JOURNAL OF BACTERIOLOGY, Oct. 1994, p. 6221–6228
0021-9193/94/$04.00+0

Vol. 10, No. 20

Involvement of *Escherichia coli* DNA Polymerase II in Response to Oxidative Damage and Adaptive Mutation

MONICA ESCARCELLER,[1] JAMES HICKS,[2] GUDMUNDUR GUDMUNDSSON,[1] GARY TRUMP,[2] DANIELE TOUATI,[3] SUSAN LOVETT,[4] PATRICIA L. FOSTER,[5] KEVIN McENTEE,[6] AND MYRON F. GOODMAN[1]*

Department of Biological Sciences[1] and Craniofacial Biology Program,[2] University of Southern California, Los Angeles, California 90089-1340; Institut Jacques Monod, CNRS-University of Paris VII, 75251 Paris Cedex 05, France[3]; Rosenstiel Basic Medical Sciences Center, Brandeis University, Waltham, Massachusetts 02254[4]; Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts 02118[5]; and Department of Biological Chemistry and the Molecular Biology Institute, University of California at Los Angeles School of Medicine, Los Angeles, California 90024[6]

Received 27 December 1993/Accepted 7 August 1994

DNA polymerase II (Pol II) is regulated as part of the SOS response to DNA damage in *Escherichia coli*. We examined the participation of Pol II in the response to oxidative damage, adaptive mutation, and recombination. Cells lacking Pol II activity (*polBΔ1* mutants) exhibited 5- to 10-fold-greater sensitivity to mode 1 killing by H_2O_2 compared with isogenic *polB*$^+$ cells. Survival decreased by about 15-fold when *polB* mutants containing defective superoxide dismutase genes, *sodA* and *sodB*, were compared with *polB*$^+$ *sodA sodB* mutants. Resistance to peroxide killing was restored following P1 transduction of *polB* cells to *polB*$^+$ or by conjugation of *polB* cells with an F′ plasmid carrying a copy of *polB*$^+$. The rate at which Lac$^+$ mutations arose in Lac$^-$ cells subjected to selection for lactose utilization, a phenomenon known as adaptive mutation, was increased threefold in *polB* backgrounds and returned to wild-type rates when *polB* cells were transduced to *polB*$^+$. Following multiple passages of *polB* cells or prolonged starvation, a progressive loss of sensitivity to killing by peroxide was observed, suggesting that second-site suppressor mutations may be occurring with relatively high frequencies. The presence of suppressor mutations may account for the apparent lack of a mutant phenotype in earlier studies. A well-established *polB* strain, a *dinA* Mu d(Ap^r *lac*) fusion (GW1010), exhibited wild-type (Pol II$^+$) sensitivity to killing by peroxide, consistent with the accumulation of second-site suppressor mutations. A high-titer anti-Pol II polyclonal antibody was used to screen for the presence of Pol II in other bacteria and in the yeast *Saccharomyces cerevisiae*. Cross-reacting material was found in all gram-negative strains tested but was not detected in gram-positive strains or in *S. cerevisiae*. Induction of Pol II by nalidixic acid was observed in *E. coli* K-12, B, and C, in *Shigella flexneri*, and in *Salmonella typhimurium*.

Escherichia coli contains three DNA polymerases, polymerase I (Pol I), Pol II, and Pol III (33). Clear biological roles for Pol III and Pol I have been well established on the basis of studies using *dnaE* (17) and *dnaQ* (14, 51, 52) mutant genes, corresponding to the polymerase and proofreading exonuclease components of the Pol III holoenzyme complex (43), and *polA* mutants, corresponding to the structural gene for DNA polymerase I (13, 33). Pol III holoenzyme consists of 10 subunits and has an essential role in DNA replication and in modulating the occurrence of spontaneous mutations (43, 49, 50). Pol I is involved primarily in DNA repair following exposure of cells to damaging agents such as UV light (33). A role for Pol II in either replication or repair has not been unambiguously established, even though this enzyme was identified more than 23 years ago (31, 34, 35, 45).

Recently, the gene encoding DNA polymerase II has been cloned (3, 11, 27), and its structural gene was identified as the SOS damage-inducible *dinA* gene (3, 27). Pol II is part of the SOS regulon, having a LexA repressor operator site present in the promoter of the gene (3, 27). Earlier, we had shown that Pol II could bypass a single site-directed abasic lesion in vitro, and levels of the enzyme in vivo exhibited a sevenfold increase in specific activity following induction of SOS by nalidixic acid (4). There is recent in vivo evidence suggesting that Pol II may be required for SOS-induced mutagenic bypass of abasic lesions when the heat shock proteins GroES and GroEL are present at uninduced levels (56). In this study, we investigated the involvement of Pol II in oxidative damage survival and adaptive mutation (6), using a well-characterized Pol II null mutant.

* Corresponding author. Mailing address: University of Southern California, Department of Biological Sciences, University Park, Los Angeles, CA 90089-1340. Phone: (213) 740-5190. Fax: (213) 740-8631.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Media, buffers, and reagents. K medium contains 1% glucose, 1% Casamino Acids (Difco Laboratories, Detroit, Mich.), 1 μg of thiamine hydrochloride per ml, 1 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $CaCl_2$, and M9 salts (44). M9, MacConkey, and LB liquid media and/or plates were prepared as described previously (44). M9-glycerol and M9-lactose minimal media were prepared as described previously (6). Media were supplemented with leucine (0.3 mM), threonine (0.3 mM), and thiamine (1 μg/ml) if required. Arabinose was added at a concentration of 1% when indicated; glucose and lactose were added at described concentrations for the medium used. Transductional recombination assays for arginine prototrophy were done with 56/2 minimal medium (62) supplemented with the required amino acids at 50 μg/ml. Antibiotics were used at

TABLE 1. *E. coli* strains used

Strain	Relevant genotype or description	Reference or source
AB1157	F⁻ λ⁻ *thi-1 hisG4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 rfbD1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31 rac*	1
BW6160	*zdh-57*::Tn*10* derivative of Hfr Broda 8	60
BW6166	*zhf-721*::Tn*10* derivative of Hfr J4	60
C600	F⁻ λ⁻ *tonA21 thr-1 leuB6 lacY1 supE44 rfbD1 thi-1*	D. J. Galas
C600.1	C600/F'101(F' episome: from *leu* to *uxuBA*)	D. J. Galas
CAG18477	*zij-501*::Tn*10 metF159*	54
FC40	*ara* Δ(*lac-pro*)$_{xiii}$ Rif^r *thi*/F' *lacI33*::*lacZ pro*$^+$	6
GW1000	*lac*Δ(U169) *recA441*(*tif-1*) *sfiA11 thr-1 leuB6 his-4 argE3 lacY1 galK2 str-31*	30
GW1010	GW1000 *dinA1*::Mu d(Ap^r *lac*)	30
HMS83	*polA1 polB1 leu lys thyA lacZ rha*	8
JC7623	AB1157 *recB21 recC22 sbcB15 sbcC201*	22
MC1061	*hsdR araD139* Δ(*araABC-leu*)*7679 galU galK* Δ*lacX74 rpsL thi*	K. McEntee
ME101	C600 *polB*Δ*1*::Ω Sm-Sp	This study
ME101.1	ME101/F'101(*polB*$^+$)	This study
ME101.2	ME101/F'Lac(*lac*$^+$)	This study
ME101.3	ME101/F$^+$	This study
ME1AB	C600 *sodA sodB*	This study
ME2AB	ME1AB *polB*Δ*1*::Ω Sm-Sp	This study
ME102	*polB*$^+$ *ara*$^+$ transductant of ME101	This study
PFB60	FC40 *polB*Δ*1*::Ω Sm-Sp	This study
QC773	F⁻ Δ(*lac-arg*) *rpsL sup*$^+$ in(*rrnD-rrnE*) *sodB*Δ*2* Km^r Sm^r	D. Touati
QC781	F⁻ Δ(*lac-arg*) *rpsL sup*$^+$ in(*rrnD-rrnE*) *sodA25* Cm^r Sm^r	D. Touati
RD5042	X7026 λ⁻ *thi-1 relA1* Δ(*pro-lac*) *supE44 recA56*/F'Lac(*lac*$^+$)	R. Deonier
S90C	*ara* Δ(*lac-pro*) *thi* Sm^r	J. H. Miller
SC301	C600 *polB*Δ*1*::Ω Sm-Sp	12
SC301.1	SC301/F'101(*polB*$^+$)	12
SH2101	S90C *polB*Δ*1*::Ω Sm-Sp	C. Bonner
STL678	F$^+$::mini-Tn*10*Tc^r *sbcB15 endA* Δ(*pnc-xth*) *gal thi thyA*	S. Lovett
STL1334	AB1157 *polB*Δ*1*:Cm^r Sp^r *recB21 recC22 sbcB15 sbcC201*	This study
STL1336	AB1157 *polB*Δ*1*:Cm^r Sp^r *rec*$^+$	This study

the following concentrations: chloramphenicol, 20 μg/ml; kanamycin, 30 μg/ml; spectinomycin, 50 or 70 μg/ml; streptomycin, 10 μg/ml; and tetracycline, 15 μg/ml. Hydrogen peroxide was purchased as a 30% aqueous solution from Mallinckrodt, Inc. (St. Louis, Mo.). Catalase was from Sigma Chemical Co. (St. Louis, Mo.) and added at a final concentration of 2 μg/ml.

Genetic procedures and treatments. (i) Construction of the *polBΔ1* null mutant strain SH2101. All DNA manipulation, plasmid transformations, and related techniques were performed as described elsewhere (48). A 4.8-kb *Pst*I-*Pvu*II fragment from the λ phage 7H9 (32), shown to contain a portion of the *araD* gene and the entire *polB* gene (3), was cloned into pT7T3 19U (Pharmacia LKB) to generate pSH100 (21). The pSH100 (Amp^r) plasmid was restricted with *Bgl*II to generate a deletion that extended from the last 680 bp of the *araD* gene and 1,100 bp into *polB*. Plasmid pHP45Ω (46) was restricted with *Bam*HI to isolate the Ω element (Sm^r Sp^r), followed by subsequent ligation into the *Bgl*II-cut pSH100 vector. The resulting plasmid was linearized and transformed into *E. coli* JC7623 (*recB21 recC22 sbcB15*) (22), using calcium chloride. Cells were plated on streptomycin-spectinomycin medium, and colonies were checked for ampicillin sensitivity. Southern hybridization was used to confirm the presence of the construct in the genome. The mutation was P1 transduced into *E. coli* S90C, and colonies selected on spectinomycin medium. Strain SH2101 obtained in this way is a *polBΔ1* null mutation with roughly 40% of the N-terminal coding region deleted, including the LexA box and *polB* promoter.

(ii) P1 transduction. *polBΔ1*, *sodB*, and *sodA* derivatives of strain C600, using P1 grown on strains SH2101, QC773, and QC781, respectively, were constructed by transduction as described previously (55), selecting for resistance to the correspondent drug marker in K medium (LB and M9 were also used). When restoring the *polB*$^+$ genotype, we used selection in M9 medium supplemented with 1% arabinose. Superoxide dismutase-negative transductants were screened with superoxide dismutase activity gels (2). *polB*$^+$ and *polBΔ1* transductants were screened by Western blotting (immunoblotting) with a highly specific polyclonal antibody against DNA Pol II.

A *polBΔ1* derivative of FC40 (6) was constructed by transducing the parent strain, FC36 (15), with a P1 lysate grown on SH2101, selecting for resistance to spectinomycin on LB plates. Most such transductants could not grow in minimal medium, even when supplemented with proline and leucine. Several spontaneous mutants able to grow on minimal medium supplemented with proline were isolated and used for further experimentation. The F' episome carrying the *lacI33*::*lacZ* allele was mated into these mutants as described previously (15). The phenotypes of the majority of these strains were identical, and results from only one, PFB60, are given in Fig. 5. *polB*$^+$ derivatives of PFB60 were constructed by transducing its parent, PFB59, to *ara*$^+$ with a P1 lysate grown on a strain wild type for that region (BW367; obtained from B. Weiss). Four independent transductants were isolated, the F' episome was mated in, and the transductants were used for the experiment shown in Fig. 5. The presence and absence of *polB* were confirmed by Western blotting.

(iii) Conjugation. Conjugation by liquid mating was performed as described previously (44). The pedigrees of the strains are given in Table 1. The presence of F plasmids was checked by M13 infection and by corresponding markers; F$^+$ (from STL678) was checked by tetracycline resistance, F'101

(from C600.1) was checked by omitting leucine and threonine in M9 medium supplemented with 1% arabinose, and F'Lac (from RD5042) inheritance was scored by plating on lactose-MacConkey plates.

(iv) H_2O_2 sensitivity. Liquid cultures were grown in K medium at 37°C. Cells were challenged with H_2O_2 at a density of 1×10^7 to 4×10^7 CFU/ml in 1 ml of K medium for 15 min at 37°C with shaking at 150 rpm. The challenge was terminated either by dilution into M9 salts or addition of catalase. For survival studies, cells were plated onto K plates (LB and M9 were also tried), and colonies were counted after 24 to 48 h. For testing of starved cells, 5 ml of K medium culture was incubated at 37°C for 7 (or more) consecutive days. Before testing of H_2O_2 sensitivity, a fresh 5-ml K medium culture was inoculated and grown overnight. The process continued as described above. When multiple subcultured cells were tested, cells were subcultured in 5 ml of K medium 21 times in 21 days (fresh medium every time). Before testing for H_2O_2 sensitivity, a fresh culture of 5 ml in K medium was inoculated and grown overnight. The process continued as described above.

(v) Mutagenesis. Spontaneous mutation rates to Lac^+ with FC40 derivatives were determined as described previously (6). For the experiment shown in Fig. 5, 20 independent 1-ml cultures of FC40, PFB60, and four *polB*$^+$ derivatives of PFB60 were grown in M9–0.01% glycerol medium, in which the cells saturated at a density of 2×10^8 cells per ml. Each culture was plated with a 10-fold excess of scavenger cells (FC29) in 2.5 ml of top agar onto a M9-lactose plate, and Lac^+ colonies were counted every day. The results from seven cultures, which contained jackpots, were eliminated. Results with the four *polB*$^+$ transductants were identical and have been combined in Fig. 5.

Measurement of recombination proficiency. Quantitation of recombination efficiency by conjugation or P1 transduction was performed as described previously (37) with freshly transduced *polBΔ1* derivatives.

Immunization of mice. Eight-week-old female Swiss Webster mice (Simonson Laboratories, Gilroy, Calif.) were initially immunized intraperitoneally with 50 μg of DNA Pol II (0.25 ml) emulsified in an equal volume of complete Freund's adjuvant (Gibco). They were boosted at monthly intervals with the immunogen (25 μg) in incomplete Freund's adjuvant.

ELISAs. Anti-DNA Pol II binding activity was measured by using serial twofold serum dilutions in an antibody-trapping enzyme-linked immunosorbent assay (ELISA) (19, 58). Reagents were added in 50-μl volumes. Wells were coated with enzyme (5 μg/ml) in 50 mM carbonate buffer (pH 9.6) and then blocked with 3% bovine serum albumin in carbonate buffer. Antisera were diluted in phosphate-buffered saline–3% bovine serum albumin (pH 7.4). The secondary antibody (13.8 μg/ml) was goat anti-mouse immunoglobulin G (IgG) plus IgM (M30700; Caltag Laboratories, South San Francisco, Calif.). The detecting antibody (1:3,000) was an alkaline phosphatase-conjugated swine anti-goat IgG (G5008; Caltag Laboratories). The chromagen was *p*-nitrophenyl phosphate (1 mg/ml; Sigma). Mouse antipolymerase could be detected at a 1:10,000 dilution within 7 days following the second boost.

ECL Western blotting. Various *E. coli* strains were grown in 500 ml of LB broth with vigorous shaking to an optical density (A_{600}) of 0.6 to 0.8. Nalidixate (40 μg/ml) was added at an optical density of 0.6, and growth continued for an additional 90 min. Cells were harvested by centrifugation and lysed as described previously (4). Cell lysates were clarified by centrifugation, and the supernatants were concentrated by Centriprep ultrafiltration (41) before polyacrylamide gel electrophoresis (PAGE) (36). For PAGE, the amount of protein extract loaded per gel lane varied from 400 to 800 μg; 0.5 μg of purified Pol II was run as both size marker and control. Samples were separated by electrophoresis at constant current at room temperature. Following electrophoresis, the gel was blotted (29) overnight at 40 V (Bio-Rad submersible blotting apparatus) onto a Hybond-ECL nitrocellulose membrane (Amersham International, Amersham, England). The membrane was blocked for 1 h with 10 ml of TBS-Tween (20 mM Tris base, 137 mM NaCl, 0.1% Tween 20) containing 5% powdered milk in a rolling cylinder (57), rinsed in TBS-Tween (without milk), incubated with mouse anti-Pol II sera (1:10,000) in TBS–Tween–5% milk for 1 h, rinsed again with TBS-Tween, and then incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse IgG (1:5,000; Caltag Laboratories) in TBS–Tween–5% milk. Following a final rinse in TBS-Tween, the membrane was rinsed with a mixture (10 ml of each) of ECL Western blotting detection reagents 1 and 2 (Amersham). The paper was briefly drained onto paper towels, wrapped in cellophane, and exposed to Hyperfilm-ECL (Amersham). Exposure times varied from 30 s to 1 h, and several exposures could be made from one paper.

FIG. 1. DNA Pol II is not present in *polB* null mutant strains of *E. coli*. Cell extracts were prepared as described in Materials and Methods. Lane 1, 0.2 μg of purified Pol II; lane 2, *E. coli* C600; lane 3, *E. coli* HMS83; lane 4, *E. coli* MC1061; lane 5, *E. coli* GW1010; lane 6, *E. coli* GW1010 containing 0.2 μg of purified Pol II; lane 7, *E. coli* SC301 (*polBΔ1*); lane 8, *E. coli* SC301 (*polBΔ1*) containing 0.2 μg of purified Pol II; lane 9, *E. coli* SC301.1. Approximately 400 μg of total protein was used from each cell extract. *E. coli* strains are listed in Table 1.

RESULTS

Absence of intact Pol II in *polB* null mutants. *polB* null mutants were used to examine possible biological roles for DNA Pol II in vivo. A null mutant of Pol II, strain SH2101 (see Materials and Methods), was constructed by replacement of the N-terminal half of the *polB* gene with a DNA fragment, Ω element, containing two selectable drug resistance markers, Sm^r and Sp^r, that are flanked by strong transcriptional and translational termination sequences (46). To characterize these null mutants constructed by gene disruption, polyclonal anti-Pol II antibodies were prepared in a mouse and used for Western blot analysis. This antibody preparation appears to be highly specific and reacts with a single band in Western blots (Fig. 1, lane 2); occasionally, trace bands were observed at other positions on the gel, and these may represent proteolysis products (data not shown). The absence of full-length Pol II was verified in *polBΔ1* disruption mutants by Western blot analysis (Fig. 1, lane 7). However, DNA Pol II was readily detected in *polBΔ1* cells after introduction of the F′ factor F′101 (38), which carries the gene for Pol II (Fig. 1, lane 9).

Antibody prepared against purified Pol II was used to examine crude extracts prepared from three other *polB* mutants. Normal levels of Pol II were found for strain HMS83 (Fig. 1, lane 3), an *E. coli* mutant shown previously to be devoid of DNA Pol II enzyme activity, using either a standard polymerase incorporation assay (8) or an in situ polyacrylamide gel assay (4). Pol II cross-reacting material was absent in strains MC1061 (Fig. 1, lane 4) and GW1010 (Fig. 1, lane 5). Strain MC1061 contains a deletion extending beyond the *araD*

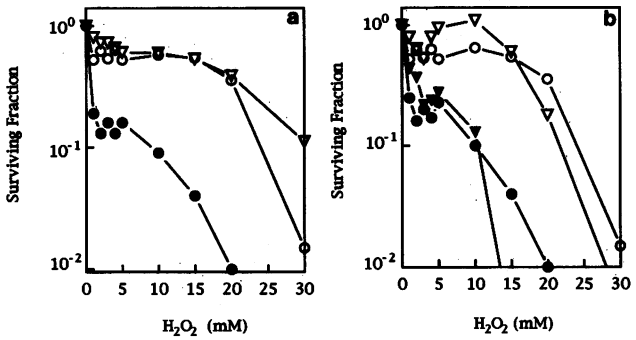

FIG. 2. Comparison of the survival of *polBΔ1* and *polB*$^+$ cells following exposure to hydrogen peroxide. (a) Symbols: ○, strain C600 (*polB*$^+$); ●, strain ME101 (*polBΔ1*); ▽, strain ME102 (*polB*$^+$). Values are means of 11 independent transductants of strain ME101 (*polBΔ1*) to *polB*$^+$. (b) Symbols: ○, C600 (*polB*$^+$); ●, strain ME101 (*polBΔ1*) subcultured seven times; ▽, strain ME101.1 (*polBΔ1*) containing F'101 (*polB*$^+$); ▼, strain ME101.3 (*polBΔ1*) containing an F$^+$ plasmid. Seven subcultures were required to isolate strains carrying F episomes and for the hydrogen peroxide sensitivity tests. Exponentially growing cells were exposed to H_2O_2 for 15 min as described in Materials and Methods.

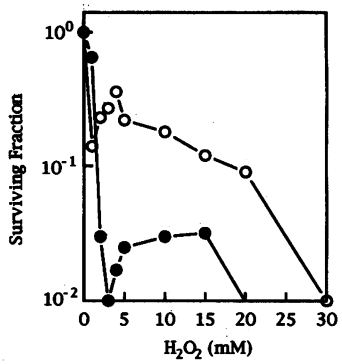

FIG. 3. Comparison of the survival of superoxide dismutase mutants carrying normal and mutant *polB* alleles following exposure to hydrogen peroxide. Symbols: ○, *sodA sodB* (*polB*$^+$) strain ME1AB; ●, *sodA sodB* (*polBΔ1*) strain ME2AB. Exponentially growing cells were exposed to H_2O_2 for 15 min as described in Materials and Methods.

gene (9) that removes the *polB* gene, whose promoter is immediately adjacent at the C terminus of *araD* (3, 27). Strain GW1010 contains a Mu d(Ap^r *lac*) element fused approximately halfway into the Pol II structural gene (3, 30). Purified Pol II, added to extracts of a *polBΔ1* strain and GW1010 (Fig. 1, lanes 6 and 8, respectively), was readily detected by antibody probing, demonstrating that the absence of a detectable Pol II band in extracts of the strains was probably not caused by proteolysis of the enzyme or by other factors that might interfere with anti-Pol II antibody binding.

Sensitivity of Pol II$^-$ cells to killing by hydrogen peroxide. *E. coli* exhibits two modes of killing by hydrogen peroxide: mode 1 killing occurs at low H_2O_2 concentrations (~1 to 5 mM); mode 2 killing occurs at peroxide concentrations above 10 mM (25). Mode 1 killing requires active metabolism, while mode 2 killing does not (25). The absence of Pol II in cells conferred greater sensitivity to peroxide at all concentrations examined (Fig. 2a). At the peak of mode 1 killing (2 mM H_2O_2), *polBΔ1* cells were approximately fivefold more sensitive than *polB*$^+$ cells (Fig. 2a). Reintroduction of *polB*$^+$ into the *polB* null mutant by P1 transduction restored resistance to killing to wild-type levels (Fig. 2a). Eleven independent *polB*$^+$ transductants were analyzed, and all exhibited wild-type levels of survival at concentrations up to 20 mM H_2O_2 (Fig. 2a), a result consistent with *polB* being involved in the repair of oxidative damage.

Reversal of the peroxide-sensitive phenotype of *polBΔ1* cells was also observed when the F'101 plasmid carrying the *polB*$^+$ gene was introduced by conjugation into a *polBΔ1* background, strain ME101.1 (Fig. 2b). F$^+$ plasmid alone had no significant effect on survival of *polBΔ1* cells (strain ME101.3). Partial restoration of peroxide resistance resulted from introduction of F'Lac into the *polBΔ1* mutant, strain ME101.2 (data not shown), but this F'Lac plasmid carries an uncharacterized catalase gene, *katC* (1), which may also act to reduce peroxide damage, thus increasing the fraction of surviving cells in the absence of Pol II.

Increased sensitivity to peroxide killing in *polB* mutants lacking superoxide dismutase. The sensitivities of *polBΔ1* and *polB*$^+$ cells to peroxide were compared in the absence of superoxide dismutase by transducing *sodA sodB polB*$^+$ cells to *polBΔ1*. The strains were constructed first by transduction of *sodA* into C600, then transduction of *sodB*, and finally transduction of *polBΔ1* into the *sodA sodB* double mutant. We also constructed the triple mutant by first transducing *sodB* into C600, then transducing *sodA*, and finally transducing *polBΔ1*. The two constructs gave similar results. Introduction of the *polBΔ1* mutation in a Sod$^-$ background increased mode 1 killing by 15-fold (Fig. 3), indicating that H_2O_2-induced lesions may be repaired by Pol II. This result further supports a role for Pol II in response to oxidative damage (24).

Restoration of the wild-type phenotype following starvation or multiple passaging of *polB* cells. We encountered difficulties while attempting to establish a *polB* phenotype because the sensitivity of Pol II$^-$ cells to various DNA-damaging agents decreased over time. For example, we observed that the null Pol II mutant, strain SC301, which was initially sensitive to killing by peroxide, was later indistinguishable from wild-type strain C600 (data not shown). It therefore seemed possible that the *polB* phenotype was relatively unstable because of the accumulation of extragenic suppressor mutations. Pol II null mutants, ME101 (C600-derived) strains freshly constructed by P1 transduction, were found to have decreased viability in the presence of peroxide (Fig. 2). Freshly prepared ME101 cells were starved for extended periods or passaged for approximately 21 days in K medium, and the cultures were tested for sensitivity to H_2O_2. Using either protocol, starvation or subculturing, we found that the Pol II$^-$ cultures were significantly less sensitive to peroxide killing compared with freshly prepared (i.e., recently transduced) mutants of Pol II (Fig. 4). Further characterization of the putative extragenic suppressors is in progress. We examined strain GW1010 [a *dinA* Mu d(Ap^r *lac*) fusion], which lacked Pol II as judged from Western blot analysis (Fig. 1, lane 5), for its sensitivity to H_2O_2 and found that it did not differ significantly from the wild-type (*polB*$^+$). In the light of the results mentioned above concerning second-site suppressors of the Pol II deletion mutant, it seems plausible that the absence of an oxidative damage-sensitive phenotype for strain GW1010 may also be caused by the presence of one or more suppressor mutations that have accumulated in this strain.

Pol II null mutants behave as mutators in adaptive mutation. FC40 was used to investigate the effect of the loss of *polB* on spontaneous mutation. The *lac* allele carried by FC40 reverts to Lac$^+$ during exponential growth at about 10^{-9} per

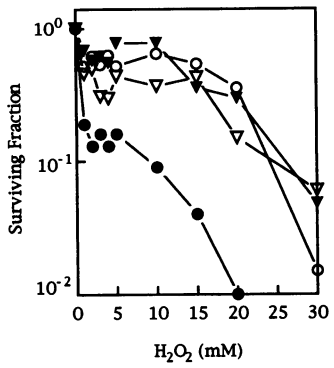

FIG. 4. Effect of starvation and passaging of *E. coli* strains carrying a null mutation of *polB* on survival following exposure to hydrogen peroxide. Symbols: ○, strain C600 (*polB*$^+$); ●, strain ME101 (*polBΔ1*); ▽, strain ME101 (*polBΔ1*) passaged for 21 days; ▼, strain ME101 (*polBΔ1*) starved. Experimental conditions are described in Materials and Methods.

cell per generation; in addition, with continued incubation on minimal lactose plates, Lac$^+$ revertants continue to arise for several days after plating. Because these postplating mutants do not appear if lactose is absent, the phenomenon is referred to as adaptive mutation (6, 15). As shown in Fig. 5, the absence of *polB* had little effect on mutations arising during nonselective growth (which appear as mutants on day 2) but increased the postplating mutation rate of FC40 by about threefold. When the cells were transduced back to *polB*$^+$, the mutation rates returned to wild-type levels (Fig. 5). Adaptive mutations in FC40 are dependent on the major recombination pathway, RecABC, but are independent of the SOS mutagenesis proteins UmuD′ and UmuC (6, 20). Likewise, the mutator effect in static cells of the *polBΔ1* was lost if the cells were *recA* but unaffected if the cells were *umuC* (data not shown).

Effects of *polB* on genetic recombination. The *polBΔ1* allele was introduced into two genetic backgrounds, *rec*$^+$ (strain STL1336) and *recBC sbcBC* (strain STL1334). The latter genetic background was used to reveal potential defects in a secondary recombination pathway, the RecF pathway (40). Efficiency of recombination was measured by inheritance of genetic markers after conjugation with an Hfr strain or after P1 transduction. Strains carrying the *polBΔ1* mutation exhibited no reduction in recombinational efficiency, determined by the number of selected recombinants relative to the *polB*$^+$ control strain, in either the *rec*$^+$ or *recBC sbcBC* genetic background (Table 2). The *polBΔ1* mutation did not significantly alter the coinheritance of a second unselected linked marker in the P1 cross in either genetic background (data not shown). Therefore, by these assays, the *polBΔ1* mutation did not appreciably alter the efficiency or nature of homologous genetic recombination.

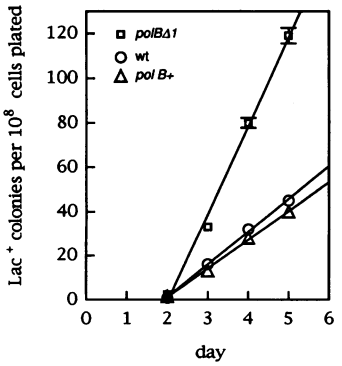

FIG. 5. Reversion of *E. coli* FC40 to Lac$^+$ in the presence or absence of *polB*. Symbols: ○, FC40 (wild type [wt]); □, *polBΔ1* strain; △, transductant carrying the *polB*$^+$ allele. Error bars correspond to 1 standard error of the mean.

A survey of Pol II diversity and induction during SOS. Several bacterial and microbial eukaryotes were assayed for the presence of Pol II, using both Western and Southern hybridization (Table 3). Pol II was found to be present in *E. coli* K-12, C and B, and increased levels of Pol II were observed following treatment with nalidixic acid to induce the SOS response. A Pol II-like protein was present in *Salmonella typhimurium*, and its levels increased following treatment with nalidixic acid (data not shown).

DISCUSSION

DNA Pol II was identified initially in mutants of *E. coli* deficient in Pol I activity (31). Subsequently it was shown that a third DNA polymerase, Pol III, was required for replication of the bacterial genome, while Pol I was involved in DNA repair (for a review, see reference 33). However, the role of Pol II as a possible participant in either replication or repair remained undefined. Recently Pol II was cloned and sequenced (3, 10, 27) and found to contain five conserved sequence motifs characteristic of group B (α-like) polymerases, including mammalian cellular DNA polymerase α, eukaryotic viral polymerases, and bacteriophage T4 polymerase (3). In common with Pol I, Pol II has polymerase and proofreading exonuclease activities present on a single polypeptide chain. However, unlike Pol I, Pol II has no detectable 5′-to-3′ exonuclease activity.

A striking property of Pol II is that its specific activity is increased by sevenfold when cells are exposed to UV light or to nalidixic acid (4). We and others have shown that expression of the *polB* (*dinA*) gene is regulated by the LexA repressor as part of the SOS operon (3, 27). A null mutant of Pol II, designated strain SH2101 (*polBΔ1*) (see Materials and Methods), was constructed with the N-terminal half of the *polB* gene replaced by a DNA fragment, Ω-element (46), containing transcriptional and translational termination signals and drug selectable markers (Sm^r and Sp^r). In this study, we used strains carrying this null mutation to investigate possible roles for Pol II in the repair of oxidative damage and in adaptive mutation. The absence of native Pol II in *polBΔ1* strains was verified by Western blot analysis showing no detectable Pol II in crude soluble cell extracts (Fig. 1, lane 7).

There are two cellular survival modes to oxidative damage caused by exposure to hydrogen peroxide (25). Mode 1 killing occurs in a range from about 1 to 10 mM peroxide, wherein wild-type cells show a loss in viability at low peroxide levels (~1 to 5 mM H_2O_2) followed by a recovery phase at higher peroxide levels (~5 to 10 mM). Mode 2 killing occurs at peroxide concentrations above 10 mM and is characterized by a dose-dependent (monotonic) decrease in cell viability. It has been reported that hydrogen peroxide induces the SOS response (18, 26, 59), but peroxide-induced mutagenesis may not require the action of *umuC* (18, 26).

The absence of Pol II appears to result in a peroxide-sensitive phenotype because *polBΔ1* appeared to be at least fivefold less viable than *polB*$^+$ during mode 1 killing (Fig. 2a). The sensitivity to peroxide in the absence of Pol II is similar to the effect of *recF* or *recN* mutations on cell survival (26), which

TABLE 2. Relative recombination proficiencies of *polB* derivatives

Recipient	Genotype	Relative recombination frequency[a]			
		× Hfr BW6166 (*zhf-721*::Tn*10*)	× Hfr BW6160 (*zdh-57*::Tn*10*)	× P1 CAG18477	
				(*zij-501*::Tn*10*)	(*argE*$^+$)
STL1336	*rec*$^+$ *polBΔ1*	1.1	1.1	1.9	1.0
STL1334	*recBC sbcBC polBΔ1*	0.92	0.93	0.90	0.99

[a] Frequency of recombination in parallel Hfr or P1 crosses relative to control strain AB1157 for STL1336 or JC7623 for STL1334. Hfr crosses were performed for 1 h with a 10:1 recipient-to-donor cell ratio. Recombination frequencies (the number of Tc^r Sm^r progeny per donor cell) for AB1157 and JC7623 were 9.2×10^{-4} and 2.4×10^{-4}, respectively, for donor BW6166 and were 9.2×10^{-2} and 1.0×10^{-1}, respectively, for donor BW6160. P1 transductions were performed by infection at a multiplicity of infection of ≤0.1 with P1 grown on CAG18477. Cells were washed and plated with selection for Tc^r or Arg$^+$. Inheritance frequencies per PFU for *zij-50*::Tn*10* were 3.2×10^{-5} and 2.3×10^{-4} for AB1157 and JC7623, respectively; for *argE*$^+$, they were 8.4×10^{-5} and 7.4×10^{-4} for AB1157 and JC7623, respectively.

is moderate compared with peroxide sensitivity in the absence of Pol I, RecA, or exonuclease III (*xthA*) (25). The enhanced sensitivity to peroxide was reversed in 11 independent *polB*$^+$ transductants (Fig. 2a), suggesting that *polB* rather than another closely linked gene is responsible for the increase in survival. Longer exposures to peroxide led to increased killing of *polBΔ1* and *polB*$^+$ cells, but the reduction in survival in a *polBΔ1* genetic background remained 5- to 10-fold lower than in the wild-type background (data not shown). Both *polBΔ1* and *polB*$^+$ cells exhibited increased sensitivity to peroxide in the absence of superoxide dismutases SodA and SodB (Fig. 2b). In a Sod$^-$ background, the relative difference between *polBΔ1* and *polB*$^+$ is approximately 15-fold. Thus, the absence of Pol II appeared to cause slight enhancement, at most threefold, in H_2O_2 killing in a *sodA sodB* background.

polBΔ1 cells that either were starved or had undergone extensive passaging in liquid culture showed reduced sensitivity to peroxide-induced killing (Fig. 4). This loss of damage-sensitive phenotype appears to have a genetic rather than physiological basis, although physiological protective effects of starvation to stress have been documented (42), including protection against exposure to hydrogen peroxide (26) and heat shock (28). We found in Western blot analysis that Pol II was absent in strain GW1010, a Mu d(Ap^r *lac*) gene fusion (Fig. 1, lane 5). However, strain GW1010 and wild-type cells exhibited similar survival at all peroxide concentrations (data not shown). We suggest, therefore, that strain GW1010 has acquired extragenic suppressors that compensate for the absence of Pol II. We tested more than 20 freshly prepared *polBΔ1* transductants, strains ME101, for sensitivity to peroxide during mode 1 killing and found that the transductants were typically 5- to 10-fold less viable than the wild type. However, the transductants became more resistant to exposure to peroxide following periods of starvation or long-term passaging (Fig. 4). These data showing a loss of peroxide-sensitive phenotype following starvation or passaging of the freshly prepared *polBΔ1* mutants are consistent with the notion that there are compensatory mutations that effectively mask phenotypes conferred by the absence of Pol II. The possible appearance of suppressor mutations could be affecting sensitivity to peroxide but perhaps not the effect of the absence of *polB* on the increase in adaptive mutations.

Pol II may play an important role in the fidelity of DNA replication and repair. We found that *polBΔ1* cells exhibit moderate mutator phenotypes during forward mutagenesis to trimethoprim resistance (*thyA*) and for reversion of a *leuB6* point mutation (data not shown). Although it is possible that *polBΔ1* also modestly increases the spontaneous reversion of the *lacI33*::*lacZ* allele during exponential growth, loss of *polB* resulted in a highly reproducible increase in the rate of postplating reversion to Lac$^+$ (Fig. 5). This finding suggests that Pol II is active in these static cells and that in its absence, a less accurate polymerase substitutes for it. The *lac* allele derives from an in-frame fusion of the *lacI* and *lacZ* genes but has a +1 frameshift mutation in the *lacI* region that is polar on *lacZ* (7). Although a variety of different mutations, including deletions and duplications, revert this allele during exponential growth, the mutations recovered during lactose selection are nearly all −1-bp deletions, and 90% are at runs of iterated bases (16, 47). Preliminary data indicate that in the *polBΔ1* strain, the spectrum of the late arising mutations may be still predominantly −1-bp deletions. We also have preliminary data indicating that the presence of a mutated *polB* gene causing a significant reduction in 3′-exonuclease proofreading activity increases the postplating mutation rate of the *polBΔ1* strain. These results suggest that Pol II may be involved in the production of the mutations that arise in static cells.

It was shown in vitro that subunits of the Pol III holoenzyme complex can stimulate Pol II activity (4, 23, 61). We have shown that Pol II interacts with the β and γ complex subunits of the Pol III holoenzyme to perform highly processive DNA synthesis (5). These in vitro data suggest that Pol II may also share subunits with Pol III in vivo. Although the current mutagenesis data cannot be used to precisely define a cellular role for Pol II, the observation that absence of Pol II confers a

TABLE 3. DNA Pol II homologs in bacterial and yeast species

Organism	Strain[a]	Hybridization	
		Southern	Western
Bacteria			
Gram negative			
Escherichia coli K-12	C600	+	+
E. coli B		+	+
E. coli C		+	+
Haemophilus influenzae	ATCC 35056	−	ND[b]
Klebsiella pneumoniae	ATCC 13883	+	+
Neisseria flava	ATCC 14221	−	ND
Proteus mirabilis		+	ND
Pseudomonas aeruginosa		+	+
Salmonella typhimurium	ATCC 14028	+	+
Serratia marcescens	ATCC 8100	+	ND
Shigella flexneri	ATCC 12022	+	ND
Gram positive			
Bacillus subtilis	ATCC 21332	−	−
Micrococcus cryophilus		−	ND
Staphylococcus aureus	ATCC 25923	−	−
Yeast (*Saccharomyces cerevisiae*)	M12B	−	−

[a] All strains were obtained from the Department of Biological Sciences, University of Southern California strain collection.
[b] ND, not determined.

mutator phenotype in adaptive mutation is circumstantial evidence that Pol II may be involved in DNA repair synthesis and possibly replication.

Experiments from our laboratory (3) and from Shinagawa and coworkers (53) demonstrated that expression of Pol II is controlled by the SOS regulon, and we found that purified Pol II can bypass abasic lesions in vitro (4). A recent experiment by Tessman and Kennedy provides evidence that Pol II may be required for bypass of abasic lesions in the absence of heat shock induction (56). The importance of understanding the function of DNA Pol II is not confined to *E. coli*. Using Southern hybridization (with the entire *polB* gene as a probe) and Western blotting (with an anti-Pol II polyclonal antibody), we have identified Pol II homologs in several close relatives of *E. coli* K-12, including *Shigella* and *Salmonella* spp., and the more distantly related gram-negative bacterium *Pseudomonas aeruginosa* (Table 3). An increase in the levels of Pol II were observed in *E. coli* strains and *S. typhimurium* in response to SOS induction using nalidixic acid. The Southern and Western analyses were complementary; for each strain examined, both showed either the presence or absence of *polB* and its homolog (Table 3).

Two other gram-negative strains, *Neisseria flava* and *Haemophilus influenzae*, did not hybridize to the *polB* DNA probe, indicating their more distant relationships to *E. coli* suggested by 16S rRNA sequence data (63). None of the gram-positive strains nor the *Saccharomyces* strain showed any reaction with the *polB* probe, but this does not mean that Pol II homologs are absent from these species. Three DNA polymerases were found in *Bacillus subtilis* and contain properties roughly analogous to those of the three *E. coli* polymerases (39).

The data presented in this report and the results of others (56) suggest that Pol II may provide the cell with redundant but biologically important functions for repair of DNA damage and for control of spontaneous and damage-induced mutagenesis. Our data may also suggest that the longstanding absence of a discernible phenotype associated with a deficiency in Pol II might be traced to the presence of suppressor mutations that were effective in masking potential *polB* phenotypes. The *polBΔ1* mutant described in this report can be conveniently transduced into a variety of genetic backgrounds. Thus, it should now be feasible to undertake a systematic investigation of the possible involvement of *E. coli* DNA Pol II during DNA replication and repair.

ACKNOWLEDGMENTS

This work was supported by grants GM 42554 and GM 29558 from the National Institutes of Health.

REFERENCES

1. **Bachmann, B. J.** 1987. *In* J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
2. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal. Biochem. **44:**276–287.
3. **Bonner, C. A., S. Hays, K. McEntee, and M. F. Goodman.** 1990. DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**7663–7667.
4. **Bonner, C. A., S. K. Randall, C. Rayssiguier, M. Radman, R. Eritja, B. E. Kaplan, K. McEntee, and M. F. Goodman.** 1988. Purification and characterization of an inducible *Escherichia coli* DNA polymerase capable of insertion and bypass at abasic lesions in DNA. J. Biol. Chem. **263:**18946–18952.
5. **Bonner, C. A., T. Stukenberg, M. Rajagopalan, R. Eritja, M. O'Donnell, K. McEntee, H. Echols, and M. F. Goodman.** 1992. Processive DNA synthesis by DNA polymerase II mediated by DNA polymerase III accessory proteins. J. Biol. Chem. **267:**11431–11438.
6. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:**695–701.
7. **Calos, M. P., and J. H. Miller.** 1981. Genetic and sequence analysis of frameshift mutations induced by ICR-191. J. Mol. Biol. **153:**39–66.
8. **Campbell, J. L., L. Soll, and C. C. Richardson.** 1972. Isolation and partial characterization of a mutant of *Escherichia coli* deficient in DNA polymerase II. Proc. Natl. Acad. Sci. USA **69:**2090–2094.
9. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
10. **Chen, H., S. K. Bryan, and R. E. Moses.** 1989. Cloning the *polB* gene of *Escherichia coli* and identification of its product. J. Biol. Chem. **264:**20591–20595.
11. **Chen, H., Y. Sun, T. Stark, W. Beattie, and R. E. Moses.** 1990. Nucleotide sequence and deletion analysis of the *polB* gene of *Escherichia coli*. DNA Cell Biol. **9:**631–635.
12. **Creighton, S.** 1993. DNA polymerization: microscopic mechanics and evolutionary utility. Ph.D. thesis. University of Southern California, Los Angeles.
13. **DeLucia, P., and J. Cairns.** 1969. Isolation of an *Escherichia coli* strain with a mutation affecting DNA polymerase. Nature (London) **224:**1164–1166.
14. **DiFrancesco, R., S. K. Bhatnagar, A. Brown, and M. J. Bessman.** 1984. The interaction of DNA polymerase III and the product of the *Escherichia coli* mutator gene, mutD. J. Biol. Chem. **259:**5567–5573.
15. **Foster, P. L., and J. Cairns.** 1992. Mechanisms of directed mutation. Genetics **131:**783–789.
16. **Foster, P. L., and J. M. Trimarchi.** 1994. Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions at homopolymeric runs. Science **265:**407–409.
17. **Gefter, M. L., Y. Hirota, T. Kornberg, J. A. Wechsler, and C. Barnoux.** 1971. Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Natl. Acad. Sci. USA **68:**3150–3153.
18. **Goerlich, O., P. Quillardet, and M. Hofnung.** 1989. Induction of the SOS response by hydrogen peroxide in various *Escherichia coli* mutants with altered protection against oxidative damage. J. Bacteriol. **171:**6141–6147.
19. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Harris, R. S., S. Longerich, and S. M. Rosenberg.** 1994. Recombination in adaptive mutation. Science **264:**258–260.
21. **Hays, S., M. McEntee, and M. F. Goodman.** Unpublished data.
22. **Horii, Z. I., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway of genetic recombination in *E. coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80:**327–344.
23. **Hughes, A. J., S. K. Bryan, H. Chen, R. E. Moses, and C. S. McHenry.** 1991. *Escherichia coli* DNA polymerase II is stimulated by DNA polymerase III holoenzyme auxiliary subunits. J. Biol. Chem. **266:**4568–4573.
24. **Imlay, J., and S. Linn.** 1988. DNA damage and oxygen radical toxicity. Science **240:**1302–1309.
25. **Imlay, J. A., and S. Linn.** 1986. Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **166:**519–527.
26. **Imlay, J. A., and S. Linn.** 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **169:**2967–2976.
27. **Iwasaki, H., A. Nakata, G. Walker, and H. Shinagawa.** 1990. The *Escherichia coli polB* gene, which encodes DNA polymerase II, is regulated by the SOS system. J. Bacteriol. **172:**6268–6273.
28. **Jenkins, D. E., J. E. Schultz, and A. Matin.** 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. **170:**3910–3914.
29. **Johnstone, A., and R. Thorpe.** 1982. Immunochemistry in practice, p. 37–42. Blackwell Scientific Publications, Oxford.
30. **Kenyon, C. J., and G. C. Walker.** 1980. DNA-damaging agents

stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:2819–2823.
31. **Knippers, R.** 1970. DNA polymerase II. Nature (London) **228**: 1050–1053.
32. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
33. **Kornberg, A., and T. A. Baker.** DNA replication, 2nd ed. W. H. Freeman & Company, New York.
34. **Kornberg, T., and M. Gefter.** 1970. DNA synthesis in cell-free extracts of a DNA polymerase-defective mutant. Biochem. Biophys. Res. Commun. **40**:1348–1355.
35. **Kornberg, T., and M. L. Gefter.** 1971. Purification and DNA synthesis in cell-free extracts: properties of DNA polymerase II. Proc. Natl. Acad. Sci. USA **68**:761–764.
36. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
37. **Lovett, S. T., C. A. Luisi-DeLuca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics **120**:37–45.
38. **Low, B.** 1968. Formation of merodiploids in matings with a class of Rec^- recipient strains of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **60**:160–167.
39. **Low, R. I., S. A. Rashbaum, and N. R. Cozzarelli.** 1976. Purification and characterization of DNA polymerase III from *Bacillus subtilis*. J. Biol. Chem. **251**:1311–1325.
40. **Mahajan, S. K.** 1988. Pathways of homologous recombination in *Escherichia coli*, p. 87–140. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
41. **Marshall, T., and K. M. Williams.** 1993. Centriprep ultrafiltration for fractionation of serum and urinary proteins before electrophoresis. Clin. Chem. **39**:1558.
42. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor KatF has a central role in development of starvation-mediated resistance in *Escherichia coli*. J. Bacteriol. **173**:4188–4194.
43. **McHenry, C. S.** 1988. DNA polymerase III holoenzyme of *Escherichia coli*. Annu. Rev. Biochem. **57**:519–550.
44. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Moses, R. E., and C. C. Richardson.** 1970. A new DNA polymerase activity of *Escherichia coli*. I. Purification and properties of the activity present in *E. coli polA1*. Biochem. Biophys. Res. Commun. **41**:1557–1564.
46. **Prentki, P., and H. M. Krisch.** 1984. *In vitro* insertional mutagenesis with a selectable DNA fragment. Gene **29**:303–313.
47. **Rosenberg, S. M., S. Longerich, P. Gee, and R. S. Harris.** 1994. Adaptive mutation by deletions in small mononucleotides. Science **265**:405–407.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Schaaper, R. M.** 1988. Mechanisms of mutagenesis in the *Escherichia coli* mutator *mutD5*: role of DNA mismatch repair. Proc. Natl. Acad. Sci. USA **85**:8126–8130.
50. **Schaaper, R. M., and M. Radman.** 1989. The extreme mutator effect of *Escherichia coli mutD5* results from saturation of mismatch repair by excessive DNA replication errors. EMBO J. **8**:3511–3516.
51. **Scheuermann, R., S. Tam, P. M. Burgers, C. Lu, and H. Echols.** 1983. Identification of the epsilon-subunit of *Escherichia coli* DNA polymerase III holoenzyme as the *dnaQ* gene product: a fidelity subunit for DNA replication. Proc. Natl. Acad. Sci. USA **80**:7085–7089.
52. **Scheuermann, R. H., and H. Echols.** 1984. A separate editing exonuclease for DNA replication: the ε-subunit of *Escherichia coli* DNA polymerase holoenzyme. Proc. Natl. Acad. Sci. USA **81**: 7747–7751.
53. **Shinagawa, H., H. Iwasaki, Y. Ishino, and A. Nakata.** 1991. SOS-inducible DNA polymerase II of *Escherichia coli* is homologous to replicative DNA polymerases of eukaryotes. Biochemie **73**:433–435.
54. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53**:1–24.
55. **Sternberg, N. L., and R. Maurer.** 1991. Bacteriophage-mediated generalized transduction in *Escherichia coli* and *Salmonella typhimurium*. Methods Enzymol. **204**:18–43.
56. **Tessman, I., and M. A. Kennedy.** 1993. DNA polymerase II of *Escherichia coli* in the bypass of abasic sites *in vivo*. Genetics **136**:439–448.
57. **Thomas, N., C. N. Jones, and P. L. Thomas.** 1988. Low volume processing of protein blots in rolling drums. Anal. Biochem. **170**:393–396.
58. **Tijssen, P.** 1985. Practice and theory of enzyme immunoassays, p. 333–347. *In* R. H. Burdon and P. H. Knippenberg (ed.), Laboratory techniques in biochemistry and molecular biology. Elsevier, Amsterdam.
59. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
60. **Wanner, B. L.** 1986. Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K-12. J. Mol. Biol. **191**:39–58.
61. **Wickner, S., and J. Hurwitz.** 1974. Conversion of ϕX174 viral DNA to double-stranded form by purified *Escherichia coli* proteins. Proc. Natl. Acad. Sci. USA **71**:4120–4124.
62. **Willetts, N. S., A. J. Clark, and B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**:244–249.
63. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.