BIOLOGIJA. 2006. Nr. 3. P. 12–15

RegB endoribonuclease is involved in the processing of transcripts from *mobD* gene cluster of bacteriophage T4

Aurelija Zajančkauskaitė*,

Eglė Gineikienė,

Giedrė Petkūnienė,

Rimas Nivinskas

Institute of Biochemistry, Mokslininkų 12, LT-08662 Vilnius, Lithuania

Bacteriophage T4 encoded endoribonuclease RegB introduces cuts in the middle of GGAG/U motifs of early phage mRNAs. Analysis of DNA sequence revealed 18 potential cleavage sites for T4 RegB nuclease in genes *mobD.5*-*mobD* comprising the *mobD* gene cluster. Primer extension sequencing of the transcripts from this genomic region showed the existence of seven 5' ends generated by RegB-dependent cleavage. All but one of them occurred in the middle of the GGAG tetranucleotide. Three newly identified RegB nuclease targets are located in the SD sequences of genes *mobD.5*, *mobD.2* and *mobD.1*, while the other four are situated within the coding sequences of transcripts for genes *mobD.4*, *mobD.1* and *mobD*. The primer extension analysis also indicates that most of GGAU motifs escape the processing by RegB. The results show that RegB nuclease efficiently cleaves the transcripts from the *mobD* gene cluster and thus plays a substantial role in the degradation of transcripts from this genomic region.

Key words: bacteriophage T4, endoribonuclease RegB, *mobD* gene cluster

INTRODUCTION

The development of bacteriophage T4 relies on the sequential expression of three classes of genes, early, middle and late, which are transcribed by host RNA polymerase subsequently modified by phage-encoded proteins. Three types of promoters (early, middle and late) activated in a time-dependent manner ensure the temporally regulated transcription program of T4 [1]. Although transcriptional regulation is a predominant factor in T4 gene expression, the rapid shift of gene expression from early to middle and late stages also requires mRNA degradation or stabilization in a stage-dependent manner. Both phage and host encoded nucleases cooperate in determining the levels of T4 mRNAs. *Escherichia coli* endoribonuclease E appears to be the major determinant of mRNA turnover throughout infection [2–6]. *E. coli* RNase III contributes to the processing and degradation of a few T4 mRNAs [7, 8]. Recently, a novel *E. coli* endoribonuclease RNase LS has been shown to play a role in phage T4 metabolism [9, 10] under the control of T4 gene *dmd* product, which is required for the regulation of mRNA stability in a stage-dependent manner [6, 11–13].

Bacteriophage T4 itself encodes a sequence-specific endoribonuclease RegB which post-transcriptionally regulates the expression of T4 early genes. The T4 RegB endoribonuclease, produced shortly after infection, cleaves specifically in the middle of the GGAG motif or, in a few cases, in the GGAU, with a strong bias towards those found in intergenic regions, and in many cases these are Shine–Dalgarno (SD) sequences of early genes. The RegB targets found in coding sequences are for the most part poorly recognized [14–17]. Out of 25 processing sites identified [17–19], 12 occur in SD sequences, thus rendering the ribosome-binding site nonfunctional. Most middle and all late transcripts escape RegB processing. Thus, RegB is required for the normal turnover of T4 early mRNAs, but not for middle and late mRNAs, and it regulates the translation of several pre-replicative mRNAs [20].

Seven genes comprising the *mobD* gene cluster are situated within the T4 genomic region which contains early and middle promoters [1, 21]. Based on microarray analysis [22], the genes of the *mobD* gene cluster were classified as having a complex pattern of transcription: the transcripts appeared early, disappeared during the middle period, and reappeared later. It has been suggested that RegB nuclease could be involved in the regulation of expression of genes from this genomic cluster. Here we have identified seven new RegB endoribonuclease target sites within the *mobD* gene cluster. We present experimental evidence showing that the sequence-specific T4 RegB nuclease is involved in the

* Corresponding author. E-mail: aurelija@bchi.lt

degradation process of these transcripts. The results also clearly shows that RegB nuclease exhibits a strict specificity towards the GGAG motif.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *Escherichia coli* strain B^E (*sup*^{0}) was kindly provided by Dr L. W. Black. This strain was used for the propagation of bacteriophages T4D wild-type and T4 *regB*$^-$ (*regBL52*), as well as for isolation of the total RNA. Bacteriophage T4D wild-type was a gift from Dr W. B. Wood. Bacteriophage T4 *regB*$^-$ (T4 *regBL52*) was kindly supplied by Dr M. Uzan.

RNA preparation and primer extension analysis of phage mRNA. Total RNA from phage-infected *E. coli* cells was phenol-extracted and analyzed by primer extension under conditions of primer excess, using avian myeloblastosis virus reverse transcriptase as described [14, 21]. Eight synthetic oligonucleotides were used to prime reverse transcriptase: Pr.1, 5'-GTAACTTCATCCATGTCGACG, complementary to nt 63–83 of the *mobD.5* gene; Pr.2, 5'-GCGTGTTAGCATACTTAAACTCTCG, complementary to nt 173–183 of the *mobD.4* and 14 nt downstream of the termination codon of this gene; Pr.3, 5'-GAACATTCATAGTCAGAAACTGC, complementary to nt 55–77 of the *mobD.2a* gene; Pr.4, 5'-GTTTCGCCTCGCTCATCATTACA, complementary to nt 1–19 of the *mobD.1* gene and 4 nt upstream of the initiation codon of this gene; Pr.5, 5'-GTCATCTTCTTGCGTCAAACG, complementary to nt 85–105 of the *mobD.1*; Pr.6, 5'-GTCTTTAGTGCAATAGCTTCCTC, complementary to nt 484–506 of the *mobD.1* gene; Pr.7, 5'-GGAAGACTTAATGTTATCGAACG, complementary to nt 376–398 of the *mobD* gene; Pr.8, 5'-GTCTTGCCAATGTTTACCAGTTC, complementary to nt 596–618 of the *mobD* gene. Oligonucleotides were 5'-end-labeled with [γ-^{32}P]ATP (Amersham Biosciences).

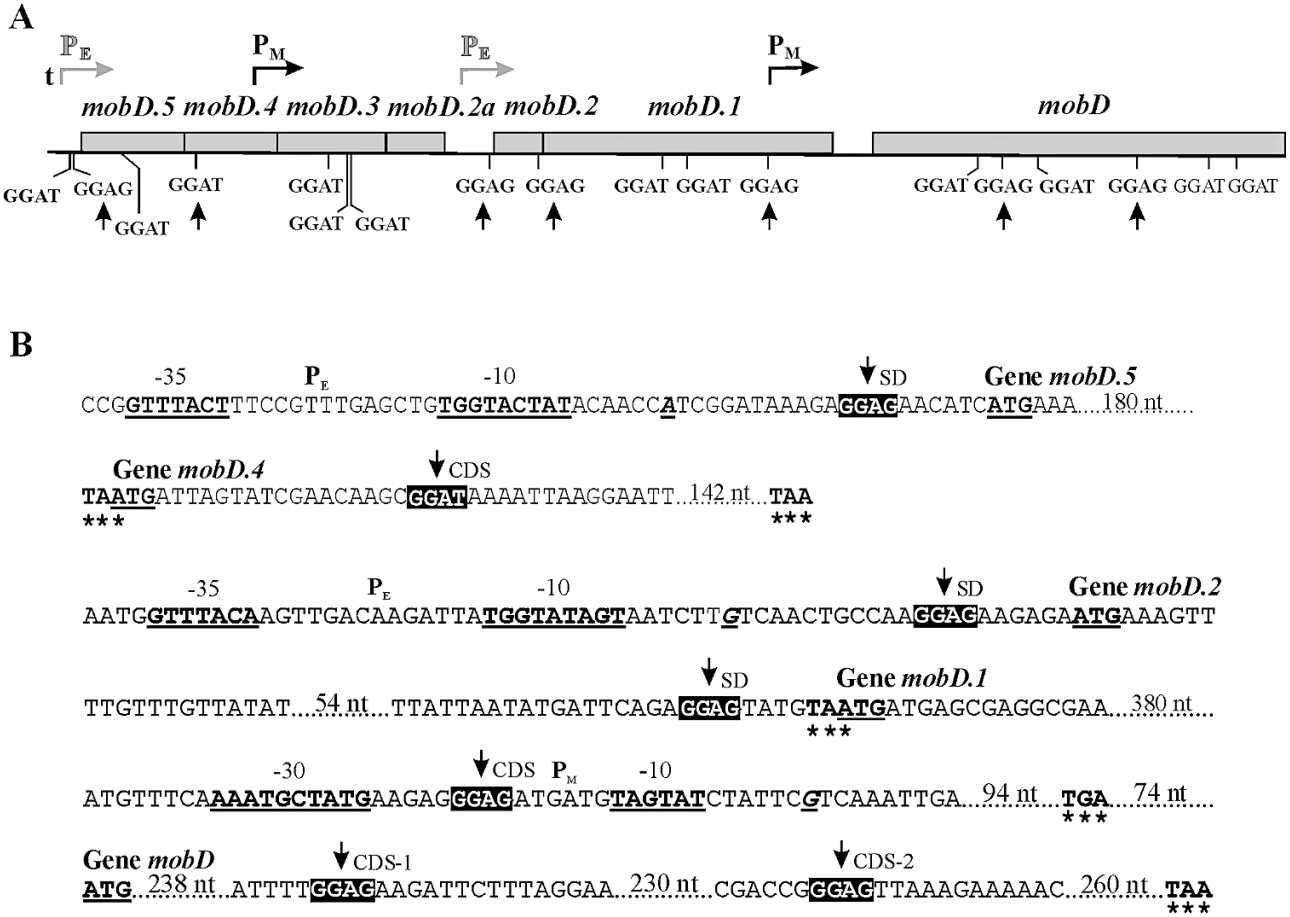

Fig. 1. The *mobD* gene cluster of bacteriophage T4. (A) The positions of potential RegB cleavage sites in the genomic region between genes *mobD.5* and *mobD* of T4. Shown are the locations of terminator (t), early (P_E) and middle (P_M) promoters. The GGAG and GGAU motifs are shown in bold. Vertical arrows show sites susceptible to RegB cleavage. (B) Nucleotide sequences flanking RegB cleavage sites of the genes *mobD.5*, *mobD.4*, *mobD.2*, *mobD.1* and *mobD* transcripts. The –35 and –10 regions of early promoters, the –30 and –10 regions of the middle promoter, the start nucleotides for the transcripts, as well as the initiation codons are in boldface and underlined. The GGAG and GGAU motifs within the SD sequences and in the coding sequences are shown on black background. Asterisks indicate the termination codons for the upstream genes. Vertical black arrows show the positions of RegB cleavages

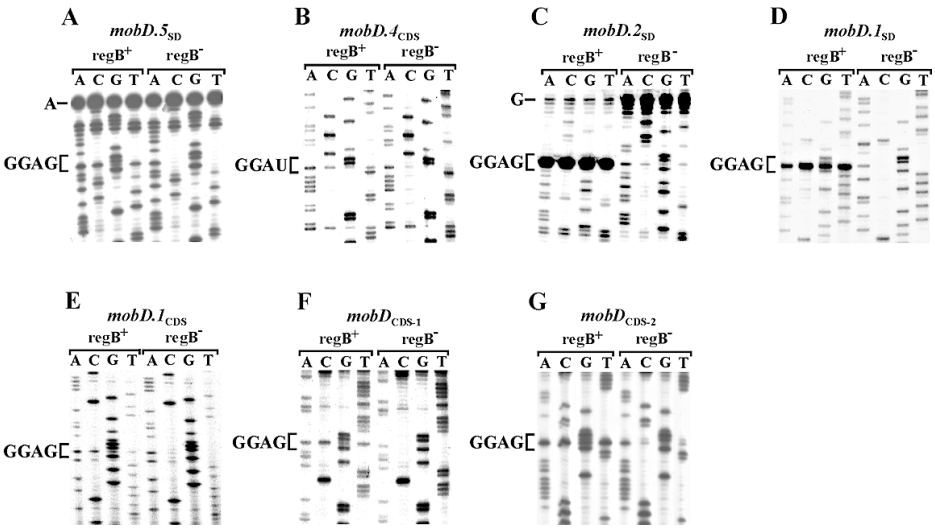

Fig. 2. Primer extension sequencing of the transcripts for genes *mobD.5* (A), *mobD.4* (B), *mobD.2* (C), *mobD.1* (D, E) and *mobD* (F, G) of bacteriophage T4. Primer extension sequencing reactions were done on RNA isolated from *E. coli* B^E cells at four minutes post-infection at 30 °C with T4D wild-type or T4 *regB*$^-$ (T4 *regBL52*). Sequencing lanes are labeled with dideoxynucleotides used in sequencing reactions. The initiating nucleotides for transcripts *mobD.5* and *mobD.2*, as well as GGAG or GGAU motifs within SD sequences and in the coding sequences are noted

RESULTS AND DISCUSSION

Seven genes comprising the *mobD* gene cluster are situated within the genomic region which has been shown to contain a transcription terminator, two early promoters [1], as well as two middle promoters [21]. In order to examine the possible role of T4-encoded RegB nuclease in the degradation of the transcripts from this genomic region, we searched for the putative RegB cleavage sites. DNA sequence analysis revealed 18 potential targets for this nuclease. Most of them have been detected in the coding sequences of the genes, while only three motifs were situated in the intergenic regions just upstream of the genes *mobD.5* and *mobD.2* (Fig. 1A). The activity of the RegB nuclease towards the GGAG and GGAU motifs has been tested by primer extension analysis of RNA isolated from the *E. coli* cells after infection with T4D$^+$ or T4 *regB*$^-$ mutant, T4 *regBL52*. Primer extension analysis revealed even nine bands reflecting the reverse transcriptase (RT) stops. The 5' ends of the transcripts truncated in the middle of the SD sequence motif GGAG occurred in the transcripts for the genes *mobD.5*, *mobD.2* and *mobD.1* (Fig. 2 A, C, D). In the case of gene *mobD.1*, the second RegB-dependent cut was observed in the distal part of the gene *mobD.1* mRNA (Fig. 2 E). The RT stops corresponding to the mRNA truncated at the middle of the GGAU motif located within the *mobD.4* transcript have been also observed (Fig. 2 B). Finally, two 5' ends assigned to the RegB cleavage event have been detected within the coding sequence of the gene *mobD* transcript (Fig. 2 F, G). The analysis of RNA isolated from *E. coli* cells infected with T4 *regB*$^-$ mutant revealed the absence of truncated transcripts in all the cases studied. Only transcriptional start sites from early promoters were detected as 5' ends in the cases of genes *mobD.5* and *mobD.2* (Fig. 2 A, C). It should be noted that the GGAU motifs located within the coding sequences of genes *mobD.5*, *mobD.3*, *mobD.1*, *mobD*, as well as the GGAU motif upstream gene *mobD.5* (Fig. 1 A) were resistant to RegB nucleolytic activity (data not shown). Thus, the primer extension analysis indicated the existence of seven RegB cleavage sites located within the *mobD* gene cluster.

As mentioned above, a detailed analysis of DNA sequence of this gene cluster revealed 18 potential targets for RegB nuclease – six GGAG and twelve GGAT motifs. However, only seven of them have been shown to be the substrates for the RegB nuclease attack. It should be noted that all six GGAG motifs were shown to be susceptible to the nucleolytic attack of RegB. In contrast, we identified only one GGAU RegB processing site located within the coding sequence for the transcript of gene *mobD.4*. This result is in agreement with the fact that RegB exhibits a rather strict specificity towards the GGAG motif [17, 18]. In T4, only two exceptions were found so far, where the RegB cleaved in the middle of the GGAU motif. These RegB-dependent events were observed within the GGAU tetranucleotide located in the SD region of T4 gene *motB.2*, as well as in the GGAU motif situated within the coding sequence of the transcript for the gene *segF=69* [17, 18]. Recently, we have examined the primary structures and functional properties of RegB ribonucleases encoded by T4-related phages. Interestingly, in the course of these studies we have established that the RegB nuclease encoded by phage RB49 controls its biosynthesis by attacking its own mRNA within the GGAU motif in the SD region [23].

The results of this study show that the T4 genomic region carrying the *mobD* gene cluster is susceptible to the nucleolytic attack of the RegB nuclease. Out of seven genes comprising this cluster, five genes, *mobD.5*, *mobD.4*, *mobD.2*, *mobD.1* and *mobD*, have been shown to contain RegB target sites. The most efficient cleavages in the middle of the SD sequences were observed in the cases of transcripts for genes *mobD.2* and *mobD.1* (Fig. 2 C, D). The transcript for gene *mobD.5* also represents an example of mRNA efficiently recognized

by RegB (Fig. 2 A). Thus, these results are consistent with the fact that RegB sites situated within the SD sequences are efficiently recognized by RegB nuclease [17, 18].

Here we identified T4 RegB targets located within coding sequences of the transcripts for genes *mobD.4*, *mobD.1* and *mobD*. According to the data of other authors [17, 18], not all RegB sites are processed equally by phage-encoded RegB enzyme, i.e. GGAG motifs, as well as the GGAU that lie in coding sequences are generally processed with a lower efficiency than the sites located in the SD regions. The results obtained in this work show that RegB targets situated within the coding sequences are cut with different efficiencies. The GGAU and GGAG motifs located in the coding sequences of *mobD.4* and *mobD.1* transcripts, respectively, are processed at low levels (Fig. 2 B, E). On the other hand, the *mobD* transcript that contains two GGAG motifs within the coding sequence has been shown to be a good substrate for RegB (Fig 2 F, G). Taken together, these results show that some RegB targets located within the coding sequences could be efficiently processed by RegB nuclease.

In this study, we have determined the extent of RegB processing in the *mobD* gene cluster showing that RegB nuclease plays a substantial role in the degradation of the transcripts for genes *mobD.5*, *mobD.4*, *mobD.2*, *mobD.1* and *mobD*. Thus, the rapid appearance and disappearance of the transcripts during the middle period of phage infection [22] could be associated with an efficient degradation of mRNA from this genomic region. Moreover, the results of this study extend the list of bacteriophage T4 mRNAs containing the targets for RegB enzyme, and now we know 32 RegB cleavage sites located in the phage T4 genome.

ACKNOWLEDGMENTS

We appreciate Fermentas AB for kindly supplied reagents for molecular biology. This work was supported in part by the Lithuanian State Science and Studies Foundation.

Received 12 February 2002
Accepted 4 June 2006

References

1. Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Rüger W. Microbiol Mol Biol Rev 2003; 67: 86–156.
2. Mudd EA, Prentki P, Belin D, Krish HM. EMBO J 1988; 7: 3601–7.
3. Carpousis AJ, Mudd EA, Krish HM. Mol. Gen Genet 1989; 219: 39–48.
4. Mudd EA, Carpousis AJ, Krish HM. Genes Dev 1990; 4: 873–81.
5. Loyaza D, Carpousis AJ, Krisch HM. Mol Microbiol 1991; 5: 715–25.
6. Otsuka Y, Ueno H, Yonesaki T. J Bacteriol 2003; 185: 983–90.
7. Pragai B, Apirion D. J Mol Biol 1982; 154: 465–84.
8. Barth KA, Powell G, Trupin M, Mosig G. Genetics 1988; 120: 329–43.
9. Otsuka Y, Yonesaki T. Genetics 2005; 169: 13–20.
10. Yamanishi H, Yonesaki T. Genetics 2005; 171: 419–25.
11. Kai T, Selick EH, Yonesaki T. Genetics 1996; 144: 7–14.
12. Kai T, Ueno H, Yonesaki T. Virology 1998; 248: 148–55.
13. Ueno H, Yonesaki T. Genetics 2001; 158: 7–17.
14. Uzan M, Favre R, Brody E. Proc Natl Acad Sci USA 1988; 85: 8895–99.
15. Ruckman J, Parma D, Tuerk C, Hall DH, Gold L. New Biol 1989; 1: 54–65.
16. Sanson B, Uzan M. J Mol Biol 1993; 233: 429–46.
17. Sanson B, Uzan M. FEMS Microbiol Rev 1995; 17: 141–50.
18. Uzan M. Methods Enzymol 2001; 342: 467–80.
19. Truncaite L, Zajančkauskaite A, Arlauskas A, Nivinskas R. Virology 2006; 344: 378–90.
20. Sanson B, Hu RM, Troitskaya E, Mathy N, Uzan M. J Mol Biol 2000; 297: 1063–74.
21. Truncaite L, Piešiniene L, Kolesinskiene G, Zajančkauskaite A, Driukas A, Klausa V, Nivinskas R. J Mol Biol 2003; 327: 335–46.
22. Luke L, Radek A, Liu X, Campbell J, Uzan M, Haselkorn R, Kogan Y. Virology 2002; 299: 182–91.
23. Piešiniene L, Truncaite L, Zajančkauskaite A, Nivinskas R. Nucleic Acids Res 2004; 32: 5582–95.

A. Zajančkauskaitė, E. Gineikienė, G. Petkūnienė, R. Nivinskas

RegB ENDORIBONUKLEAZĖ INAKTYVUOJA T4 BAKTERIOFAGO *mobD* SRITIES GENŲ TRANSKRIPTUS

S a n t r a u k a

T4 bakteriofagas koduoja RegB endoribonukleazę, kuri kerpa ankstyvąsias fago informacines RNR ties Šaino-Dalgarno sekos GGAG motyvo viduriu. Analizuojant *mobD.5-mobD* genų nukleotidų sekas buvo aptikta 18 potencialių RegB nukleazės skėlimo taikinių. Atlikus šioje genomo srityje esančių transkriptų analizę pradmens ilginimo metodu buvo rasti dėl RegB nukleazės skėlimo susidarę nauji transkriptų 5' galai. Daugelis šių transkriptų buvo perskelti ties GGAG sekos viduriu ir tik vienas jų – GGAU motyvo viduryje. Trys naujai identifikuoti RegB skėlimo taikiniai yra *mobD.5*, *mobD.2* ir *mobD.1* genų transkriptų Šaino-Dalgarno sekose, tuo tarpu kiti keturi – *mobD.4*, *mobD.1* ir *mobD* genų transkriptų koduojančiose dalyse. Pradmens ilginimo analizė taip pat rodo, kad RegB nukleazė atpažįsta tik vieną iš dešimties tirtų GGAU motyvų. Taigi šiuo darbu buvo nustatyta, kad RegB nukleazė efektyviai skaldo *mobD* genų srities transkriptus ir atlieka svarbų vaidmenį šios genomo srities transkriptų degradacijoje.