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Isolation of an altered form of DNA polymerase I from *Escherichia coli* cells induced for *recA/lexA* functions

(SOS repair/mutagenesis/fidelity of DNA synthesis)

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ABSTRACT **A novel form of DNA polymerase I (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, DNA nucleotidyltransferase, EC 2.7.7.7) activity has been isolated from *Escherichia coli* cells that had been activated for expression of the DNA damage-inducible genes. Induction was by treatment of normal cells or cells carrying the *spr-51* and *tif-1* mutations with nalidixic acid. This activity, DNA polymerase I*, seems to be a form of DNA polymerase I because it is insensitive to *N*-ethylmaleimide, is inhibited by antibody to DNA polymerase I, and does not appear in a *polA1* strain. DNA polymerase I* activity sediments through sucrose gradients as a broad peak with $s_{20,w}$ = 6.6–10.5, compared with an $s_{20,w}$ = 4.8–5.5 for DNA polymerase I. The fidelity during polymerization reactions of DNA polymerase I* is relatively low with a variety of synthetic templates and deoxynucleoside triphosphates, although the enzyme appears to have a normal level of 3′->5′ exonuclease. Polymerase I* has properties that might implicate it in some form of mutagenic DNA repair.**

Mutations induced in *Escherichia coli* by UV radiation or by a variety of chemicals are believed to often be the consequence of an error-prone DNA repair pathway controlled, along with other SOS functions, by the *recA* and *lexA* genes (1–3). This pathway is evidently induced by agents that block DNA replication either by interfering with replicative enzymes or by modifying bases so as to affect base recognition in the DNA template. Blocks due to base damage are presumably mediated by constraints that regulate replicative fidelity by ensuring the synthesis only of properly base-paired DNA. Models have been proposed for overcoming such replicative blocks that predict the transient appearance of error-prone forms of DNA polymerase that can polymerize random nucleotides opposite template damage (2, 4). Because such hypotheses are consistent with much of the available data on mutagenic DNA repair (5), we have sought to identify these predicted forms of DNA polymerase. We report here the observation of an error-prone form of DNA polymerase I that is associated with the induction of *recA/lexA* functions in *E. coli*.

MATERIALS AND METHODS

Bacterial Strains. Extracts were prepared from *E. coli* K-12 strains DM1187 (*tif-1, spr-51, lexA3, sfiA11, his-4, strA31*) (6), AB1157 (*argE3, his-4, leu-6, proA2, thr-1, ara-14, galK2, lacY1, mtl1, xyl5, thi-1, tsx-33, supE44, sup-37, str-31*) (7), and P3478 (*polA1, thyA36*), a derivative of W3110 (5).

Materials. Synthetic polymers and unlabeled dNTPs were from P-L Biochemicals, radioactive nucleotides were from Amersham, and electrophoretically homogeneous DNA polymerase I was a gift from Arthur Kornberg, Stanford University, or Lawrence Loeb, University of Washington. Antibody against DNA polymerase I was provided by I. R. Lehman, Stanford University. Polymin-P was from Miles; agarose (type II) was from Sigma; DNA-agarose was prepared by the method of Schaller *et al.* (8), except that DNA had been denatured by heating to 95°C.

Sucrose Gradient Sedimentation of DNA Polymerase I*. Active fractions from the DNA-agarose column (see Fig. 1) were pooled and concentrated 20-fold by precipitation with ammonium sulfate (0.42 g/ml), dialyzed against 0.05 M Tris·HCl, pH 7.5/0.5 mM dithiothreitol/0.2 mM EDTA/2% sucrose/200 mM NaCl, and then stored in liquid nitrogen or sedimented directly for 11.5 hr at 50,000 rpm in an SW50.1 rotor through 5–20% sucrose gradients in 0.02 M Tris·HCl, pH 7.5/0.5 mM dithiothreitol/0.2 mM EDTA/200 mM NaCl. Bovine serum albumin and catalase were sedimented in a separate tube as sedimentation markers. Sucrose gradient fractions were stable for 1 to 2 weeks when stored at 0°C and were used as described below unless otherwise noted.

DNA Polymerase Assays. Reaction mixtures were 50 mM Tris·HCl, pH 7.5/10 mM MgCl/0.1 M KCl/0.5 mM dithiothreitol containing bovine serum albumin at 0.5 mg/ml, 0.13 μmol (nucleotide residues) of "activated" salmon sperm DNA, and 5 nmol each of dATP, dGTP, dCTP, and dTTP. One triphosphate was labeled with ^3H at 50–100 cpm/pmol and assays were carried out as described (9). Fidelity assays were as described (10), except that 10 mM $MgCl_2$ always provided the divalent cation.

RESULTS

Isolation of DNA Polymerase I*. Cell-free lysates were prepared, fractionated with polyethyleneimine to remove nucleic acids, and then chromatographed on single-stranded DNA-agarose. Expression of the *recA* gene is accompanied by the appearance of a new peak of DNA polymerase activity, DNA polymerase I*, that elutes at 150 mM NaCl (Fig. 1). The peak of material absorbing at 280 nm that cochromatographs with polymerase I* appears to be largely recA protein as judged by its appearance during *recA* induction, the high level of DNA-dependent ATPase activity appearing in these fractions, and the presence of an intense band at the recA protein position during $NaDodSO_4$/polyacrylamide gel electrophoresis. In the absence of *recA* gene expression, DNA polymerase activity does not appear at 150 mM but elutes from DNA-agarose as a single peak near 500 mM NaCl and in the column flow through (Fig. 1*B*).

The activity of the polymerase I* peak as well as that of legitimate polymerase I eluting near 500 mM or in the flow through are inactivated by antibody to DNA polymerase I (Table 1). DNA polymerase I* was obtained from *E. coli* DM1187, a strain that is constitutive for the expression of the *recA* gene because it carries the *spr-51* mutation in the *lexA* gene and the *tif-1* mutation in the *recA* gene (6). DM1187 was induced with

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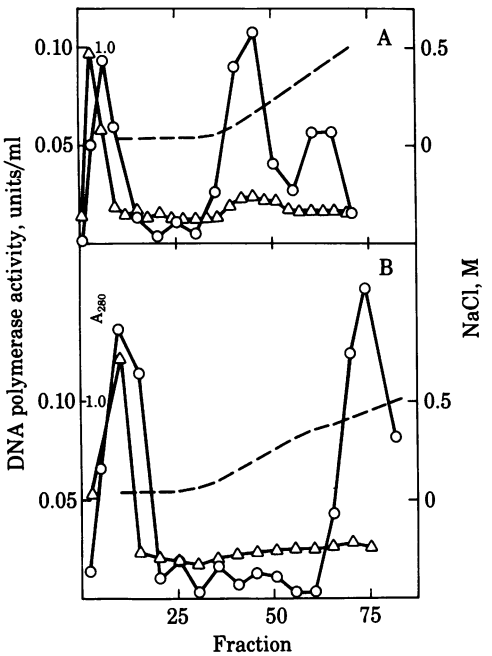

FIG. 1. DNA-agarose chromatography of material from *E. coli* DM1187 induced for SOS repair (*A*) and from uninduced AB1157 (*B*). *E. coli* K-12 cells were grown in 200 liters of Hershey broth at 37°C with aeration. For SOS induction, nalidixic acid was added (final concentration, 40 μg/ml) when the cell density reached 2×10^8/ml (OD_{550} = 0.75). After 45 min, the culture was chilled to 0°C within 20 min and cells were harvested in a refrigerated centrifuge. Cells were lysed as described by Wickner *et al.* (11), except that they were suspended in 0.05 M Tris·HCl, pH 7.5/0.5 mM dithiothreitol/0.2 mM EDTA/10% sucrose (buffer A) before freezing and centrifugation after lysis was for 60 min at 44,400 × *g*. The lysate was adjusted to 0.75% dimethyl sulfoxide/0.5 mM phenylmethylsulfonyl fluoride. NaCl was added (final concentration, 0.20 M) and 10% Polymin-P, pH 7.5, was slowly added (final concentration, 0.9%) while mixing in a Waring Blendor. After 5 min, the suspension was centrifuged. (All centrifugations were for 10 min at 13,700 × *g*, unless otherwise noted.) The pellet was extracted with 500 ml of buffer A/0.50 M NaCl and then with 500 ml of buffer A/1.0 M NaCl. To the last supernatant was added solid ammonium sulfate slowly with stirring (0.42 g/ml). After 5 min at 0°C, the precipitate was collected by centrifugation and washed with 500 ml of buffer A/ammonium sulfate (0.42 g/ml)/50 mM NaCl to remove residual polyethyleneimine. Finally, the washed precipitate was dissolved in 25 ml of buffer A/50 mM NaCl and the solution was dialyzed overnight against the same buffer. The dialyzed material was centrifuged and could be quickly frozen for storage in liquid nitrogen. The dialyzed material was applied to a 100-ml DNA-agarose column equilibrated with buffer A/4 mM $MgCl_2$ and then washed with 300 ml of the same buffer. The column activity was eluted with a linear gradient of 0–0.60 M NaCl in buffer A/4 mM $MgCl_2$ (700 ml). DNA polymerase I* (○) activity eluted near 150 mM. △, A_{280}; ----, NaCl concentration. The activity peaks near 500 mM NaCl were at the same ionic strength, although the two columns had different numbers of fractions.

nalidixic acid under conditions that increase the yield of *recA* protein ≈2-fold. Polymerase I* has also been obtained in comparable yield from *E. coli* AB1157 (which has no *recA* or *lexA* mutations) after induction with nalidixic acid.

DNA polymerase activity eluting at 150 mM NaCl was concentrated and sedimented through 5–20% sucrose gradients in 200 mM NaCl to remove traces of DNA that might interfere with fidelity measurements. A broad peak of DNA polymerase activity corresponding to $s_{20,w}$ values of 6.6–10.5 was obtained. On the other hand, DNA polymerase activity eluting from DNA agarose at 500 mM, homogenous *E. coli* DNA polymerase I purified by standard procedures, and DNA polymerase I large fragment sedimented as single sharp peaks with an $s_{20,w}$ value

Table 1. Inhibition of DNA polymerase activities

Experiment	Addition	DNA polymerase activity, pmol of nucleotide incorporated I*	I	HeLa α
I	None	8.9		
	Antibody (25 μl)	0.0		
II	None	3.8	1.3	
	Antibody (3 μl)	2.4	0.8	
III	None	41.1	39.9	19.5
	Antibody			
	3 μl	38.2	27.1	24.1
	10 μl	26.0	15.0	24.1
	25 μl	20.4	10.9	25.1
	N-Ethylmaleimide (3 mM)	33.5	33.8	0.0
IV	None		1.6	
	Antibody (25 μl)		0.0	
V	None		6.0	
	Antibody (25 μl)		0.2	

Experiment III used different preparations of the polymerases than experiments I or II. HeLa polymerase α was prepared as reported for human fibroblast enzyme (10), except that the enzyme was further purified by chromatography on heparin-agarose. Antibody was diluted and heated before use. In experiment IV, the flow-through fraction from the DNA-agarose column of Fig. 1*A* was used and, in experiment V, the activity eluting at 0.5 M NaCl was used.

of 4.8–5.5 on these gradients. The amount of DNA polymerase I* obtained as sucrose gradient fraction was ≈0.1% of the total DNA polymerase in the fraction I cell-free lysate. It is currently not known what fraction of that lysate activity was polymerase I*.

DNA Polymerase I* Is Related to DNA Polymerase I. DNA polymerase I* activity has roughly the same sensitivity to low levels of antibody to DNA polymerase I as does legitimate DNA polymerase I and could be essentially totally inhibited by high levels of antibody that had no effect on HeLa polymerase α (Table 1). Relatively slight differences in degree of inhibition relative to polymerase I were noted from preparation to preparation, probably because of variations in the amount of inactive crossreacting enzyme. Polymerase I* shows the same insensitivity to *N*-ethylmaleimide inhibition as does DNA polymerase I (Table 1). [DNA polymerases II and III would have been very sensitive to this level of the sulfhydryl blocking agent (12) and α-polymerase was completely inhibited by it.] Finally, DNA polymerase I* activity has not been detected in the *polA1* mutant strain, P3478, indicating that this enzyme is associated with the *polA* gene.

DNA Polymerase I* Is Relatively Unfaithful. An abnormally high frequency of misincorporation of nonhomologous nucleotide by DNA polymerase I* has been observed with a variety of polydeoxyribonucleotide templates and dNTPs (Table 2). The highest misincorporation frequencies were in reactions involving the homopolymer pair poly(dA)·poly(dT), and the error frequency for misincorporation of dGMP was approximately the same whether the complementary triphosphate in the reaction mixture was dTTP or dATP. Evidently, the misincorporation of dGMP by DNA polymerase I* is equally frequent when copying either the poly(dA) strand or the poly(dT) strand. When dTTP was present, most—but not all—of the dGMP was incorporated into the poly(dT) strand (see Fig. 3). Similarly, with dATP present, most—but not all—of the dGMP was incorporated into the poly(dA) strand (data not shown, but see below).

The incorporation of dGMP into poly(dI)·poly(dC) permits the misincorporation of either dTMP or dAMP at approximately

Table 2. Misincorporation frequencies of DNA polymerases with synthetic polydeoxyribonucleotide polymers

Template	Enzyme	Nucleotide incorporated: Complementary dNMP	Complementary pmol	Noncomplementary dNMP	Noncomplementary fmol	Misincorporation frequency
poly(dA)·poly(dT)	DNA polymerase I*	dAMP	19.8	dGMP	39.0	1/510
		dTMP	27.3	dGMP	33.0	1/830
	DNA polymerase I	dAMP	33.3	dGMP	<4	<1/8,300
		dAMP	61.8	dGMP	<5	<1/12,000
	DNA polymerase I†	dAMP	59.5	dGMP	<7	<1/8,500
		dTMP	34.7	dGMP	<5	<1/6,900
		dTMP	99.4	dGMP	<5	<1/20,000
poly(dA·dT)	DNA polymerase I*	dAMP/dTMP	67.0	dGMP	20	1/3,400
	DNA polymerase I	dAMP/dTMP	113	dGMP	6	<1/19,000
poly(dI)·poly(dC)	DNA polymerase I*	dGMP	187	dTMP	39.0	1/4,800
		dGMP	33.7	dAMP	10.0	1/3,400
		dGMP	187	dAMP	57.0	1/3,300
	DNA polymerase I	dGMP	57.8	dTMP	<9	<1/6,400
		dGMP	466	dTMP	<11	<1/42,000
		dGMP	3390	dTMP	<11	<1/308,000
		dGMP	57.8	dAMP	<9	<1/5,800
		dGMP	3390	dAMP	280	1/12,000

Each reaction contained either 25 μl of sucrose gradient fraction DNA polymerase I* or 4–130 ng of apparently homogeneous *E. coli* DNA polymerase I (except where noted). After incubation for 4 hr at 37°C [except 2 hr for poly(dI)·poly(dC) reactions], polymers were precipitated and collected on glass fiber filters as described (9).
† DNA polymerase activity eluting from DNA-agarose at 500 mM and purified by sucrose gradient sedimentation.

equal frequencies, suggesting that DNA polymerase I* will misincorporate either dTMP or dAMP while copying poly(dC) (Table 2). Finally, a relatively high misincorporation frequency for polymerase I* was also observed with the alternating copolymer poly(dA·dT).

Depending on the primer/template and nonhomologous triphosphate present, the misincorporation frequency of DNA polymerase I* was 4-fold to at least 64-fold that of legitimate DNA polymerase I. This higher frequency was not due to contamination of either the misincorporated nucleotide or the template primer; if this were the case, DNA polymerase I would have given the same high misincorporation. As an additional control, normal DNA polymerase I was isolated by the procedure used for polymerase I* (except that the cells were not treated with nalidixic acid) and tested with poly(dA)·poly(dT) (Table 2). This enzyme did not have the error-prone characteristic of polymerase I*.

The measurements of incorporation of noncomplementary nucleotides into synthetic primer/template were by published procedures (9, 13, 14). Blank values for misincorporation were 0.001–0.01% of total radioactivity present and were reproducible on a given day to within 20%, although they tended to increase with the age of the dNTP preparation. Levels of misincorporation <50% above the blank were considered to be insignificant. Blank values with polymer omitted were never greater than with enzyme omitted, ruling out incorporation of label into template endogenous to DNA polymerase preparations as a cause of high misincorporation. In addition, reactions containing single-stranded synthetic polydeoxyribonucleotide in place of duplex primer/templates gave no detectable misincorporation above the blank.

The sucrose gradient fraction that was used to determine fidelity of polymerization contains a significant amount of recA protein, and we have noted that this protein can hydrolyze dATP as well as ATP to the diphosphate in the presence of DNA. Because decreasing the dATP/dGTP ratio in a misincorporation assay can increase the apparent error frequency (15), it was important to verify that the concentration of dATP was not significantly altered by hydrolysis during the assay. Fig. 2 shows an example of a time course of misincorporation of dGMP into poly(dA)·poly(dT) with dATP as the complementary triphosphate in which the concentration of dATP was monitored by thin-layer chromatography. Significant dATP breakdown was not observed, thus demonstrating that the high error frequency (1/1000) throughout this time course was not due to changes in relative dNTP concentrations.

The products of misincorporation reactions were also analyzed in alkaline CsCl density gradients to ensure that the observed misincorporation represented authentic DNA synthesis (Fig. 3). For DNA polymerase I, the amount of dGMP present in the poly(dT) peak was very small, corresponding to an error frequency of 1/913,000. In contrast, for DNA polymerase I*, the level of dGMP in the poly(dT) peak corresponded to an error frequency of 1/6840, ≈130 times higher. Because poly(dA) is not extended by incorporation of dTMP in this experiment, it is not meaningful to calculate a separate error frequency for the dGMP banding at the density of poly(dA). This dGMP could represent addition of a single dGMP to poly(dA) termini. In a separate experiment, alkaline CsCl gradient analysis of a misincorporation reaction containing dGTP, dATP, and poly(dA)·poly(dT) showed that 80% of the misincorporated dGMP was associated with poly(dA) (data not shown).

Polymerase I* Appears to Have Normal 3′->5′ Exonuclease Activity. The increased error frequency of DNA polymerase I* could, in principle, be due to inhibition or inactivation of the 3′->5′ "proofreading" exonuclease (*E. coli* exonuclease II) or to a reduction in the specificity of triphosphate selection by the enzyme. Both situations have been observed for mutant forms of phage T4 DNA polymerase (16, 17). However, no significant difference in the exonuclease II activity/polymerase activity ratio for polymerase I* versus polymerase I could be detected. The exonuclease II activity/DNA polymerase activity ratio, both using poly(dA·dT), was 0.3 to 0.4 for the two polymerase preparations. That exonuclease II activity

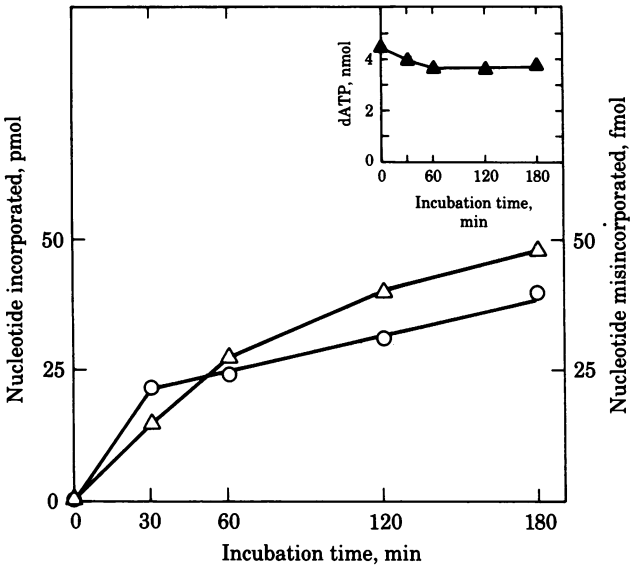

FIG. 2. Time course with poly(dA)·poly(dT) primer/template. △, Incorporation of dAMP; ○, misincorporation of dGMP. (*Inset*) dATP (▲) present in each incorporation reaction as determined by thin-layer chromatography on polyethyleneimine plates in 1.2 M LiCl.

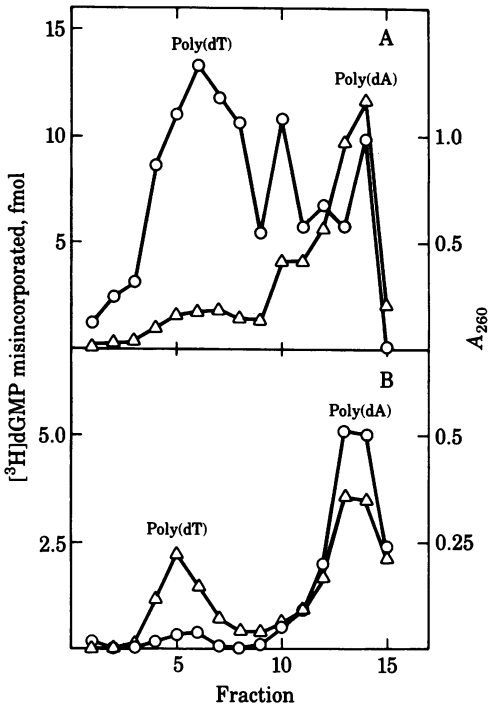

FIG. 3. Isopycnic centrifugation in alkaline CsCl of deoxyguanylate misincorporated during the replication of poly(dA)·poly(dT) by DNA polymerase I* (*A*) and DNA polymerase I (*B*). Reactions with dTTP present were for 30 min as described, except that the amount of template was increased 5-fold; the reaction volume was 3.0 ml for DNA polymerase I* and 0.1 ml for the polymerase I reaction. The poly(dA)·poly(dT) had been purified by centrifugation on an alkaline CsCl density gradient and then dialyzed extensively against 5 mM Tris·HCl, pH 7.5, whereas [^{3}H]dGTP had been purified by ion-exchange chromatography (9). Reactions were stopped by adding EDTA (final concentration, 20 mM); additional poly(dA)·poly(dT) was added to the smaller DNA polymerase I reaction, and both samples were dialyzed extensively against 1 M NaCl/10 mM Tris·HCl, pH 8.2/1 mM EDTA and then against 1 mM Tris·HCl, pH 8.2. To each sample was added 3 μmol of EDTA and 160 μmol of NaOH per 0.10 ml of reaction mixture, and the DNA polymerase I* mixture was evaporated to 2 ml with dry nitrogen. The solution weight of each sample was adjusted to 2.20 g with distilled water and then 2.73 g of solid CsCl was added. The solutions (final vol, 2.80 ml) were transferred to polyallomer tubes, topped off with mineral oil, and centrifuged for 43 hr at 38,000 rpm in a Spinco SW50.1 rotor. Drops were collected from the tube bottom, and individual fractions were diluted to 0.44 ml to determine A_{260} (△) and then neutralized with HCl, precipitated, and filtered for the misincorporation assay (○). Poly(dT) peaks contained 1063 cpm (88.6 fmol of dGMP) and 16 cpm (1.3 fmol of dGMP) for DNA polymerase I* and DNA polymerase I, respectively. Simultaneous 0.10-ml reaction mixtures in which dTTP was labeled showed the equivalent of 606 pmol of polymer synthesized in the DNA polymerase I* assay (adjusted for the larger volume) and 1196 pmol of synthesis by DNA polymerase I. Error frequencies would therefore be 1/6840 and 1/913,000 for the respective assays taking into account the 72% recovery of the synthesized polymer.

was being specifically measured was determined by checking-sensitivity of the nuclease activities to inhibition by DNA polymerase I antiserum.

As a separate measure of exonuclease II activity, triphosphate turnover during synthesis was quantitated. With activated DNA primer/template under our polymerase assay conditions, the ratio of free dGMP formed to free dGMP formed/dGMP incorporated (16) was in the same range for the polymerase I* as for the polymerase I—0.4 to 0.5. Hence, it appears that polymerase I* is not abnormally error prone by virtue of a change in 3′->5′ exonuclease. This finding is consistent with the observations of Loeb *et al.*, who found a minimal contribution of the exonuclease to fidelity when Mg^{2+} was present (18).

DISCUSSION

An error-prone DNA polymerase activity has been hypothesized to be responsible for the fixation of mutations in *E. coli* that are induced by UV light or some chemicals and are dependent on the expression of the *recA* and *lexA* genes (3, 4). This hypothesis is consistent with a wide range of experimental data (5), the most direct *in vivo* evidence (19–21) indicating that DNA polymerase III genes and a factor whose appearance is inhibited by chloramphenicol are essential. Indeed, polymerase I is considered not to play a major role in error-prone repair, which is inducible by UV light and inhibited by chloramphenicol, because such mutagenesis appears to be roughly normal in strains carrying *polA* mutations (22, 23). These conclusions may be somewhat qualified, however, by the necessity to use secondary mutations to overcome survival effects (23), by unknown factors that necessitate the use of minimal growth media (23, 24), and by the dependence of the effects observed on the particular DNA damaging agent used (25).

What, then, might be the role of polymerase I*? Perhaps it is induced and normally can act to form these mutations but is replaceable by analogous forms of polymerase II or III (or both). Just as normal polymerase I synthesizing activity is dispensible under many growth conditions, so might polymerase I* be efficiently replaceable by modified forms of other polymerases. Conversely, polymerase I* might act in a chloramphenicol-resistant "constitutive" pathway (25). Although not requiring protein synthesis, such a pathway might still require a DNA damage-induced activation of preexisting proteins in the cell by *lexA*/*recA* functions. Finally, polymerase I* might be involved in another repair function induced by DNA damage or replication cessation. The determination of the number of such functions and their characterization is still preliminary (26), although inducible long-patch repair is one possibility (27).

A good correlation exists between *in vivo* mutation frequency and the *in vitro* error frequency of purified mutator DNA polymerase as measured by incorporation of nonhomologous nu-

cleotides into synthetic DNA homopolymers (15). With this assay, DNA polymerase I* has a lower fidelity than a partially purified preparation of polymerase I obtained analogously or electrophoretically homogeneous DNA polymerase I. The biochemical basis of this decreased fidelity is unknown and the involvement of recA protein cannot be ruled out because preparations of purified polymerase I* contain significant amounts of recA protein. However, we have not been able to generate polymerase I* activity from purified polymerase I and recA protein. The determination of what role, if any, recA protein might have in generating polymerase I* activity will probably be determined only after relatively large amounts of purified polymerase I* activity are obtained.

In conclusion, the relatively unfaithful DNA polymerase I* obtained from induced cells could be involved in an inducible *recA/lexA*-dependent DNA repair pathway, but it should be noted that the *in vitro* fidelity experiments were not carried out under conditions of repair synthesis. In this regard, the capacity of DNA polymerase I* to copy abnormal bases could be tested by using DNA sequence analysis techniques that measure the exact extent and nature of replication of template DNA containing damaged bases (28, 29). In addition, the genetic regulation of the appearance of polymerase I* should be studied to determine what role, if any, the activity might play in DNA repair.

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1. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
2. Radman, M. (1975) in *Molecular Mechanisms for Repair of DNA*, eds. Hanawalt, P. C. & Setlow, R. B. (Plenum, New York), pp. 355–367.
3. Caillet-Fauquet, P., Defais, M. & Radman, M. (1977) *J. Mol. Biol.* **117**, 95–112.
4. Witkin, E. (1967) *Brookhaven Symp. Biol.* **20**, 17–55.
5. Mount, D. W. & Hall, J. D. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **25**, 53–126.
6. Mount, D. W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 300–304.
7. Bachman, B. (1972) *Bacteriol. Rev.* **36**, 525–557.
8. Schaller, H., Nusslein, C., Bonhoeffer, F. J., Kurz, C. & Nietzman, I. (1972) *Eur. J. Biochem.* **26**, 474.
9. Linn, S., Kairis, M. & Holliday, R. (1976) *Proc. Natl. Acad. Sci. USA* **255**, 2818–2822.
10. Krauss, S. W. & Linn, S. (1980) *Biochemistry* **19**, 220–228.
11. Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 965–969.
12. Kornberg, A. (1980) in *DNA Replication*, (Freeman, San Francisco), pp. 169–170.
13. Murray, V. & Holliday, R. (1981) *J. Mol. Biol.* **146**, 55–76.
14. Hall, Z. W. & Lehman, I. R. (1968) *J. Mol. Biol.* **36**, 321–333.
15. Agarwal, S. S., Dube, D. K. & Loeb, L. A. (1979) *J. Biol. Chem.* **254**, 101–106.
16. Muzyczka, N., Poland, R. L. & Bessman, J. J. (1972) *J. Biol. Chem.* **247**, 7116–7122.
17. Hershfield, M. S. (1973) *J. Biol. Chem.* **248**, 1417–1423.
18. Loeb, L. A., Dube, D. K., Beckman, R. A., Koplitz, M. & Gopinathan, K. P. (1981) *J. Biol. Chem.* **256**, 3978–3987.
19. Bridges, B. A. & Mottershead, R. P. (1978) *Mol. Gen. Genet.* **162**, 35–41.
20. Bridges, B. A. & Mottershead, R. P. (1976) *Mol. Gen. Genet.* **148**, 125–130.
21. Sedgwick, S. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2753–2757.
22. Kondo, S., Ichikawa, H., Iwo, K. & Kato, T. (1970) *Genetics* **66**, 187–217.
23. Witkin, E. M. & George, D. L. (1973) *Genetics Suppl.* **73**, 91–108.
24. Barfknecht, T. R. & Smith, K. C. (1978) *Mol. Gen. Genet.* **167**, 37–41.
25. Bridges, B. A. (1978) in *DNA Repair Mechanisms*, eds. Hanawalt, P. C., Friedberg, E. C. & Fox, C. F. (Academic, New York), pp. 345–348.
26. Kenyon, C. J. & Walker, G. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2819–2823.
27. Hanawalt, P. C., Cooper, P. K. & Smith, C. A. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **26**, 181–196.
28. Moore, P. & Strauss, B. S. (1979) *Nature (London)* **278**, 664.
29. Moore, P. D., Bose, K. K., Rabkin, S. D. & Strauss, B. S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 110–114.