THE EFFECT OF VARIOUS TEMPLATES AND OLIGONUCLEOTIDE PRIMERS ON RNA AND POLY(A) SYNTHESIS BY *E. COLI* AND T7 RNA POLYMERASES

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1. Introduction

Comparative studies of RNA polymerases isolated from different sources represent an approach to establishing the relationship between the structure and function of these enzymes. *E. coli* RNA polymerase is a large oligomeric protein which can initiate RNA synthesis on a variety of promoters [1,2] while the enzyme coded by gene I of T7 phage is monomeric and utilizes only 'late' promoters of the phage chromosome [3].

Our results indicate that T7 enzyme is more specific than *E. coli* RNA polymerase with respect to not only template and initial nucleosidetriphosphate but also to oligonucleotide primer.

2. Materials and methods

Dinucleosidemonophosphates were obtained from M. Grunberg-Manago and Sigma Chemical Company, oligo(dT), oligoribonucleotides, [^{3}H]UTP were kindly provided by Yu. A. Berlin and Z. A. Shabarova, S. M. Zhenodarova [4] and N. F. Myasoedov. $(dA)_3(dT)_3(dA)_3$ was synthesized by us using the phosphodiester method [5]. T2 and T7 phages and their DNA were isolated by the conventional phenol technique [6], where indicated the DNA was heat-denatured. Holo and core RNA polymerases from *E. coli* were isolated as in [7]; T7 RNA polymerase was isolated as in [8].

RNA synthesis with T7 RNA polymerase was effected in 50 μl 0.05 M Tris-acetate (pH 8.0), 0.02 M $MgCl_2$, 0.1 mM dithiothreitol, 0.5 mM of each ATP, GTP, CTP and UTP, one of which was labeled ([^{3}H]ATP, 100 mCi/mmol, or [^{3}H]UTP, 96 mCi/mmol), 50 μg/ml DNA (see below), 600 μg/ml bovine serum albumin and 50 μg/ml rifampicin. Poly(A) was synthesized in the same mixture except that $MgCl_2$ was made 0.01 M and only [^{3}H]ATP (100 μg/ml, 1 μCi per tube) was added. For the *E. coli* enzymes, the incubation mixture was supplemented with 0.05 M KCl and rifampicin was omitted. The samples were incubated for 20 min at 30°C and fixed with 5% trichloroacetic acid.

3. Results and discussion

As shown in table 1, T7 RNA polymerase synthesizes RNA only with native or denatured T7 DNA as template while poly(A) is produced with denatured T2 DNA even more intensively than with T7 DNA. *E. coli* enzyme is similar to T7 RNA polymerase in the latter respect but for RNA synthesis it equally utilizes both T2 and T7 DNAs. Both enzymes produce poly(A) several times more actively on $(dT)_{12}$ than on DNA as template. *E. coli* RNA polymerase can utilize denatured T7 DNA for poly(U) synthesis in the presence of UTP without other nucleosidetriphosphates while the T7 enzyme completely lacks this ability (data not shown). The phage polymerase requires a shorter template for poly(A) synthesis than does *E. coli* RNA polymerase: the respective minimum lengths of oligo(dT) are 3 and 6 bases (the latter figure agrees with the data in [9]). The T7 enzyme

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Table 1
Template specificity of poly(A) and RNA synthesis by *E. coli* and T7 RNA polymerases

Exp. no.	Template	*E. coli* holo enzyme		T7 enzyme	
		RNA	poly(A)	RNA	poly(A)
	Native T7 DNA	26 339	175	3432	707
	Denatured T7 DNA	15 068	9641	1894	2393
1	Native T2 DNA	26 125	5695	641	972
	Denatured T2 DNA	8704	22 032	435	4369
	$(dT)_{12}$		105 297		17 997
	$(dT)_3$		0.4		3.5
	$(dT)_6$		4.8		23
	$(dT)_9$		20		103
2	$(dT)_{12}$		96		183
	$(dT)_{15}$		132		200
	$(dA)_3(dT)_3(dA)_3$		0.3		36
	Same + 0.5 mM UTP		0.3		6.7

[^{3}H]ATP incorporation is expressed as cpm in exp. 1 and as % [^{3}H]ATP incorporation with denatured T2 DNA as a template in exp. 2. 100% corresponds to 21785 cpm for the *E. coli* enzyme and 4894 cpm for the T7 enzyme. Concentration of oligonucleotides was 12 μM

synthesizes poly(A) much faster with $(dA)_3(dT)_3$-$(dA)_3$ than with $(dT)_3$ as template while for the *E. coli* enzyme both are ineffective. Thus both the length of oligo(T) sequence capable of being transcribed and the total template length are important for reiterative poly(A) synthesis with T7 RNA polymerase. For the *E. coli* enzyme $(dT)_3$ seems to be too short irrespective of the template length.

Only a high concentration of GTP results in stimulation of RNA synthesis when the concentrations of other nucleosidetriphosphates are low (table 2). Thus in accord with [10], the T7 enzyme initiates RNA synthesis on native T7 DNA only with GTP. Of all 16 dinucleosidemonophosphates only those containing 3′-G can partially substitute for high GTP concentration. Hence, the T7 enzyme can add nucleotides to dinucleosidemonophosphate only beginning with the 3′-OH group of guanosine, the only initiator of RNA chains on native T7 DNA. It appears that dinucleosidemonophosphate primers in the case of T7 enzyme cannot shift the initiation site to bases adjacent to the normal initiation pair dC:dG of the template T7 DNA. By contrast, addition of dinucleosidemonophosphate complementary to neighbouring bases of the template can shift the initiation site for *E. coli* RNA polymerase [2,11,12].

Dinucleosidemonophosphates can be arranged in the following orders according to the extent of poly(A) synthesis stimulation on denatured T7 DNA as a template. ([^{3}H]AMP incorporation without dinucleosidemonophosphates is taken as 1, accuracy ± 10%.)

T7 RNA polymerase (1 = 749 cpm):

ApA (5.6) > GpA (3.9) > UpA (2.4) ≥ ApG (2.1) > CpA (1.6) > UpU (1.1) = CpC (1.1) = CpU (1.1) = ApU (1.1) ≥ CpG (1.0) = UpG (1.0) = ApC (1.0) = UpC (1.0) ≥ GpC (0.9) > GpU (0.7) > GpG (0.5).

Table 2
The effect of various nucleosidetriphosphates and dinucleosidemonophosphates on RNA synthesis by T7 RNA polymerase with T7 DNA as a template

Nucleotides added	Relative radioactivity
ATP	2.2
GTP	46
CTP	1.8
ATP + GTP + CTP	81
ApG	8.3
GpG	4.9
CpG	4.1
UpG	3.7
ApA, ApU, ApC, GpA, GpU, GpC, CpA, CpU, CpC, UpA, UpU, or UpC	0.7–1.2

Relative incorporation of [^{3}H]UMP (spec. act. 2.4 Ci/mmol, 1.2 μCi/test tube). Radioactivity of the control containing only the background level of 10 μM of each 4 nucleosidetriphosphates (1857 cpm) is taken for 1. Nucleosidetriphosphates and dinucleosidemonophosphates were added to 0.5 mM and 0.2 mM, respectively. Average of 2 experiments

Holoenzyme of *E. coli* (1 = 1823 cpm):

GpA (5.3) > ApA (4.1) > ApG (3.2) > UpA (2.0) > CpA (1.6) ≥ CpG (1.5) > UpG (1.3) > ApU (1.1) = GpG (1.1) ≥ UpC (1.0) = CpU (1.0) ≥ GpC (0.9) = UpU (0.9) = CpC (0.9) ≥ ApC (0.8) = GpU (0.8).

The difference between the enzymes is apparent. The T7 RNA polymerase is markedly stimulated by all dinucleosidemonophosphates with 5′-A and by ApG and is inhibited by all dinucleosidemonophosphates with 3′-G except GpA. The bacterial enzyme is also primed by 5′-A dinucleosidemonophosphates and also by 5′-G dinucleosidemonophosphates although the latter are less efficient. It appears that the *E. coli* enzyme is more tolerant to A → G substitutions and, therefore, is stimulated by a larger set of dinucleosidemonophosphates. Both enzymes can synthesize poly(A) chains only beginning with the 3′-A end of the primer.

The following order represents the priming efficiency of several dinucleosidemonophosphates in poly(A) synthesis with T7 RNA polymerase and $(dT)_{12}$ as a template (1 = 3811 cpm):

ApA (5.8) > GpA (5.1) >> ApG (2.2) ≥ UpA (2.0) ≥ CpA (1.9) > GpC (1.3) ≥ ApC (1.1) ≥ 1.

As expected, the most effective primer is ApA. 5′ substitutions decrease the priming efficiency of ApA to a lesser extent than do 3′ substitutions; the same holds for A→G as compared to A→U and A→C. These data agree with those on poly(A) synthesis with denatured T7 DNA as a template (see above). The situation reminds one of the pairing ambiguity ('wobbling') in translation [13], it appears that the same phenomenon can take place in transcription. Even if the dinucleosidemonophosphate imitating the 3′ terminus of synthesized RNA is not completely complementary with the template, it can still be bound by the enzyme and serve as an acceptor for the next nucleotide attachment.

The priming efficiency of various oligonucleotides in poly(A) synthesis with $(dT)_{12}$ as a template corresponds to the following orders.

T7 RNA polymerase (1 = 1434 cpm):

ApA (4.8) ⩽ ApApA (5.0) ⩾ ApApApA (4.5) >

CpApApA (3.7) > CpApA (2.2) > ApCpA (1.3) =

CpA (1.3) ⩾ ApApC (1.2) > 1 > CpCpA (0.7) =

ApC (0.7).

Holoenzyme of *E. coli* (1 = 16938 cpm):

ApApApA (5.2) > ApApA (3.3) > CpApA (2.8) >

ApA (2.5) > CpA (1.5) > ApApC (1.3) ⩾ ApCpA

(1.2) > 1 > CpCpA (0.8) ⩾ ApC (0.7).

Core enzyme of *E. coli* (1 = 3071 cpm):

ApApApA (41) = CpApApA (42) > ApApA (31) >>

CpApA (11) > ApA (7.8) > CpA (5.7) ⩾ ApApC (5.4)

> ApCpA (3.6) > ApC (1.3) ⩾ CpCpA (1.2) ⩾ 1.

It is apparent that in the case of T7 RNA polymerase the 2-base primer has already maximum activity since stimulation by $(A)_2$, $(A)_3$ and $(A)_4$ is nearly equal. This could suggest a very small enzymatic site that is in contact with the 3′-OH terminus of RNA product. However, CpApA and CpApApA are less active than the oligo(A) primers. It follows that the viral enzyme contacts with at least 4 bases of the RNA 3′-OH terminus as if proofreading them. If incorrect bases are present, the rate of elongation drops. For efficient priming, the T7 enzyme thus requires not the presence of the 3rd and 4th bases but rather the absence at these positions of unpaired bases (since CpApA and CpApApA < $(A)_2$, $(A)_3$ and $(A)_4$).

The holo and core enzymes of *E. coli* do not differ in the efficiency of their priming with the different oligonucleotides studied; however, the core enzyme is stimulated by them to a much greater extent. By contrast to the T7 enzyme, increasing the primer length with both *E. coli* enzymes enhances its efficiency ($(A)_4 > (A)_3 > (A)_2$) and can even partially compensate the inhibitory effect of non-complementary 3′ terminal nucleoside (ApApC>>ApC). The primer length is so important that addition of a non-complementary nucleoside to the 5′ terminus may increase the priming efficiency (CpApA > ApA; CpApApA > ApApA).

The farther the non-complementary nucleotide from the 3′ terminus the less the primer inactivation (CpApA >> ApCpA). All these data suggest that the primer (3′ terminus of RNA product) is held in the *E. coli* RNA polymerase molecule by two kinds of bonds. Firstly, the primer binds to the template with complementary bases while non-complementary ones weaken the binding. Secondly, the primer binds to the protein directly, the strength of this bond being a function of nucleotide number, i.e., primer length rather than its nucleotide composition.

It can be concluded that the T7 enzyme as compared to bacterial RNA polymerase more strictly sorts out mispaired primers, i.e., 3′ termini of RNA that contain bases non-complementary to the template. As has been shown above, the viral enzyme is also more critical in choosing the template and initiator nucleosidetriphosphate. The observed difference between the two RNA polymerases is most probably related to the fact that the bacterial enzyme is able to utilize a much greater variety of promoters than the viral enzyme.

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