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Effect on DNA transcription of nucleotide sequences upstream to T7 promoter

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ABSTRACT

The T7 RNA polymerase-dependent transcription was studied as a function of nucleotide sequence structures positioned upstream of the T7 promoter. Model double-stranded DNA templates were constructed for this purpose. They contained a target sequence of 485 base pairs (cDNA fragment of *Venesuelian encephalomyelitis equine* virus genome), T7 promoter consensus and different extra base sequences upstream of the T7 promoter. The level of the target sequence transcription was clearly determined by the extra base sequence. The presence of one extra base pair G·C ensured the most pronounced effect, transcription was increased one order of magnitude in comparison with template which has only a canonical T7 promoter sequence at the 5′-end.

T7 RNA polymerase-dependent transcription was studied as a function of nucleotide sequence structures positioned upstream of the T7 promoter. Model double-stranded DNA templates were constructed for this purpose by means of PCR using *Tte* DNA polymerase (from *Thermus thermophilis* strain 635, purchased by SibEnzyme, Novosibirsk). They contained a target sequence of 485 base pairs [cDNA fragment of *Venesuelian encephalomyelitis equine* virus genome between positions 947 and 1431 (1)], a T7 promoter consensus (2) and different extra base sequences upstream of the T7 promoter.

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                  –17                 +1
                   |                   |
I     5′-          TAATACGACTCACTATAGGG
II    5′-        AT                    GGG
III   5′-        CT                    GGG
IV    5′-     GATTT                    GGG
V     5′-CGGAATTCT                    GGG
VI    5′-    GCAGCT                    GGG
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Template transcription

Reactions were carried out at 37°C. A reaction volume of 25 µl contained: 40 mM Tris–HCl (pH 8.0 at 25°C), 25 mM NaCl, 10 mM DTT, 10 mM $MgCl_2$, 2 mM spermidine, 1 mM each ribonucleoside triphosphate, 6 ng of corresponding template and 6 U T7 RNA polymerase. The template concentrations were determined on a Perkin-Elmer spectrophotometer model 550 at 260 nm. Transcription products were separated by electrophoresis in a 5% polyacrylamide gel containing Tris-borate–EDTA system and were visualized by ethidium bromide treatment. The gel photonegatives obtained were scanned by 'Ultrascan XL' (LKB, Sweden) and relative levels of full-size RNA synthesis were calculated.

Figure 1 and Table 1 demonstrate that the yield of transcription product depends significantly on the template used. The lowest level is obtained using template I, which has no extra bases upstream of the T7 canonical promoter sequence (–17 to +3). The addition of one A·T pair before canonical sequence increased significantly the efficiency of the transcription. Further extension of the extra base sequence from 4 to 8 nucleotide pairs (template IV and V correspondingly) has little effect on the yield of transcription products in comparison with template II. At the same time, the presence of one extra G·C base pair ensured the most pronounced effect (template III), transcription was increased one order of magnitude in comparison with template I. Contrary to the expectations, template V, which also contains the G·C base pair immediately anterior to the promoter sequence and three additional G·C pairs in position –23 to –25 yielded a moderate level of transcription.

Table 1. Synthesis of RNA transcripts from templates with different pre-promoter regions

Template	Relative RNA quantities[a]	
	10 min incubation	30 min incubation
I	1.0 ± 0.1	1.0 ± 0.07
II	2.0 ± 0.14	2.5 ± 0.08
III	12 ± 0.17	6.8 ± 1.0
IV	2.7 ± 0.08	3.5 ± 0.2
V	2.7 ± 0.1	2.8 ± 0.1
VI	3.2 ± 0.13	4.1 ± 0.37

[a]The mean values from five independent determinations are presented. Several different PCR template preparations were used for each sequence assayed.

The nucleotide sequences upstream of the T7 promoter listed in Table 1 have almost identical effects on the transcription of other fragments derived from VVE cDNA. The length of these templates varied approximately from 300 to 600 base pairs. It is

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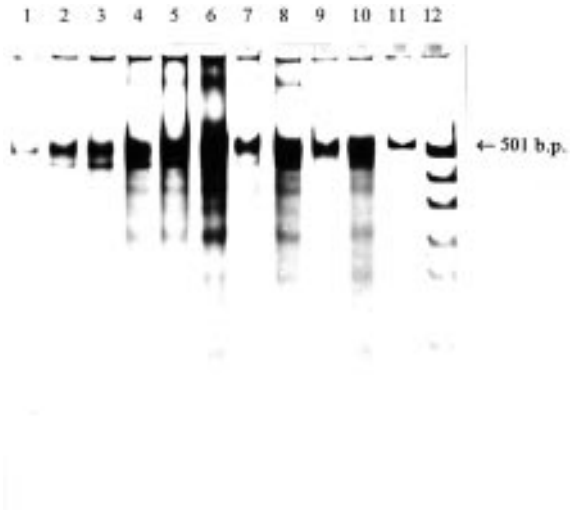

Figure 1. Comparison of *in vitro* transcription of template DNAs with different pre-promoter regions. Lanes 1 and 2, template I; lanes 3 and 4, template II; lanes 5 and 6, template III; lanes 7 and 8, template IV; lanes 9 and 10, template V (odd lanes, 10 min incubation; even lanes, 30 min incubation). Lane 11, template V DNA, 15 ng; lane 12, pUC19 DNA after restriction endonuclease *Msp*I treatment.

known that the recognition of promoter determinants by RNA polymerases is associated with local melting of the DNA duplex (3,4). The displacement of the enzyme in the course of transcription initiation sets free the 5′-end of the promoter sequence which must again to be drawn into the double-stranded structure. The presence of extra base sequences may favour the local duplex restitution which may have a peristaltic effect of a sort on the mobile open complex. However, the proposed peristaltic effect on transcriptional bubble would have to be rather sensitive to some other peculiarities of an extra base sequence structure. For example, the less effective template V, which has a longer extra base sequence enriched with G·C pairs, supports this conclusion. Possibly, it is somehow connected with different molecular dynamic characteristics of localized DNA structure.

When using synthetic promoters that produce a short five-base message (5) or a 24-base message (6), the data for these templates demonstrated that the presence of two additional A·T pairs upstream of position –17 provides no significant benefit to the rate of transcription. However, it was not determined whether or not an extra G·C pair would improve these rather inefficient short templates.

With circular plasmid templates our results for templates V and VI predict a moderate effect of the extra upstream G·C pair on the level of transcription. The most effective nucleotide sequence upstream of the T7 promoter must be specially investigated for this template type.

The present work illustrates the potential of an extra upstream G·C pair to stimulate significantly the transcription of the double-stranded templates that may be of interest in different experimental situations including, for example, isothermal amplification system (7) and gene expression in a cell-free system on a preparative scale (8).

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