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Identification of middle promoter P_M30.2 of T4-related bacteriophage KC69

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During infection, bacteriophage T4 regulates three sets of genes: early, middle, and late. Temporal control of T4 gene expression is achieved mainly at the stage of transcription initiation by sequential activation of different classes of promoters. The consensus sequence of T4 middle promoters is defined by the MotA box (a/t)(a/t)TGCTT(t/c)A centered at position –30 and the *E. coli* σ^{70} consensus sequence TAnnnT centered at –10. Our previous studies identified two new T4 middle promoters: promoter P_M30 which is located just upstream of gene *30* and promoter P_M30.2 located in the coding region of gene *30.3*. Now we have detected the middle promoter P_M30.2 in the coding region of gene *30.3* of phage KC69. The central motive CGGTC of the –30 region of the middle promoter P_M30.2 of the T4-related phage KC69 differs from that of T4, TGGTC, while the –10 region matches very well. The intensity of the bands in the sequencing reaction of the transcripts from KC69 infection was significantly lower than that of the transcripts from T4 infection, indicating that the deviation in a highly conserved position of the MotA box affects the efficiency of transcription.

Key words: bacteriophage T4, bacteriophage KC69, gene *30.3*, middle promoter, MotA box

INTRODUCTION

Expression of the middle genes of bacteriophage T4 starts approximately two minutes after infection. T4 middle genes can be transcribed either from an adjacent middle promoter or by elongation from distant early promoters, or by both [1, 2]. The host RNA polymerase is capable of transcribing early genes, but middle transcription requires the T4-encoded transcriptional activator, MotA protein, which binds a DNA sequence (a MotA box) centered at position –30, (a/t)(a/t)TGCTT(t/c)A, and the T4 coactivator, AsiA protein, both of which bind to the sigma 70 (σ^{70}) subunit of RNA polymerase. The T4 middle promoters are characterized by a standard *E. coli* σ^{70} consensus sequence TAnnnT centered at –10, the MotA box consensus sequence centered at the position –30 and the spacing of 11–13 bp between them [2–6]. The –10 region is highly conserved among all known middle promoters, while the –30 sequence of some middle promoters deviates from the MotA box consensus sequence even at the most highly conserved positions. Although the MotA box of the P_M30.2 AGTGGTCTA showed a weak similarity to the –30 consensus sequence, the results of primer extension sequencing of RNA isolated from T4D *motA*$^+$ and T4D *motA*$^-$ infections confirmed the presence of the middle promoter [6].

Bacteriophage KC69 is a member of the T-even phage subgroup [7]. The aim of our current investigation is to demonstrate the presence of the middle promoter P_M30.2 in the coding region of gene *30.3* of bacteriophage KC69.

MATERIALS AND METHODS

Phages and bacterial strains. Bacteriophage T4D wild-type was a gift from Dr. W. B. Wood. Bacteriophage KC69 was from Dr. K. Carlson. Both phages were grown in *Escherichia coli* B^E (sup^0) provided by Dr. L W. Black.

RNA isolation. *E. coli* B^E (sup^0) cells were grow at 30 °C to a density 3×10^8 cells/ml in LB medium before infection with bacteriophage T4 wild-type at a m.o.i. of 10. RNA was extracted essentially as described [9].

Primer extension analysis. Two synthetic oligonucleotides were used to prime reverse transcriptase: Pr. 1, a 26-mer, 5'-GCTTAAACATACTCCATCA ATATCAG, complementary to nucleotides 20–45 of gene *30.2* coding sequence of phage T4; Pr. 2, a 26-mer, 5'-GCTTAAACATACACCATCAATATCAG, complementary to nucleotides 20–45 of the gene *30.2* coding sequence of phage KC69. The oligonucleotides were 5'-end labeled by T4 polynucleotide kinase with [γ-^{32}P]ATP (Amersham Biosciences) and sepa-

rated from the labeled nucleotides by precipitation with ethanol in the presence of 2 M amonium acetate. Primer extension and RNA sequencing were carried out essentially as described by Uzan et al. [8].

RESULTS AND DISCUSSION

Figure 1 summarizes the positions of bacteriophage T4 genes, *30.7*, *30.6*, *30.5*, *30.4*, *30.3*, *30.3*', *30.2*, and *30.1* located between early promoter P_E128.2 and T4 DNA ligase gene *30*. Detailed analysis of the nucleotide sequence of gene *30.3* of bacteriophage KC69 as well as primer extension experiments confirmed the presence of the middle promoter located just upstream of gene *30.2* in the coding region of gene *30.3*. Promoter P_M30.2 of phage T4 contains the sequence TGGTC in a highly conserved position of the –30 region, while P_M30.2 of phage KC69 contains the sequence CGGTC in the same position. Although the MotA box sequence of the P_M30.2 of phage KC69 differs from that of T4 and from the middle promoter consensus sequence as well (Fig. 1), the primer extension sequencing of RNA isolated from KC69-infected cells confirmed the presence of a promoter just upstream of gene *30.2*. The 5' ends of transcripts directed from P_M30.2 of bacteriophages T4 and KC69 start at nucleotides C and G positioned 6–7 bp downstream from the –10 sequence TACAAT (Fig. 2(a,b)). The intensity of the bands in the sequencing reaction of the transcripts from KC69 infection was significantly lower than that of the transcripts from T4 infection, indicating that the deviation in a highly conserved position of the MotA box affects transcription efficiency. The kinetics of 5' end accumulation in the cells infected with phage KC69 show very weak bands, indicating the 5' ends of transcript at two minutes after infection (Fig. 2(c)). The intensities of the bands increase up to six to eight minutes after infection, and this is a characteristic feature of middle promoters. The kinetics of the 5' ends accumulation in KC69-infected cells is similar to that of T4-infected cells [6].

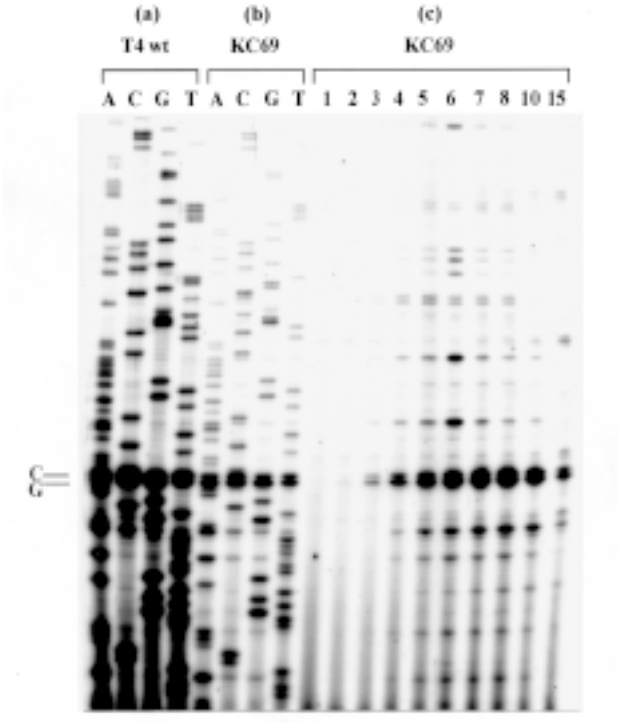

Fig. 2. Primer extension analysis of gene *30.2* mRNA using RNA isolated from *E. coli* B^E cells infected with KC69: (*a*) primer extension sequencing of RNA isolated at six minutes post infection from cells infected at 30 °C with T4D wild-type; (*b*) primer extension sequencing of RNA isolated at six minutes post infection from cells infected at 30 °C with KC69. The sequencing lanes are labeled with the dideoxynucleotides used in the sequencing reactions. The initiating nucleotides, C and G, are noted; (*c*) primer extension reactions on RNA isolated at 1 to 15 minutes post infection from cells infected with KC69 at 30 °C. The time (minutes) of post infection that each RNA was isolated is noted at the top of the figure

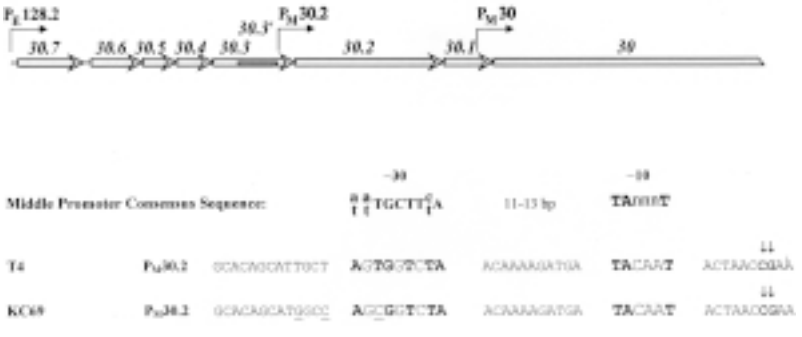

Fig. 1. Genes and promoters in the genomic region between early promoter P_E128.2 and the DNA ligase gene *30* of phage T4. The schematic outline of T4 DNA fragment carrying overlapping genes *30.3* and *30.3*' is presented at the top of the figure. Shown are the positions of genes, as well as the positions of the early and middle promoters. The consensus sequence of T4 middle promoters is given below. The middle promoter sequence of phage KC69 is shown at the bottom of the figure. The matches between given promoter sequence and the consensus sequence of T4 middle promoters are shown in bold face type. Initiation nucleotides for the transcripts directed from T4 P_M30.2 are indicated by vertical arrows. The nucleotides that differ from T4 are underlined

It should be noted that the previous transcription studies revealed middle promoters with –30 sequence, which deviate from the MotA box consensus sequence. These promoters contain changes within the MotA box center motif: AGGTC sequence in

promoter P_M55 [5], TGGTT in $P_M$td [5], GGCTA in P_M55.9 [5], TGCAT in P_M55.8 [5], CCAAA in $P_M$nrdA [5], TACTT in P_M46 [3], AGCTT in P_M30 [6], P_M39 [2] and P_M47 [2], CGCTT in P_MrIIA [2], GGCTT in P_M43 [3] and TGGTC in promoter P_M30.2 [6]. Thus, our results indicate that P_M30.2 of bacteriophage KC69 containing the MotA box sequence motive CGGTC, which has not been seen before, acts as a middle promoter. Our data support the prediction of Marshall et al. [5] that there is more flexibility in the sequence requirements for MotA than previously appreciated.

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References

1. Brody E, Rabussay D, Hall DH. In Bacteriophage T4 1983; 174–83.
2. Stitt B, Hinton D. In Mol Biol of Bacteriophage T4 1994; 142–60.
3. Guild N, Gayle M, Sweeney R, Hollingsworth T, Moodeer T, Gold L. J Mol Biol 1988; 199: 241–58.
4. Nivinskas R, Raudonikiene A, Guild N. Mol Biol (Moscow) 1989; 23: 739–49.
5. Marshall P, Sharma M, Hinton D. J Mol Biol 1999; 285: 931–44.
6. Truncaite L, Zajanckauskaite A, Nivinskas R. J Mol Biol 2002; 317: 179–90.
7. Tetart F, Desplats C, Kutateladze M, Monod C, Ackermann HW, Krisch HM. J. Bacteriol 2001; 183: 358–66.
8. Uzan M, Favre R, Brody E. Natl Acad Sci USA 1988; 85: 8895–9.

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T4 GIMININGO KC69 BAKTERIOFAGO VIDURINIO PROMOTORIAUS P_M 30.2 NUSTATYMAS

S a n t r a u k a

Mūsų ankstesniuose tyrimuose buvo nustatyti du nauji T4 fago viduriniai promotoriai: P_M30, kuris yra išsidėstęs šiek tiek aukščiau *30* geno, ir P_M30.2, esantis *30.3* geno koduojančioje srityje. T4 fago viduriniai promotoriai turi *E. coli* σ^{70} promotoriams būdingą konservatyvią –10-os srities seką TATAAT ir konservatyvią seką –30 srityje –(a/t)(a/t)TGCTT(t/c)A. Šiame darbe mes nustatėme vidurinį promotorių P_M–30.2, esantį KC69 fago *30.3* geno koduojančioje srityje. KC69 fago šio promotoriaus 30-os srities centrinis motyvas CGGTC skiriasi nuo T4 fago P_M30.2 MotA srities motyvo – TGGTC, tuo tarpu, kai –10 sritis, TACAAT, visiškai atitinka T4 –10 seką. KC69 bakteriofago infekcijos atveju transkriptų sekvenavimo intensyvumas buvo daug silpnesnis nei T4 infekcijos atveju, ir tai rodo, kad skirtumai, esantys MotA srityje, veikia transkripcijos efektyvumą. Taigi mūsų tyrimai patvirtina prielaidą, kad MotA sekoje galimi didesni pakitimai, nei buvo manyta anksčiau.