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Deoxyribonucleic Acid Polymerase II Activity in an *Escherichia coli* Mutator Strain

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The *polB* gene encoding deoxyribonucleic acid (DNA) polymerase II has been located close to a mutator gene, *mutT1*, in *Escherichia coli*. We find the DNA polymerase II prepared from *mutT1*, strains to be normal in reaction requirements, heat stability, and ability to remove mismatched bases at termini. Recombinants formed from a mutant defective in DNA polymerase II (*polB100*) and *mutT1* are deficient in polymerase II and have the same mutator phenotype as *mutT1*. Our linkage analysis indicates that *mutT1* and *polB100* are not isoallelic.

The first report of a mutator gene in bacteria resulted from its detection in a highly mutagenized *Escherichia coli* K-12 stock by Treffers et al. (18). This mutator gene, *mutT1*, is located between the leucine and azide loci (8). Recently, the *polB* locus, specifying deoxyribonucleic acid (DNA) polymerase II, was mapped in the same chromosomal region as the *mutT1* gene (3, 11). This raised the possibility that the mutator gene might specify an altered DNA polymerase II.

At the present time the only phenotypic characteristic of the *polB*$^-$ strain is an absence of DNA polymerase II, though the following characteristics have been examined (3, 10): (i) phage growth, (ii) F and R plasmid replication, (iii) temperature sensitivity, (iv) X-ray sensitivity, (v) recombinational capacity, (vi) ultraviolet sensitivity, and (vii) sensitivity to alkylating agents.

The *mutT1* allele is recessive to the wild-type allele (8). It can increase mutation rates in infecting episomes and various phages and requires DNA replication for expression (6, 7, 8). This gene specifically causes an adenosine-thymidine to cytidine-guanosine transversion mutation (7, 21). The increase in mutation rate for *mutT1*-containing strains is 100- to 1,000-fold over wild type, depending on the particular gene being assayed.

This report demonstrates the presence of DNA polymerase II in *mutT1* strains, the characteristics of this enzyme, and fine mapping data of the *mutT1 polB* region of the chromosome, identifying these genes as two separate loci.

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MATERIALS AND METHODS

Strains. *E. coli* strain 58-278* was obtained from E. C. Siegel. It is the original mutator strain from Treffers et al. (18) and has the following genotype: *mutT1*$^-$ *bio*$^-$ *phe*$^-$. HMS83 was obtained from C. C. Richardson and has the following genotype: *polA*$^-$ *polB*$^-$ *lys*$^-$ *thy*$^-$ *str*^{r}. This *polB*$^-$ allele has recently been designated *polB100* (3). W3110 *thy*$^-$ was obtained from J. Gross. P1*vir* was obtained from M. Oishi.

Media. L-broth medium contained 1.0% tryptone, 1.0% NaCl, 0.5% yeast extract, 1.0% glucose, and 50 μg of thymine per ml. Minimal medium (VB medium) was that of Vogel and Bonner (19). When required, L-amino acids were included at 50 μg/ml and pyrimidines at 10 μg/ml. When used in plates, 1.5% agar was included. All incubations were done at 37 C.

Penicillin selection. One milliliter of an overnight culture of HMS83 grown in VB medium supplemented with leucine was used to inoculate 20 ml of VB medium not supplemented with leucine. When the culture had grown to a cell density of 2×10^8 cells/ml, penicillin G (Sigma) was added to a final concentration of 20,000 U/ml. The culture was incubated for an additional 2 to 3 h and then centrifuged for 1 h at 27,000 × *g*. The supernatant fluid was decanted, and the collected cells were washed and resuspended in VB medium containing leucine. After overnight growth, the above-mentioned penicillin selection procedure was repeated. A *leu*$^-$ derivative was identified by replica plating isolated colonies on VB medium with and without leucine.

Transduction. P1 transduction was carried out as described by Lennox (12). For P1 transduction L-broth medium was supplemented with 2.5 mM $CaCl_2$. P1 phage stocks were obtained by growing the phage twice on *E. coli* strain 58-278* and were titered on the same strain using an L-broth soft (0.7%) agar overlay technique. One milliliter (2×10^9 cells) of the recipient strain was mixed with P1 phage at a multiplicity of 0.01. After 20 min of incubation, 10 ml of VB medium was added. The cells were sedimented

and suspended in minimal medium for plating on VB medium without leucine.

mutT1 detection. The presence of the *mutT1* gene was assayed by streaking isolated transductant colonies on a 5-mm square of an L-broth plate containing 100 μg rifampin (Sigma) per ml. Wild-type colonies showed no growth on such a plate after 24 h, whereas mutator strains showed 15 or more colonies. Quantitative mutation frequencies were assessed by determining the number of rifampin-resistant colonies in a logarithmic-phase culture of *E. coli*.

DNA polymerase II screening. A 5-ml overnight bacterial culture was concentrated, and the cells were suspended in 0.1 ml of a 1% toluene emulsion in 50 mM tris(hydroxymethyl)aminomethane-chloride, 5 mM ethylenediaminetetraacetic acid (pH 7.6). The cell suspension was kept at room temperature for 8 min and then frozen and thawed twice at −70 C. Ten microliters of 10 mg of lysozyme per ml was added, followed by incubation for 5 min at 37 C (4). After addition of 0.1 ml of 25% Triton X-100 and chilling at 0 C, the suspension was passed through a 1/2-inch, 27-gauge needle three times. After a subsequent incubation at 0 C for 1 h, 0.75 ml of a 50% (vol/vol) suspension of diethylaminoethyl-cellulose (DE-52, Whatman) in 0.6 M potassium phosphate (pH 7.4), 1 mM ethylenediaminetetraacetic acid, and 1 mM β-mercaptoethanol was added to the lysed cell suspension. The mixture was spun at 10,000 × *g* for 10 min, and a 25-μl sample of the supernatant fluid was assayed for DNA polymease II activity.

DNA polymerase II purification and assay. DNA polymerase II was purified and assayed as previously described (14, 15). After purification by phosphocellulose chromatography, the enzyme was concentrated by pressure dialysis against 20 mM potassium phosphate buffer (pH 6.5) containing 0.1 mM dithiothreitol and 1.0 mM ethylenediaminetetraacetate. Such a preparation is over 3,000-fold purified and is free from detectable endonucleolytic activity.

The following modification in the assay procedure was used when transductants were screened for polymerase II activity. After 30 min of incubation at 37 C, 2.5 ml of 10% (wt/vol) trichloroacetic acid-0.1 M sodium pyrophosphate was added. The tubes were incubated for 10 min at 0 C and then spun at 10,000 × *g* for 10 min. The pellet was suspended in 0.2 ml of 0.1 N NaOH and incubated for 10 min at room temperature, and 0.2 ml of 0.1 M HCl was added. After mixing, 2.5 ml of 10% trichloroacetic acid-0.1 M sodium pyrophosphate was added, and the tubes were incubated for 10 min at 0 C. The filtrate was collected and treated as previously described.

One unit of DNA polymerase activity is defined as that amount of enzyme leading to the incorporation of 10 nmol of total nucleotide into acid-precipitable material in 30 min at 37 C.

Protein was determined by the fluoresamine procedure (1).

Synthetic template assays. Reactions utilizing synthetic polymers as templates were reduced to a total volume of 0.1 ml. Synthetic polynucleotides (P-L Biochemicals) were used at the following concentrations: 0.27 mM poly(dA); 0.04 mM oligo(dT); 0.48 mM poly(dT); 0.1 mM oligo(dA); 0.28 mM poly(d[AT]). The oligonucleotides were of n = 12 to 18. In polymerase assays to study misincorporation of a non-complementary base [^{32}P]deoxyguanosine 5′-triphosphate (10^3 to 10×10^3 counts/min per pmol; New England Nuclear Corp.) and [^{3}H]thymidine 5′-triphosphate (10^3 to 4×10^3 counts/min per nmol; New England Nuclear Corp.) were used as substrate. In addition, the washing procedure described above was used to reduce background counts.

The 3′-exonucleolytic activity was measured using template and primers kindly supplied by F. J. Bollum. The primer oligonucleotide d(T) contained a 3′-terminus, tritium-labeled, matched (dTMP) or mismatched (dGMP, dCMP, dAMP) base with a specific activity of 3×10^3 to 5×10^3 counts/min per nmol. Concentrations of template [poly(dA)] and primer [oligo(dT)] were 0.1 and 0.02 mM, respectively. The oligonucleotides were of n = 47, whereas the polymer was n = 4,000. Removal of the 3′-terminal base was assayed by following the decrease in acid-precipitable radioactive material.

RESULTS

DNA polymerase II purified from a mutator strain. Since the *polB100* locus specifies a known enzyme, both an enzymological and genetic approach could be used to determine whether the *polB* and *mutT1* mutations are in the same cistron. However, prior to initiating these studies, the question that needed to be answered was whether DNA polymerase II is present in *mutT1*-containing *E. coli* strains. DNA polymerase II was found to be present in a *mutT1*-containing strain in amounts similar to those reported by Moses and Richardson (14, 15) for wild-type strains. Furthermore, the enzyme displayed the same chromatographic behavior as reported previously (14, 15) and found in this study for enzyme purified from a wild-type *E. coli* strain, eluting from a phosphocellulose column at 0.25 to 0.27 M phosphate at pH 6.5 (Fig. 1).

Attempts were made to discriminate between DNA polymerase II from mutator and wild-type strains. We have been unable to detect a difference in the synthetic activity of the enzyme purified from a mutator strain from that reported previously (14, 15, 20). The enzyme from the mutator strain requires the presence of all four deoxynucleoside 5′-triphosphates for full activity. It has a broad pH optimum around pH 7.5, as does the enzyme from the wild-type strain. Optimum reaction requirements are the same for both enzymes, as is inhibition by salt and sulfhydryl-blocking reagents. No immuno-

logical differences could be shown by precipitin formation in double diffusion tests against antibody prepared against the wild-type DNA polymerase II. The heat inactivation of polymerase II from both sources is the same (Fig. 2).

Synthetic templates. Mutator polymerases of T_4 have been characterized as demonstrating increased misincorporation of non-complementary bases on synthetic template in vitro (9, 10, 17). Therefore, enzymes from wild-type and mutator strains were tested on various synthetic templates (Table 1). On poly(dA)·oligo(dT) the wild-type enzyme has approximately 20% of the activity observed on activated salmon sperm DNA, whereas the mutator strain has approximately 5% of the activity observed on DNA. Neither of the enzymes has appreciable activity on the alternating co-polymer poly(d[AT]). No activity was detected when poly(dT)·oligo(dA) was used as template primer.

Since *mutT1* specifically causes an adenosine-thymidine to guanosine-cytidine transversion mutation, misincorporation into poly(dA)·oligo(dT) of the non-complementary base, dGMP, was followed (Table 2). The values shown in Table 2 represent a series of experiments with several enzyme preparations which have been arranged in increasing incorporation of dTMP, the complementary base. The mean deoxyguanosine/deoxythymidine ratios suggest that DNA polymerase II misincorporation is not responsible for the mutator effect. Under the same assay conditions DNA polymerase I co-purified through the phosphocellulose column produces a mean deoxyguanosine/deoxythymidine ratio of 4×10^{-4}.

Some of the T_4 DNA mutator polymerases were shown to have a decreased 3'-exonuclease/polymerase ratio (17). Since DNA polymerase II has a 3'-exonucleolytic activity (13, 20), the exonuclease activity of DNA polymerase II from mutator and wild-type strains was investigated. No difference was found in the ability of the enzymes to remove matched and mismatched 3'-terminus bases from defined templates. Mismatched bases are removed more rapidly than correctly paired bases (Fig. 3).

Linkage analysis. Since mutator strains contain DNA polymerase II, we could look at the co-transduction frequency of *polB100* and *mutT1*. However, since neither gene has a

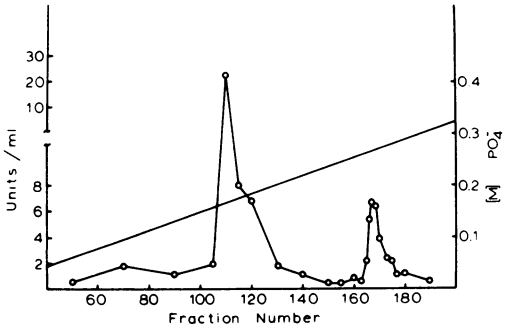

FIG. 1. *Phosphocellulose chromatography on DNA polymerase activity from a mutT1-containing E. coli strain. The left peak represents DNA polymerase I activity; the right peak represents DNA polymerase II activity.*

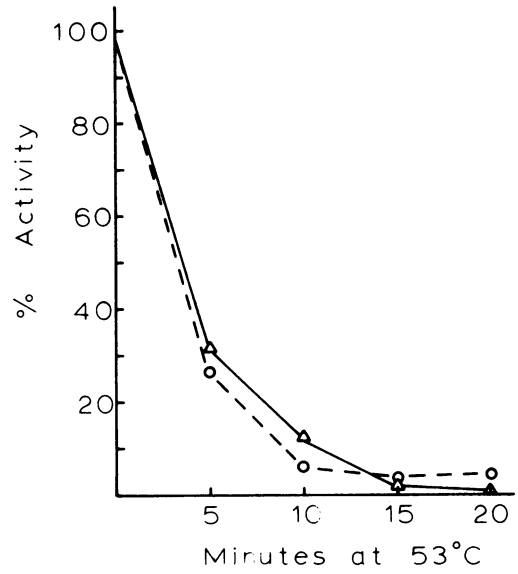

FIG. 2. *Heat sensitivity of DNA polymerase II purified from mutT1 (O) and W3110 (Δ) E. coli. One hundred percent activity is 0.093 and 0.061 nmol of [^{3}H]dTMP incorporated per reaction, and 0.63 and 0.5 μg of protein were used per reaction, respectively. The assays of activity after the indicated time at 53 C were performed at 32 C.*

TABLE 1. *DNA polymerase II activity on various templates*[a]

Template	Concn (mM)	dTMP incorporated (pmol)	
		W3110	58-278*
Activated salmon sperm DNA	0.75	60.7	97.0
Poly(dA)	0.27	11.8	5.3
Oligo(dT)	0.02		
Poly(d[AT])	0.28	1.1	0.8

[a] W3110 specific activity was 24 units/mg and 58-278* specific activity was 21 units/mg when measured at 32 C on activated DNA. Reactions were done as described in Materials and Methods.

TABLE 2. *Misincorporation of dGMP into poly(dA)·oligo(dT) by DNA polymerase II*[a]

W3110 (pmol)[b]			58-278* (pmol)		
dTMP	dGMP	G/T	dTMP	dGMP	G/T
6.3	0.011	1.7×10^{-3}	4.2	0.026	6.1×10^{-3}
7.8	0.009	1.2×10^{-3}	6.4	0.013	2.0×10^{-3}
7.9	0.038	4.8×10^{-3}	6.8	0.004	5.8×10^{-3}
9.6	0.006	6.0×10^{-4}	8.2	0.036	4.3×10^{-3}
39.0	0.049	1.2×10^{-3}	8.6	0.040	4.7×10^{-3}
50.2	0.023	4.5×10^{-4}	10.6	0.032	3.0×10^{-3}
53.6	0.060	1.1×10^{-3}	10.9	0.069	6.3×10^{-3}
57.1	0.034	5.9×10^{-3}	15.3	0.139	9.0×10^{-3}
112.6	0.080	7.1×10^{-3}	16.3	0.095	5.8×10^{-3}
135.7	0.059	4.0×10^{-4}	18.0	0.026	1.4×10^{-3}
150.8	0.104	6.0×10^{-4}	28.5	0.104	3.6×10^{-3}
151.0	0.115	7.6×10^{-4}	36.3	0.117	3.2×10^{-3}

[a] The mean guanosine thymidine (G/T) ratios were as follows: W3110, 2.2×10^{-3} (± 2.3); 58-278*, 4.6×10^{-3} (± 2.0).

[b] Template concentration was 0.27 mM poly(dA); primer concentration was 0.04 mM oligo(dT). The results are arranged in order of increasing dTMP incorporation with increasing amounts of enzyme activity. Reactions were done as described in Materials and Methods. Incorporation of both substrates increased with increasing enzyme concentration.

selectable phenotype, co-transduction with leucine was used as a primary selection technique. Workers (3, 11, 21) previously had shown that both *polB100* and *mutT1* co-transduce with leucine approximately 40% of the time. Co-transduction frequencies with an *azi* marker were not done, as such mutations proved to be unstable and azide itself was previously shown to be mutagenic (16).

The frequency of mutation in *mutT1* strains varies with the marker selected. Frequency of occurrence of rifampin resistance for the *mutT1*-containing strain is 3×10^{-3}, whereas HMS83 has a frequency of 10^{-6}. This difference in rate could easily be detected by streaking on a rifampin plate.

A spontaneous leucine auxotroph of HMS83, derived by penicillin selection, was used as the recipient strain. Besides lacking DNA polymerase II, this strain also lacks detectable DNA polymerase I and was used as the recipient in order that a short extraction scheme could be used for the detection of transfer of the *polB* gene without interference from DNA polymerase I activity. Polymerase III activity was not detected under the assay conditions employed. The recombinants scored as $polB^+$ incorporated 10 to 20 pmol of [^{3}H]dTMP (the same as the $polA^-$ $polB^+$ controls), whereas recombinants scored as $polB^-$ incorporated 1 pmol or less (the same as HMS83). One hundred and ninety-seven leucine transductants were screened for mutator and polymerase II activity (Table 3). Twenty-one of the leucine prototrophs had both activities, whereas 55 had only mutator activity and 44 had only polymerase II activity. Our data do not allow an unambiguous assignment of order for *mutT1-polB100-azi*. However, the existence of *mutT1-polB100* recombinants establishes that the genes are nonallelic. Quantitative determination showed the mutation rates for rifampin resistance in the *mutT1-polB100* recombinants to be the same as in the parent *mutT1* strain.

DISCUSSION

Since the *polB100* locus was mapped close to the position of the *mutT1* locus, it seemed reasonable to investigate the presence and nature of DNA polymerase II in *mutT1* strains.

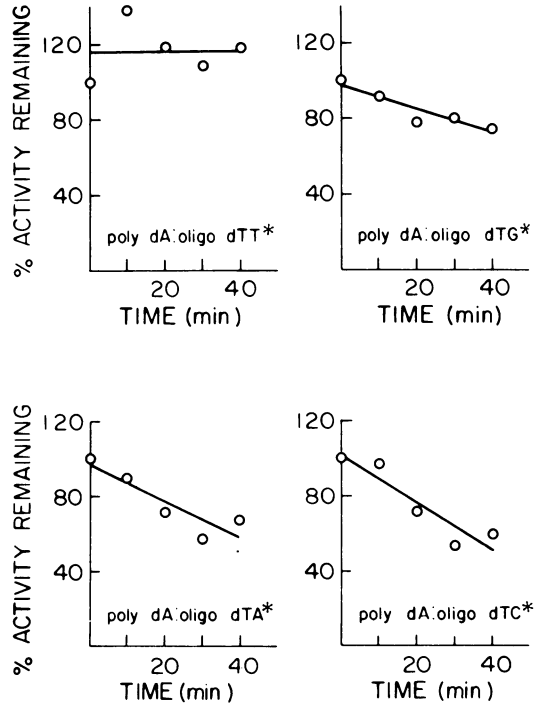

FIG. 3. *DNA polymerase II exonuclease activity on terminally matched and mismatched bases. DNA polymerase II prepared from mutT1 E. coli was incubated with homopolymers with matched or mismatched termini as described in Materials and Methods. The lines were determined by least-squares fit. DNA polymerase II was present at 0.05 unit/ml.*

TABLE 3. *Co-transduction of leu, polB100, and mutT1*[a]

Genotype	No. of *leu*$^+$ transductants
mutT1 polB$^-$	55
mutT1 polB$^+$	21
mut$^+$ *polB*$^+$	44
mut$^+$ *polB*$^-$	77

[a] Phage P1*vir* was grown on strain 58-278* (*polB*$^+$ *mutT1*$^-$) and transduction was done as described. Recipient cells (HMS83 *leu*$^-$) were selected on minimal medium for conversion to *leu*$^+$. Transductants were scored for the *polB100* marker by assay in extracts and for the *mutT1* marker by rifampin resistance, as described.

Previous studies on phage T_4 mutator polymerase have demonstrated that the expression of a mutator phenotype may require a subtle alteration in a characteristic of the enzyme rather than an absence of the DNA polymerase. Thus it was not surprising that mutants deficient in DNA polymerase II do not show an altered rate of mutation. In addition, our result showing that DNA polymerase II is present in normal amounts in the *mutT1*-containing strain does not argue against the polymerase being the basis of the mutator phenotype. However, by the criteria we applied, we could demonstrate no difference in the in vitro properties of DNA polymerase II purified in parallel from wild-type and mutator strains.

Our results do indicate that the 3'-exonucleolytic activity of DNA polymerase II is capable of removing mismatched bases from DNA duplexes. This suggests a possible "editorial" role for this enzyme, as has been shown for DNA polymerase I (2).

The construction of strains containing both a deficiency in DNA polymerase II activity and the mutator phenotype establishes that the *mutT1* and *polB100* genes are nonallelic, though co-transduction frequency does indicate close linkage. Studies cited above (3) have shown that the *polB100* locus co-transduces with *leu* at a frequency in agreement with our observations. These studies also place *polB* to the right of *leu* close to *azi*. Cox and Yanofsky have also presented data indicating that *mutT1* is closely linked to *azi* (8). However, the existence of the recombinants establishes that *mutT1* is not *polB100*. The *mutT1 polB100* double mutants do not display any apparent alteration in growth compared with the *polB100* parent.

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