T7 DNA-dependent RNA polymerase can transcribe RNA from tick-borne encephalitis virus (TBEV) cDNA with SP6 promoter

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Abstract T7 RNA polymerase is shown to recognize the SP6 promoter including 17 base pairs before the transcription start site and produce the 5′-end TBEV RNA. The yield of TBEV RNA synthesized by heterologous T7 RNA polymerase from cDNA construction with SP6 promoter is higher than the RNA production by homologous SP6 RNA polymerase. The addition of 1 pmol template DNA with SP6 17 bp promoter in transcription mixture for SP6 or T7 RNA polymerases resulted in 1–5×10^{-2} pmol RNA production.

Key words: SP6 promoter; T7 RNA polymerase; Tick-borne encephalitis virus

1. Introduction

The bacteriophage T7 grows on *Escherichia coli* cells. Bacteriophage T7 RNA polymerase (EC 2.7.7.6) is produced early in T7 infection [1]. A single-chain enzyme with a molecular weight close to 100000, T7 RNA polymerase has a stringent specificity for its own promoters and selectively transcribes DNA that has been linked to such promoters [2]. Seventeen such promoters are found in T7 DNA. Nucleotide sequence analysis revealed that 5 strong late promoters (class III) consist of 23 bp including the start site for the RNA (from −17 to +6) [3], whereas 10 weak promoters of class II contain 2–7 modified nucleotides that differ from the class III promoter sequence and do not include the transcription start [4]. Two other promoters are near the 5′ and 3′ ends of T7 DNA [5].

The bacteriophage SP6 grows only on recipient *Salmonella typhimurium* strains and not on donor strains [6]. Bacteriophage SP6 encode a single subunit RNA polymerase (EC 2.7.7.6) that is very similar to the T7 RNA polymerase [7]. Comparison of the SP6 consensus sequences with the structures of promoters recognized by T7 and T3 RNA polymerase revealed a common core sequence (5′- CACTA-3′) extending from −7 to −3 [7].

Recombinant DNA technology makes it possible to produce cDNA clones corresponding to the genomes of RNA viruses and infectious RNA by transcription in vitro [8–11]. However, the presence of nonviral nucleotides at the 5′ end of viral transcripts strongly reduces infectivity [8], so the using of commercially available in vitro transcription vectors with strong SP6 or T7 promoters leads to uninfectious RNA transcripts with additional vector-derived sequences at the 5′ end. To study TBEV infection we have constructed full-length cDNA as described previously [12]. In order to obtain recovered virus we had to introduce weak SP6 promoter by means of synthetic oligodeoxyribonucleotides upstream of the 5′ end of the full-length TBEV cDNA. The transcription of 5′ end TBEV cDNA in vitro by homologous SP6 or heterologous T7 DNA-dependent RNA-polymerases resulted in the production of radioactive infectious RNA without additional nucleotides at the 5′ end.

2. Materials and methods

2.1. Construction of full-length TBEV cDNA with SP6 promoter

The pBR322TBEVS* plasmid has been described previously [12]. The *Not*I-*Sph*I fragment of pBR322TBEVS* was replaced on the double-stranded oligodeoxyribonucleotide, containing SP6 promoter, the additional transcription-initiating G residue and the first nucleotides of TBEV cDNA (Fig. 1B). The introduction of the SP6 promoter upstream of the 5′ end of pBR322TBEVS* resulted in pS-TBEVS* plasmid construction (Fig. 1A).

2.2. [γ-^{32}P]ATP and [α-^{32}P]GTP synthesis

[γ-^{32}P]ATP and [α-^{32}P]GTP were synthesized from [^{32}P]*orto*-phosphoric acid (Obninsk Physik-energetic Institute, Russia) as described in [13].

2.3. Determination of nucleotide sequence using Taq DNA polymerase

Nucleotide sequence of promoter region and 5′ end TBEV cDNA of pS-TBEVS* plasmid has been determined by the method of Murray [14] using Taq DNA polymerase (Promix, Russia) and dideoxy-NTP (Boehringer Mannheim, Germany).

2.4. In vitro RNA transcription

The pS-TBEVS* plasmid was linearized by cleavage with *Xho*I restriction endonuclease. ^{32}P-Labeled RNA was synthesized by SP6 or T7 DNA-dependent RNA polymerases (Promix, Russia) and 50 μM [α-^{32}P]GTP (10 cpm/ml) for 3 h at 37°C as described in [15]. The labeled RNA production was estimated by incorporation of [α-^{32}P]GTP into TCA-insoluble product. The samples of reaction mixture placed on GF/C filters (Whatman, UK) were washed 4 times for 15 min each by cold 5% CCl_3COOH with 1 mM $Na_4P_2O_7$ on ice and Čerenkov counted on a 1211 MiniBeta (LKB, Sweden). The RNA products were analyzed by the denaturating 5% PAAG-7 M urea gel electrophoresis in 1×TBE buffer with subsequent autoradiography.

3. Results

Since the additional nonviral nucleotides at the 5′ end of viral RNA transcripts strongly decrease the infectivity of recovered viruses [8] we had to introduce the weak 17 bp SP6 promoter upstream of the 5′ end of pBR322TBEVS* as following (Fig. 1A). The *Not*I-*Sph*I fragment of pBR322TBEVS* was replaced by double-stranded oligonucleotide, which contained the SP6 promoter, the additional transcription-initiating G residue and fragment of the 5′ end of TBEV cDNA and was flanked by stick-ends for *Not*I and *Sph*I (Fig. 1B). Nucleotide sequences of promoter and the following 5′ end of pS-TBEVS* were determined by dideoxynucleotides according to [14].

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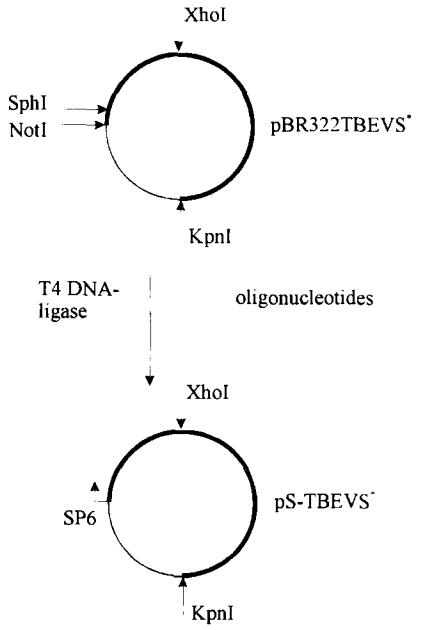
A.



B.

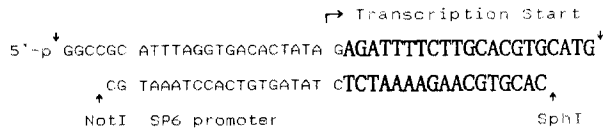


Fig. 1. A: Scheme of pS-TBEVS* plasmid construction. pBR322TBEVS* plasmid was hydrolysed by the restriction endonucleases *Not*I and *Sph*I. The double-stranded oligodeoxyribonucleotide containing promoter for DNA-dependent RNA polymerase of bacteriophage SP6 was ligated with prepared plasmid. It resulted in plasmid pS-TBEVS* construction with SP6 promoter upstream of the full-length cDNA of TBEV. B: Structure of the double-stranded oligodeoxyribonucleotide used for pS-TBEVS* plasmid construction.

The pS-TBEVS* plasmid was linearized by *Xho*I restriction endonuclease. The unique *Xho*I site is nearly 5700 bp from the 5′ end of TBEV cDNA (Fig. 1A). The TBEV RNA was synthesized in vitro in standard condition [15]. The ethidium bromide (1 μg/ml) stainning of 1% agarose gel with 0.1% SDS or 5% PAAG-7 M urea did not allow the detection of RNA transcripts (data not shown). However, RNA synthesis in the presence of [α-^{32}P]GTP [13] with subsequent denaturating electrophoresis in 5% PAAG-7 M urea and autoradiography resulted in transcription detection (Fig. 2). Therefore the RNA synthesis level is extremely low.

The incorporation of [α-^{32}P]GTP into TCA-insoluble product revealed 0.7% incorporation only. The comparison of kinetic curves (Fig. 3) showed the higher effectivity of RNA synthesis from the same template DNA with short SP6 promoter by T7 RNA polymerase than with SP6 RNA polymerase. Taking into account the [α-^{32}P]GTP specific radioactivity, the percentage of substrate incorporation into RNA product and the length of RNA, 5700 nucleotides, one can estimate the effectivity of RNA synthesis from heterologous promoter as $20–100 \times 10^{-2}$ pmol RNA from 1 pmol DNA. Therefore the TBEV 5′ end RNA yield appeared to be the same in transcription reaction with SP6 and T7 RNA polymerases.

4. Discussion

Because SP6 and T7 RNA polymerase are similar and their promoters have a consensus extending from −7 to −3, it is reasonable to expect cross-reactions in heterologous combinations between enzyme and template. However, the T7 RNA polymerase has a stringent specificity for its own promoter as shown previously by transcription reaction in vitro with subsequent 1% agarose gel electrophoresis and ethidium bromide staining of nascent RNA [2]. Only few if any promoters for T7 RNA polymerase are found in any DNA unrelated to T7. This great specificity of T7 RNA polymerase for its own promoter is extremely important for bacteriophage T7 infection and useful to biochemists. Nevertheless, SP6 and T7 bacteriophages multiply on different hosts – *Salmonella typhimurium* and *Escherichia coli*, respectively. Therefore they could never interact with one another in vivo. The similarity of SP6 and T7 RNA polymerases and their promoters might be the result of convergent evolution. The consensus sequence 5′-CACTA-3′ of SP6 and T7 promoters contains only 5 nucleotides so it might be a very weak promoter only. Indeed, the effectivity of transcription of the same DNA with SP6 promoter of 17 bp long by homologous SP6 and heterologous T7 RNA poly-

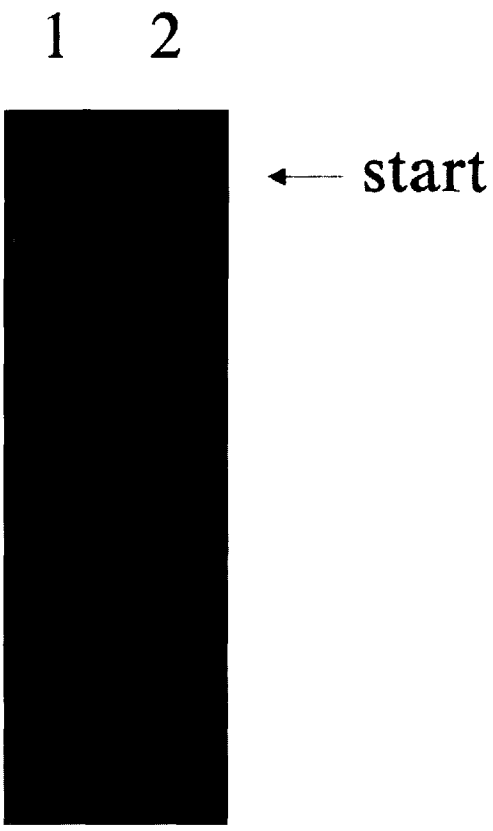


Fig. 2. Autoradiogram of RNA products after in vitro transcription of the pS-TBEVS* plasmid. Lane 1 shows the product of RNA transcription by SP6 DNA-dependent RNA polymerase, lane 2 that of T7 DNA-dependent RNA polymerase. 'Start' means the beginning of the PAAG.

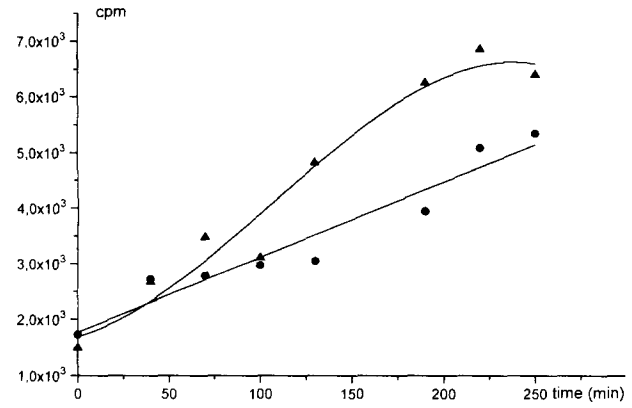


Fig. 3. Kinetic of in vitro RNA transcription determined by incorporation of α-^{32}P-labeled GTP into acid-insoluble product. ○ indicates RNA synthesis with SP6 RNA polymerase, △ indicates RNA synthesis with T7 RNA polymerase.

merases is similar. Concequently, class II promoter of bacteriophage SP6 might possibly bind with both SP6 and T7 DNA-dependent RNA polymerases.

References

[1] Studier, F.W. and Dunn, J.J. (1983) Cold Spring Harbor Symp. Quant. Biol. 47, 999–1007.
[2] Davanloo, P., Rosenberg, A.H., Dunn, J.J. and Studier, F.W. (1984) Proc. Natl. Acad. Sci. USA 81, 2035–2039.
[3] McAllister, W.T., Morris, C. and Rosenberg, A.H. (1981) J. Mol. Biol. 153, 527–544.
[4] Carter, A.D. and McAllister, W.T. (1981) J. Mol. Biol. 153, 825–830.
[5] Dunn, J.J. and Studier, F.W. (1983) J. Mol. Biol. 166, 477–535.
[6] Zinder, N.D. (1961) Science 133, 2069–2070.
[7] Brown, J.E., Klement, J.F. and McAllister, W.T. (1986) Nucleic Acids Res. 14, 3521–3526.
[8] Boyer, J.-C. and Haenni, A.L. (1994) Virology 198, 415–426.
[9] Lai, C.-J., Zhao, B., Hori, H. and Bray, M. (1991) Proc. Natl. Acad. Sci. USA 88, 5139–5143.
[10] Rice, C.M., Grakoui, A., Galler, R. and Chambers, T.J. (1989) New biologist 1, 285–296.
[11] Khromykh, A.A. and Westaway, E.G. (1994) J. Virol. 68, 4580–4588.
[12] Dobrikova, E.Yu. and Pletnev, A.G. (1995) Bioorg. Chem. 21, 528–534 (in Russian).
[13] Walseth, T.F. and Johnson, R.A. (1979) Biochim. Biophys. Acta 562, 11–31.
[14] Murray, V. (1989) Nucleic Acids Res. 17, 8889.
[15] Promega Catalogue, 1991–1992, p. 148.