ON THE FIDELITY OF TRANSCRIPTION BY *ESCHERICHIA COLI* RNA POLYMERASE

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1. Introduction

The enzymes that carry out template-directed synthesis of DNA (DNA polymerase and reverse transcriptase) were shown to participate in the selection of complementary nucleotides as proved by the effect of enzyme structure on the fidelity in copying polynucleotide templates [1,2]. Temperature-sensitive mutants affecting the structural gene of DNA polymerase are characterized by increased mutability [3–6]. A difference has been observed between nucleotide misincorporation of in vitro DNA synthesis by normal and mutant enzyme [1].

Mutational alterations of DNA-dependent RNA polymerase have not revealed such a dependence [7]. No difference in the fidelity of in vitro transcription was observed when intrinsic Zn^{2+} of RNA polymerase was substituted by Co^{2+} [8]. The data available on the accuracy of base-selection during transcription are conflicting [7,9–11]. The values of misincorporation vary substantially in different reports. In the case of poly(AU) synthesis on a poly [d(AT) X d(AT)] template the frequency of GMP incorporation was shown to be 9.5×10^{-4} [9] and 2.3×10^{-5} [10]. The frequency of misincorporation of GMP into poly(A) synthesized on a poly(dT) template varies between 3.3×10^{-2} [11] and 6.4×10^{-4} [7]. With synthetic polyribonucleotides as a template, the variations are still greater.

To study the role of RNA polymerase in ensuring the fidelity of transcription, in this work we chose RNA polymerase rifampicin resistant (rif^r) mutants of *Escherichia coli* B/r, rpoB402, rpoB403 and rpoB409 [12,13], which are characterized by increased variance within one of the phenotypic parameters (cell morphology) as compared to the wild-type strain. We analyzed the misincorporation of GMP into poly(AU) synthesized by normal and rpoB402 mutant RNA polymerase on a poly [d(AT) X d(AT)] template.

RNA polymerase from rpoB402 modified in the β-subunit as a result of the rif^r mutation, incorporates non-complementary nucleotides more frequently than the enzyme from the wild-type strain. The results indicate that RNA polymerase takes part in the selection of the complementary nucleotides and thus participates in ensuring the fidelity of transcription. Moreover, the data obtained indicate that the accuracy of base-selection during transcription can be controlled by the β-subunit of RNA polymerase.

2. Materials and methods

2.1. *Bacterial strains*

The wild-type strain of *E. coli* W12, B/r WU-36-10-11-12 (leu_{am} tyr_{oc} $supE_{oc}$) and its 3 rif^r mutants, rpoB402, rpoB403 and rpoB409 were used [12,13].

2.2. *Reagents*

All radioactive ribonucleoside triphosphates were obtained from UVVVR (Prague, Czechoslovakia) and further purified by paper chromatography on FN3 paper in two systems:

(1) Isopropyl alcohol:NH_4OH:water:ethanol (1:2:5:12) ([14] with some modifications);
(2) Isobutyric acid:0.5 M NH_4OH (10:6) [15].

The unlabeled ribonucleoside triphosphates UTP, GTP and CTP from Reanal (Hungary), were purified on Dowex 1 X 8 (Cl^-) [16]. ATP was purchased from Serva and poly [d(AT) X d(AT)] from Calbiochem.

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2.3. *Enzyme preparation and assay*

RNA polymerase holoenzyme from *E. coli* W12 and rpoB402 were purified by the method in [17]. The incorporation of complementary (AMP and UMP) and non-complementary ribonucleotides (CMP and GMP) was determined in assays that were incubated for 30 min at 37°C. Each reaction mixture contained (final vol. 0.2 ml), 0.05 M Tris–HCl (pH 8.0); 0.1 mM EDTA; 0.01 M $MgCl_2$; 0.05 M KCl; 5 mM β-mercaptoethanol; 0.1 mM ATP; 0.1 mM UTP; 0.1 mM GTP or CTP; poly [d(AT) × d(AT)] (A_{260} 0.02); and 10 μg RNA polymerase. The specific activity of [^{14}C]ATP, [^{14}C]GTP and [^{14}C]CTP was 10^8 cpm/mM and [^{14}C]UTP, 1.5×10^7 cpm/mM. The assays were terminated by the addition of 0.1 ml 0.05 M Tris–HCl (pH 8.0), 75 mM EDTA, and blue dextran to an A_{750} of 20. The non-incorporated ribonucleotides were separated from the polynucleotide product by Sephadex G-100 gel filtration as in [10].

2.4. *Morphological measurements*

The area of projection of bacterial cells on the plane was determined on the installation 'Priam' that permits morphological measurements to be carried out in cell suspension and the results to be treated in a computer with special algorithms [18].

3. Results and discussion

RNA polymerase is responsible for the phenotypic realization of the genotype. Therefore, if the fidelity of transcription is influenced by the structural state of the RNA polymerase, the errors of transcription should correlate with the degree of variance of phenotypic cell parameters and certain mutations affecting the enzyme structure would be expected to produce alterations in the stability of the phenotype. Analysis of such mutants should provide a promising approach to the control of base-selection of RNA polymerase.

Here we selected mutants which differ from the wild-type strain of *E. coli* W12 in the heterogeneity of cell size: the choice was made among rif^r RNA polymerase mutants with pleiotropic effects: rpoB402, rpoB403, and rpoB409 [12,13]. Measurements were made on the 'Priam' complex which permits intravital morphometry of a great number of cells in suspension to be carried out and thus a statistically reliable result can be obtained. The coefficient of variation (V) was a measure of the heterogeneity of the cells [19]:

$$V = \frac{\sigma}{\bar{m}} \times 100$$

where: σ is variance of projection area; $\bar{m}$ is the average projection area

From the data given in table 1 it follows that the cells of all mutants are more heterogenous in size than the cells of the wild-type strain, with the greatest difference observed with the rpoB402 mutant. Hence it appears clear that there is a correlation between the stability of the phenotype and the structural state of the RNA polymerase. The mutations of the RNA polymerase seem to result in less accurate selection of nucleotides during transcription. This was directly verified by comparing the fidelity of in vitro RNA synthesis carried out by normal and rpoB402 RNA polymerase on a poly [d(AT) × d(AT)] template. The results are presented in table 2.

One can see that the frequency of GMP misincorporation by normal enzyme is about 11.7×10^{-4}. The value is in good agreement with the results in

Table 1
Distribution of cells of *E. coli* strains W12 rpoB402, rpoB403 and rpoB409 based on the area of their projection on the plane

E. coli strain	Parameters characterizing distribution			Range of alteration of projection area (μm²)
	m (μm²)	m^2 (μm⁴)	V	
W12	7.6	4.1	26.3	7.6 ± 2
rpoB402	11.5	22.5	40.8	11.5 ± 4.7
rpoB403	8.5	9.9	36.6	8.5 ± 3.1
rpoB409	8.3	7.6	33.7	8.3 ± 2.8

Table 2
Misincorporation of GMP into poly(AU) by the holoenzyme of *E. coli* W12 and rpoB402

E. coli strain	Exp. no	[^{14}C]AMP and [^{14}C]UMP incorporation (pmol)	[^{14}C]GMP incorporation (pmol)	Misincorporation ratio
W12	1	3866	3.3 ± 0.7	8.9×10^{-4}
	2	3435	3.2 ± 0.9	9.3×10^{-4}
	3	112	0.2 ± 0.05	1.7×10^{-4}
				Mean: 11.7×10^{-4}
rpoB402	4	4520	34.0 ± 12.0	72.0×10^{-4}
	5	1242	6.9 ± 0.28	55.0×10^{-4}
	6	1110	10.2 ± 0.88	35.0×10^{-4}
				Mean: 54.0×10^{-4}

[7,9]. Mutational alteration of RNA polymerase markedly reduces the accuracy of copying the poly [d(AT) × d(AT)] template as judged by the misincorporation of GMP with the rpoB402 RNA polymerase (table 2). A similar decreased fidelity with mutant enzyme is observed using CTP as non-complementary nucleotide. The incorporation of CMP into poly(AU) by normal enzyme occurred at a frequency which was below the limit of detection, whereas misincorporation of CMP by the mutant polymerase was detectable (data not shown).

Thus, the mutant rpoB402 chosen on the basis of the greatest deviations from the mean value of a given phenotypic parameter proved to be successful to reveal the role of RNA polymerase in base-pairing selection. The data obtained indicate that the β-subunit of the RNA polymerase does participate in ensuring the fidelity of transcription.

Thus, participation in substrate selection is a property common to all enzymes that carry out template-dependent nucleic acid synthesis.

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