P_E131.7 and gene *30* is presented on the top of the figure. The positions of promoters, terminator and DNA restriction sites used for cloning are shown. Dark boxes indicate T4 DNA fragments cloned into recombinant plasmids for the homologous recombination between plasmid and phage genome. White boxes indicate *E. coli* gene *lacZ*, and gray boxes stand for genes from phage genome

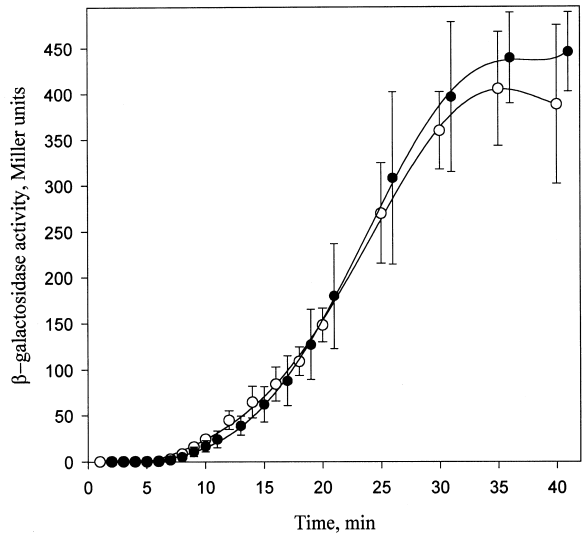

Fig. 2. β-Galactosidase activities (Miller units) in *E. coli* DH5α cells infected with T4 mutant phages T4 *rIII*::*lacZ* (filled circles) and T4 *30.9*::*lacZ* (open circles). *E. coli* DH5α cells were grown at 30 °C to A_{590} = 0.75 in LB medium. The cells were then infected with T4 mutant phages at a multiplicity 5 of infection. Samples were taken at various times after infection. The cells were harvested by centrifugation and resuspended in A medium. β-Gal activity was measured as described by Miller (1972)

the long noncoding regions upstream genes *rIII* and *30.9* imply a possible existence of negative posttranscriptional control elements such RNA stem-loop structures, it seem likely that translation of the products of genes *rIII* and *30.9* proceeds unimpeded from the transcripts directed from the early and middle promoters.

The main lysis inhibition gene *rI* is similarly regulated, though its expression has not yet been shown *in vivo*. The *rI* gene is the second gene in a three-ORF transcription unit defined by a canonical late promoter. Also, an early promoter is located 3.3 kb upstream the *rI* gene with no intervening terminator [13].

Translation of gene *rIII* product early as well as late in infection is in accordance with the requirement for gprIII in the function of lysis inhibition for the interval of time at which lysis inhibition that can be induced by secondary phage adsorption begins as early as 3 min after infection and lasts until lysis starts [14]. The expression pattern of gene *30.9* is very similar to that of the *rIII*. This result, together with the fact that T4 genes grouped into transcription units often have a related function, raises the question of whether *30.9* might also be involved in lysis inhibition. However, we observed no rapid-lysis phenotype in the case of mutant phage T4 *30.9*::*lacZ*. It therefore appears that gene *30.9* is not required for lysis inhibition, although it may still play some supportive lysis-related role.

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T4 BAKTERIOFAGO *rIII* IR *30.9* GENŲ EKSPRESIJOS TYRIMAS *in vivo*

S a n t r a u k a

Šiame darbe buvo sukonstruoti du T4 bakteriofago mutantai, kuriuose *rIII* ir *30.9* genai yra sulieti su *E. coli lacZ* β-galaktozidazės genu. Gautais mutantiniais fagais infekavus *E. coli* ląsteles, buvo tiriama βGal ekspresijos kinetika. Nustatyta, kad βGal aktyvumas tiek vieno, tiek kito fago atveju atsiranda 5–6-ą minutę po infekcijos ir 30 °C temperatūroje pasiekia maksimalią reikšmę 35-ą minutę. Šie rezultatai rodo, kad T4 bakteriofago *rIII* ir *30.9* genų ekspresiją kontroliuoja trys skirtingi fago promotoriai ir tai vyksta tiek ankstyvuoju, tiek vėlyvuoju fago infekcijos periodu.