ON THE FIDELITY OF IN VITRO POLYNUCLEOTIDE SYNTHESIS BY *E. COLI* RNA POLYMERASE

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1. Introduction

The fidelity of DNA replication in bacteria and bacteriophages is extremely high as judged by the mutation rates which are commonly thought to be due not only to complementary base pairing but also to the properties of DNA polymerase itself. Temperature-sensitive mutants affecting the structural gene of DNA polymerase in T4 bacteriophage [1,2], *B. subtilis* [3] and *E. coli* [4] are characterized by increased mutability. It is believed that alterations in the mutant enzyme result in less accurate selection of nucleotides during DNA synthesis. This hypothesis was directly verified by Hall and Lehman [5] who compared the fidelity of in vitro DNA synthesis carried out by normal and mutant T4 DNA polymerases on synthetic polydeoxyribonucleotide templates. Frequency of misincorporation of deoxynucleotides has been shown to vary from 2×10^{-6} to 3×10^{-4} depending on substrate and template used. In the case of poly(dG) synthesis on poly(dC) template the difference between misincorporation by normal and mutant enzyme was observed to be 2.4×10^{-6} and 8.3×10^{-6}, respectively. Thus, the data of Hall and Lehman suggest that DNA polymerase can select the suitable nucleotide or recognize the correct base pair in vitro.

It is of interest whether other enzymes which carry out template-directed polynucleotide synthesis also participate in the selection of complementary nucleotides. The fidelity of reverse transcription was recently investigated by Battula and Loeb [6] for the synthesis of poly(dT) on the poly(rA)oligo(dT) template by an enzyme from avian myloblastosis virus. The frequency of dC misincorporation was 1.7×10^{-3} which is close to that observed with DNA polymerase from leukemia cells but 10 times higher than with DNA polymerase from normal lymphocytes [7]. This result is in agreement with mutatory role postulated for the reverse transcriptase.

Little is known about the fidelity of RNA synthesis carried out by DNA-dependent RNA polymerase. The enzyme was shown to be able to incorporate certain nucleotide analogues provided that there are no stereochemical restrictions [8–10]. The values of incorporation vary substantially in different reports and apparently depend on the composition and secondary structure of polynucleotides used as templates [11–14]. The fact that bacterial RNA polymerase specifically transcribes only early genes of bacteriophage DNA in vitro suggests that this enzyme is capable of working with high fidelity [15].

To obtain direct data about the fidelity of in vitro transcription by RNA polymerase we analyzed the misincorporation of GMP into poly(A) synthesized on $(dT)_{12}$ template. The results indicate that the enzymes isolated from different strains of *E. coli* all take part in the selection of proper substrates and thus participate in ensuring the fidelity of transcription.

2. Materials and methods

2.1. *Bacterial strains*

Strains of *E. coli* K12 were used having different genotypes with respect to DNA dependent RNA polymerase: wild type strains 30SO (Hfr H thi lac) and AB 1450 (F^-met B arg H ilv his str-r); 18 Stl-r (F^-thr leu TI-r str-r thi stl-r 18) carrying mutation of

Table 1
Synthesis of poly(A) by RNA polymerase of *E. coli* 30SO on different templates

exp. No.	enzyme	template			
		none	$(dT)_{12}$	T2 DNA native	T2 DNA denatured
		[^{3}H]ATP incorporation, cts/min			
1.	core		12 651	284	
	core + sigma	147	13 335	4309	
2.	core		3039	286	2437
	core + sigma	62	1597	1420	3583
3.	core		7860	252	5742
	core + sigma	77	3658	3047	6030

Saturating ammounts of sigma were used. The samples were incubated for 20 min. Other conditions are described in section Materials and methods.

streptolydigin resistance; 18–35 (F⁻met B ilv his rif-r 18 str-r stl-r 18) double mutant which contains RNA polymerase resistant to rifampicin and streptolydigin; CV-5 (F⁻met B arg H ilv his cs str-r) containing the cold sensitive mutation cs which makes its RNA polymerase sensitive to decreased temperatures. The two later strains were isolated from AB 1450 in this laboratory [16].

Mutations of stl-r and cs are known to affect the elongation step of RNA synthesis and we thought they might alter the fidelity of transcription as well.

2.2. *Enzyme preparation and assay*

Bacteria were grown on synthetic medium with necessary supplements to 5×10^8 cells per ml. RNA polymerase core enzyme and sigma factor were isolated according to Burgess et al. [17]. The core preparations were tested electrophoretically and contained less than 10% of impurities. Standard assay sample contained (in 0.2 ml): 20 μM Tris buffer pH 7.9; 8 μM $MgCl_2$; 5 μM $MnCl_2$; 8 μg $(dT)_{12}$ or 20 μg T2 DNA; 100 μg ATP; 100 μg GTP; 2 μCi [^{3}H] ATP of [^{3}H]GTP; 0.04 ml of core preparation ($E_{280} \simeq 0.15$). The samples were incubated at 30°C with stirring.

2.3. *Reagents*

$(dT)_{12}$ synthesized in the Shemyakin Institute of Bioorganic Compounds was kindly supplied by Ju. A. Berlin. ^{3}H-labeled nucleotides were the gift of N.F. Myasoedov. Their specific activities were: 1.56 Ci/mmol for [^{3}H]GTP, 4.0 Ci/mmol for [^{3}H]CTP and 1.19 Ci/mmol for [^{3}H]ATP.

3. Results and discussion

The conditions used in the experiments differ from the optimal conditions of RNA synthesis in lower ionic strength. Under these conditions, core lacking sigma actively synthesized poly(A) on denatured T2 DNA or $(dT)_{12}$ (table 1). On the contrary, no poly(A) synthesis took place on native T2 DNA during 20 min incubation. However, as can be seen from table 2, at

Table 2
Synthesis of poly(A) by cores of *E. coli* 30SO on native T2 DNA

Incubation time	[^{3}H]ATP incorporation, cts/min
0	180
30 min	317
60 min	930
90 min	2279
135 min	3566
180 min	8868

The conditions are described in section Materials and methods.

Table 3
Misincorporation of G into poly(A) by core enzyme of *E. coli*

E. coli strain	exp. No.	[^{3}H]ATP incorporation, cts/min	[^{3}H]GTP incorporation in the presence of cold ATP, cts/min			Misincorporation ratio
			without $(dT)_{12}$	with $(dT)_{12}$	difference	
30SO	1.	83 746	198 ± 12	209 ± 6	+11 ± 13	1.3 (± 1.6) × 10^{-4}
	2.	60 957	189 ± 8	216 ± 7	+27 ± 11	4.4 (± 1.7) × 10^{-4}
	3.	33 785	146 ± 6	158 ± 7	+12 ± 9	3.6 (± 2.7) × 10^{-4}
	4.	32 260	126 ± 6	140 ± 6	+14 ± 9	4.3 (± 2.8) × 10^{-4}
	5.	19 022	124 ± 5	144 ± 5	+20 ± 7	10.5 (± 3.7) × 10^{-4}
	6.	102 124	274 ± 19	295 ± 15	+21 ± 24	2.0 (± 2.3) × 10^{-4}
					mean:	4.4 (± 2.6) × 10^{-4}
Stl–r18	1.	171 641	203 ± 7	289 ± 10	+86 ± 13	5.0 (± 0.8) × 10^{-4}
	2.	152 000	231 ± 14	323 ± 10	+92 ± 17	6.0 (± 1.1) × 10^{-4}
	3.	52 680	301 ± 16	359 ± 6	+58 ± 17	11.0 (± 3.2) × 10^{-4}
	4.	106 967	348 ± 22	425 ± 15	+77 ± 26	7.2 (± 2.4) × 10^{-4}
	5.	32 683	372 ± 16	378 ± 14	+ 6 ± 21	1.8 (± 6.3) × 10^{-4}
					mean:	6.0 (± 1.5) × 10^{-4}
14–50	1.	81 955	231 ± 24	239 ± 7	+ 8 ± 25	1.0 (± 3.1) × 10^{-4}
	2.	87 970	197 ± 7	277 ± 14	+80 ± 16	9.1 (± 1.8) × 10^{-4}
	3.	72 780	224 ± 7	279 ± 7	+55 ± 14	7.6 (± 1.9) × 10^{-4}
	4.	88 885	200 ± 11	261 ± 8	+61 ± 14	6.9 (± 1.5) × 10^{-4}
	5.	64 525	217 ± 7	251 ± 6	+34 ± 9	5.3 (± 1.4) × 10^{-4}
	6.	76 987	213 ± 10	267 ± 14	+54 ± 13	7.0 (± 1.7) × 10^{-4}
					mean:	6.1 (± 0.8) × 10^{-4}
CV–5	1.	62 126	400 ± 21	526 ± 20	+126±30	20.0 (± 5.0) × 10^{-4}
	2.	62 930	219 ± 19	258 ± 16	+39 ± 25	6.2 (± 4.0) × 10^{-4}
	3.	86 300	208 ± 16	269 ± 10	+61 ± 19	7.1 (± 2.2) × 10^{-4}
	4.	47 155	326 ± 9	391 ± 13	+65 ± 16	13.8 (± 3.4) × 10^{-4}
	5.	65 810	336 ± 8	413 ± 13	+77 ± 15	11.7 (± 2.3) × 10^{-4}
	6.	29 448	363 ± 17	406 ± 14	+43 ± 22	14.6 (± 7.4) × 10^{-4}
	7.	45 775	397 ± 10	406 ± 9	+ 9 ± 13	2.0 (± 2.9) × 10^{-4}
					mean:	10.8 (± 1.4) × 10^{-4}
18–35	1.	91 231	124 ± 9	145 ± 7	+21 ± 11	2.3 (± 1.2) × 10^{-4}
	2.	120 648	134 ± 6	191 ± 9	+57 ± 11	4.7 (± 0.9) × 10^{-4}
	3.	90 700	58 ± 3	76 ± 7	+18 ± 8	2.0 (± 0.9) × 10^{-4}
	4.	140 192	61 ± 6	83 ± 7	+22 ± 9	1.6 (± 0.6) × 10^{-4}
	5.	53 837	152 ± 14	214 ± 6	+62 ± 16	11.5 (± 2.9) × 10^{-4}
	6.	43 613	256 ± 8	280 ± 13	+23 ± 15	5.3 (± 3.4) × 10^{-4}
	7.	55 103	244 ± 4	276 ± 4	+32 ± 6	5.8 (± 1.0) × 10^{-4}
					mean:	4.7 (± 1.9) × 10^{-4}

There are three parallel samples for [^{3}H]ATP incorporation and nine parallel samples for [^{3}H]GTP incorporation in each experiment. The conditions are described in section Materials and methods.

longer incubation times incorporation of [^{3}H]ATP by core alone did take place.

Since the presence of sigma is not required for poly(A) synthesis on $(dT)_{12}$, in further experiments we used core enzyme alone. Misincorporation was determined as the difference between nonspecific incorporation of [^{3}H]GTP into acid insoluble material of the samples incubated without template and the incorporation of label into parallel samples containing $(dT)_{12}$. The frequency of errors was determined by dividing this difference by the amount of [^{3}H]ATP incorporated under the same conditions. The results are presented in table 3.

One can see that the frequency of misincorporation under the conditions used is about 6.4×10^{-4}. No difference between wild-type and the mutant strains used was revealed. The misincorporation of [^{3}H]CTP into poly(A) occured at a frequency less than 1×10^{-5} which was beyond the sensitivity of our assay (data not shown).

The frequency of misincorporation observed is in good agreement with the results of Hall and Lehman [5] obtained for DNA polymerase working on artifical templates in vitro. Assuming that the fidelity of in vivo transcription by RNA polymerase is the same as observed in vitro one can expect that the enzyme will make one error per 1700 nucleotides (or even less if the calculations are based on results with [^{3}H]CTP). This implies that the probability of an error in an 'average' mRNA molecule of *E. coli* (about 1000 nucleotides long) will be 0.6. This level of fidelity seems to be high enough to ensure the cell survival if one takes into account the fact that mistranscription must be counterbalanced by the ambiguity of genetic code and by the acceptibility of some amino acid replacements in protein molecules.

It is worth noting that poly(A) synthesis proceeds with high fidelity in a system containing core enzyme and synthetic template. This result provides justification for the wide use of synthetic polymers for studies on the mechanism of RNA polymerase activity.

Thus, the relatively high fidelity of in vitro polynucleotide synthesis by RNA polymerase makes it possible to suggest that participation in substrate selection may be the property common to all enzymes which carry out template synthesis of nucleic acids.

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