

Detection and characterization of mammalian DNA polymerase β mutants by functional complementation in *Escherichia coli*

(DNA replication/mutagenesis)

JOANN B. SWEASY AND LAWRENCE A. LOEB

The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology, University of Washington, Seattle, WA 98195

Communicated by Evelyn M. Witkin, February 17, 1993

ABSTRACT We have designed and utilized a bacterial complementation system to identify and characterize mammalian DNA polymerase β mutants. In this complementation system, wild-type rat DNA polymerase β replaces both the replicative and repair functions of DNA polymerase I in the *Escherichia coli* *recA718 polA12* double mutant; our 263 DNA polymerase β mutants replace *E. coli* polymerase I less efficiently or not at all. Of the 10 mutants that have been shown to contain DNA sequence alterations, 2 exhibit a split phenotype with respect to complementation of the growth defect and methylmethanesulfonate sensitivity of the double mutant; one is a null mutant. The mutants possessing a split phenotype contain amino acid residue alterations within a putative nucleotide binding site of DNA polymerase β . This approach for the isolation and evaluation of mutants of a mammalian DNA polymerase in *E. coli* may ultimately lead to a better understanding of the mechanism of action of this enzyme and to precisely defining its role in vertebrate cells.

Our understanding of the physiologic functions and catalytic mechanisms of prokaryotic DNA polymerases (Pols) has depended upon the availability of mutant enzymes (1). For example, the phenotypic, biochemical, and physical characterization of mutants of *Escherichia coli* DNA Pol I has facilitated the identification of amino acid residues involved in catalysis and elucidation of the roles of Pol I in DNA replication and repair (2). Analysis of Pol I mutations in *E. coli* strains bearing multiple genetic lesions has allowed detailed dissection of Pol I function (3).

Direct selection of mammalian DNA Pol mutants is difficult and has been limited to chromosome X-linked mutations of mouse DNA Pol α (4, 5). Only a few rationally designed mammalian DNA Pol mutants are available, and these are altered at residues that share putative homology with other DNA Pols (6, 7). We introduce here a method for isolation and characterization of mammalian DNA Pol mutants, based on the ability of a wild-type mammalian DNA Pol to substitute for a defective host Pol within bacterial cells (8). Our approach exploits functional complementation to create a collection of mutant mammalian DNA Pols whose structural and catalytic properties, assessed *in vivo*, can be correlated with defined capabilities *in vitro*. The method affords, in an alternate host, a phenotypic analysis of mutant enzymes otherwise precluded by lack of mutant mammalian cells.

The mammalian enzyme we have employed is DNA Pol β , a 38-kDa protein and the smallest DNA Pol known (9). Pol β has been considered to be the primary catalyst for DNA repair synthesis in vertebrate cells (8). However, Pol β may also function in DNA replication, because it is essential for the conversion of single-stranded to double-stranded DNA in *Xenopus* extracts (10) and because it can substitute for Pol I

in *E. coli*, where it catalyzes the joining of Okazaki fragments (8).

In our complementation system, wild-type rat Pol β replaces both replicative and repair functions of DNA Pol I in the *E. coli* *recA718 polA12* double mutant. We have mutagenized *in vitro* the cDNA for Pol β and used this complementation system to identify and characterize Pol β mutants that are less efficient in substituting for Pol I in either or both of these functions. Structural and phenotypic analysis of these mutant Pols should lead to better understanding of the catalytic mechanisms and physiologic roles of Pol β in mammalian cells.

MATERIALS AND METHODS

Bacterial Strains and Media. Strain SC18-12 is derived from *E. coli* B/r and has the genotype *recA718 polA12 uvrA155 trpE65 lon-11 sulA1* (11). It was used in the screening of the cDNA library of Pol β mutants. Minimal medium (ET) was E medium (12) supplemented with 0.4% glucose and tryptophan (20 μ g/ml). CAA medium was ET supplemented with various levels (0.01–0.4%) of vitamin-free Casamino acids (Difco). Transformants were selected on LB agar supplemented with chloramphenicol (30 μ g/ml). Rich medium was nutrient agar composed of Difco nutrient agar containing NaCl (4 g/liter). Nutrient broth was prepared according to directions from Difco.

Construction of the Library of Pol β Mutants. The Pol β rat cDNA was kindly provided by S. H. Wilson (University of Texas, Galveston), and plasmid vector pHSG576 (13) was provided by T. Hashimoto-Gotoh (National Institute of Health, Tokyo). The rat Pol β cDNA was subcloned from p β (8) into pHSG576 to create p β L. This construct placed the cDNA under regulation of the *lac* promoter, rendering its expression inducible upon the addition of isopropyl β -D-thiogalactopyranoside (IPTG) to the growth medium. The plasmid p β L is present at around five copies per cell due to its pSC101 origin of replication (13).

The cDNA for Pol β was treated with nitrous acid and then reconstructed to restrict mutations to the 3'-terminal 248 nt. The entire Pol β cDNA was amplified from the plasmid p β L using the PCR with the M13(–48) reverse sequencing primer that is complementary to bases 5' to the Pol β cDNA and the M13(–20) universal primer that hybridizes to residues 3' to the Pol β cDNA (13). The amplified DNA was ethanol-precipitated and dissolved in 40 μ l of 250 mM sodium acetate (pH 4.3) containing 1 M sodium nitrite and incubated at room temperature for 30 min. These conditions were chosen to yield \approx 1 altered residue per 500 bp (14). After treatment, the damaged DNA was used as the template in the PCR with the 3' universal primer and the internal primer 577⁺, with the sequence 5'-GCTGGATCCTGAGTACATCG-3'. The thermocycling conditions were as follows: 95°C, 30 sec; 50°C, 30

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Abbreviations: Pol, DNA polymerase; IPTG, isopropyl β -D-thiogalactopyranoside; MMS, methylmethanesulfonate.

sec; 72°C, 2 min; for 30 cycles. This was done to produce large amounts of a DNA fragment including the 3'-terminal 248 nt of mutated Pol β DNA. This DNA was purified, digested with the restriction enzymes *Cla* I and *Xba* I, and ligated into the p β L vector that had also been digested with the same enzymes, replacing the nonmutated 3'-terminal 248 residues of this vector with the 3'-terminal fragment of mutated Pol β cDNA. This construct was transformed into the *E. coli* DH5 α (15) to obtain large numbers of transformants. The plasmid DNA was isolated from \approx 10,000 pooled colonies and then used to transform the *E. coli* SC18-12 double mutant.

Pol β Mutant Screen. Individual colonies were grown overnight in nutrient broth in 96-well plates at 30°C without aeration. Cultures were spotted onto minimal medium, minimal medium plus 0.04% methylmethanesulfonate (MMS), minimal medium plus 0.04% MMS with 1 mM IPTG, rich medium, and rich medium with 1 mM IPTG. Plates were incubated at 42°C overnight. Cultures were also spotted onto a master plate consisting of minimal medium containing chloramphenicol (30 μ g/ml) and incubated overnight at 30°C.

Efficiency of Plating Assay. Cultures were grown to logarithmic phase at 30°C in nutrient broth with or without 1 mM IPTG, diluted in saline, and plated onto CAA medium containing various amounts of Casamino acids. Plates were incubated at 30°C or 42°C for 2 days and colonies were counted.

MMS Survival Curves. Cultures were grown to logarithmic phase at 30°C in ET broth in the presence of 1 mM IPTG and treated with various concentrations of MMS at 42°C with aeration for 1 h. Cultures were diluted in saline, plated onto ET medium containing IPTG, and incubated at 42°C for 2 days before colonies were counted.

Extract Assay. Extracts were prepared as described (16), after growth in the presence of 1 mM IPTG at 30°C to an OD₅₅₀ value of 0.5 followed by incubation at 42°C for 1 h. Various amounts of extracts were assayed at 37°C for 15 min in a 25- μ l reaction volume containing 25 mM Tris Cl (pH 8.4), 49 mM NaCl, 10 mM MgCl₂, 40 μ M dATP, 40 μ M dCTP, 40 μ M dGTP, and 40 μ M [α -³²P]dTTP (2.75 dpm/pmol; NEN), and activated DNA (240 μ g/ml), prepared by the method of Spanos *et al.* (17).

DNA Sequencing. DNA template was amplified from individual spots on the master plate (see above) by using the PCR. One primer (577⁺), hybridizes to nt 494–513 of the Pol β rat cDNA (operon); the other is the M13 bacteriophage (–20) universal sequencing primer (New England Biolabs) that is complementary to bases within the polylinker and 3' to the Pol β cDNA of p β L. The DNA was sequenced using the Sequenase kit (United States Biochemical) with the following modifications provided by G. Burmer (University of Washington). The double-stranded DNA was mixed with the 5'-end-³²P-labeled primer, denatured at 98°C for 5 min, and immediately placed in an ice-water bath to prevent renaturation. After combining all reagents, the sequencing reaction mixtures were incubated at 37°C for 30 min. Stop buffer was added and the reaction products were resolved on a denaturing 8% polyacrylamide gel. The sequencing primers, provided by G. Burmer, were 746⁺, which hybridizes to nt 669–695 of the Pol β cDNA and has the sequence 5'-ACTCGTTCAAAGGGTGAGACAAAGTTC-3', and 1074[–], which hybridizes to nt 1074–1052 on the opposite strand and has the sequence 5'-ACACCCAACCCTTACAT-3'. Both strands of the mutated portion of the gene were sequenced, including the junctions of the restriction sites used to subclone the mutagenized DNA into the vector. The DNA sequence of the entire gene of one of our mutants was determined and shown to contain no mutations outside of the 3'-terminal 248 nt.

Partial Purification of Pol β Antiserum. The Pol β antiserum was a generous gift from A. Matsukage (Aichi Cancer Research Institute, Nagoya, Japan). Before use in Western blot analysis, the anti-*E. coli* antibodies were removed from the Pol β antiserum by affinity chromatography by the method of de Wet *et al.* (18). The lysate that was coupled to the cyanogen bromide-activated Sepharose 4B was prepared from the SC18-12 strain.

Western Blot Analysis. Aliquots of identical extracts described above were resolved on an SDS/12.5% polyacrylamide gel. The gel was equilibrated in Towbin transfer buffer (25 mM Tris base/192 mM glycine/20% methanol) and the proteins were electroblotted to nitrocellulose at 100 V for 1 h at 4°C. After transfer, the filter was incubated overnight with affinity-purified Pol β rabbit antiserum at room temperature. The filter was then incubated with goat anti-rabbit secondary antibody at a 1:2000 dilution (Promega) for 1 h at room temperature. The bands were visualized by incubating the filter with 5 ml of western blue (Promega).

RESULTS

Overview. A collection of Pol β mutants, containing single-base substitutions within the 3'-terminal quarter of the coding sequence, was generated by nitrous acid treatment and reconstruction of the cDNA. Putative Pol β mutants were transformed into *E. coli* SC18-12, the *recA718 polA12* (SC18-12) double mutant that provides the basis for our complementation system. SC18-12 cells are unable to grow at low plating density on rich medium at 42°C and are sensitive to MMS. However, we have shown (8) that wild-type Pol β restores growth and MMS resistance to this strain by catalyzing the joining of Okazaki fragments during DNA replication. We have exploited both of these complementation functions in our screen for Pol β mutants.

Screening for Pol β Mutants. A total of 1186 SC18-12 transformants were screened for their ability to grow at low plating density on both minimal medium containing MMS and rich medium at 42°C. Of the 1186 transformants, 263 were unable to grow on rich medium or on MMS-containing medium, or both, in the presence and absence of IPTG, and were designated as putative mutants. The phenotype of all the mutants was further assessed by streaking each mutant on gradient plates with minimal medium containing 0–0.04% MMS and on gradient plates with rich medium composed of minimal medium with 0–0.4% Casamino acids, all with IPTG (results not shown). Two phenotypes were identified. Fifty-six of 263 mutants displayed a null phenotype, failing to complement the growth defect of the SC18-12 strain on both minimal medium with MMS or on rich medium. The remaining 207 mutants exhibited a split phenotype and grew on minimal medium containing 0.04% MMS and IPTG but not on medium containing high levels of Casamino acids with IPTG. DNA sequence analysis of 10 of the mutants confirmed that the mutant phenotypes resulted from single-base substitution mutations in the mutagenized coding sequence of the Pol β cDNA. The *in vivo* characterization of 3 of these Pol β mutants, 1 with a null phenotype (pbLN-1) and 2 with split phenotypes (pbLSP-1 and pbLSP-2), is presented below.

Characterization of the Mutants on Rich Medium. The ability of wild-type Pol β to promote growth of the SC18-12 double mutant on rich medium is associated with its ability to increase the rate of joining of Okazaki fragments (8). To quantitate this complementation function, we measured plating efficiency on semienriched medium (CAA) containing increasing concentration of Casamino acids. The plating efficiency assay assesses the ability of Pol β mutants to promote growth of single colonies under increasingly rigorous conditions and permits detection of even weak complementation (Table 1). Bacteria containing the pHSG576 plas-

Table 1. Efficiency of plating of Pol β mutants on various amounts of Casamino acids

| Plasmid | IPTG, mM | Plating efficiency | | | | |
|-----------------|----------|--------------------|------|------|------|------|
| | | 0 | 0.01 | 0.02 | 0.04 | 0.08 |
| pHSG576 | 0 | 68.4 | 2.4 | 1.4 | 0.57 | 0.35 |
| | 1 | 89.2 | 3.9 | 2.2 | 0.69 | 0.38 |
| p β LN-1 | 0 | 6.1 | 0.35 | 0.46 | 0.28 | 0.12 |
| | 1 | 42.0 | 1.4 | 0.43 | 0.31 | 0.10 |
| p β L | 0 | 28.2 | 5.3 | 2.4 | 0.25 | 0.14 |
| | 1 | 100.0 | 37.7 | 48.6 | 56.8 | 46.5 |
| p β LSP-1 | 0 | 12.8 | 0.58 | 0.41 | 0.26 | 0.18 |
| | 1 | 73.8 | 50.7 | 40.9 | 13.6 | 2.2 |
| p β LSP-2 | 0 | 19.6 | 1.1 | 0.92 | 0.43 | 0.44 |
| | 1 | 100.0 | 19.9 | 8.7 | 2.5 | 0.50 |

Cells were cultured in medium containing Casamino acids at 0, 0.01, 0.02, 0.04, and 0.08%. Efficiency of plating is number of colonies at 42°C/number of colonies at 30°C \times 100. The genotype of the host strain SC18-12 is *recA718 polA12 fad751::Tn10 sulA lon uvrA mal*. Data represent the average of three experiments.

mid, our negative control, grew on minimal medium in the presence and absence of IPTG but were highly sensitive to the growth inhibitory effects of 0.01% Casamino acids, the lowest concentration tested. Bacteria harboring p β LN-1, the null plasmid, exhibited only limited growth on minimal medium in the absence of IPTG and growth was inhibited by minimal amounts of Casamino acids in the presence of IPTG. p β L, the wild-type Pol β plasmid, was able to promote growth of the SC18-12 tester strain on medium containing IPTG with up to 0.08% Casamino acids or even 0.4% Casamino acids (data not shown). Bacteria containing either of the active mutant plasmids, p β LSP-1 and p β LSP-2, presented an intermediate phenotype; they grew on medium containing IPTG and low levels of Casamino acids, but not on medium with higher levels of Casamino acids. Moreover, these two mutants were quantitatively distinguishable, the p β LSP-1 plasmid conferring higher plating efficiency than p β LSP-2 at all concentrations of Casamino acids tested in Table 1.

Characterization of the Mutants on MMS. We also analyzed our mutants with respect to their ability to confer resistance

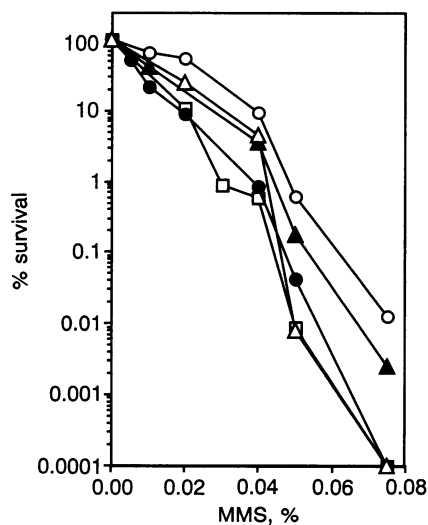


FIG. 1. Survival of MMS-treated strains containing the Pol β mutant plasmids. \circ , p β L; \blacktriangle , p β LSP-1; \triangle , p β LSP-2; \square , p β LN-1; \bullet , pHSG576. The data used to plot the curves are the average of three experiments. Separate plots of the data points from each of the three experiments show the same relationships among the mutants and between the mutants and the controls as the plot of the data averaged from the three experiments.

to MMS. Bacteria that contain a nonfunctional DNA Pol I were unable to grow in the presence of 0.04% MMS (Fig. 1). This is considered to be due to the inability of DNA Pols II and III to fill the single-stranded gap created by removal of the methylated base by the sequential action of DNA glycosylase and apurinic endonuclease (1). The expression of wild-type Pol β conferred MMS-resistance to *polA* mutants of *E. coli*, presumably by substituting for Pol I in DNA repair synthesis. Each of the three Pol β mutants was further characterized by measuring survival of plasmid-bearing SC18-12 cells after exposure to increasing concentrations of MMS (Fig. 1). Bacteria carrying the null Pol β mutant plasmid p β LN-1 were as sensitive to MMS as the negative control (pHSG576). The SC18-12 cells carrying plasmid p β L, the wild-type Pol β plasmid, displayed 10-fold greater resistance to MMS than cells carrying the pHSG576 parent plasmid. Cells carrying the Pol β mutants p β LSP-1 and p β LSP-2 exhibited intermediate resistance to MMS. Specifically, the p β LSP-1 mutant conferred 5-fold less resistance to MMS than the wild-type plasmid; the p β LSP-2 mutant plasmid conferred 5-fold less resistance to MMS than the wild-type plasmid at low doses of MMS and this strain was up to 100-fold less resistant to MMS than the p β L plasmid-containing strain at higher doses of MMS. These two Pol β mutants were differentiated once again: the p β LSP-1 plasmid conferred greater resistance to MMS than the p β LSP-2 plasmid especially at higher concentrations of MMS, in parallel with the results obtained for growth on Casamino acids (Table 1).

The Pol β Mutant Proteins Are Expressed in Equal Amounts. Fig. 2 is a photograph of a Western blot of proteins prepared from extracts containing the various plasmids and from purified rat liver Pol β . Visualization of purified Pol β using affinity-purified Pol β antisera yielded two bands corresponding to 38 kDa and a commonly observed 31-kDa proteolytic product. Two amounts of protein were analyzed from extracts of *E. coli* containing N-1, SP-2, SP-1, or the WT plasmid. No significant differences were noted in the amount of immunoreactive protein that migrated coincidentally with Pol β . In contrast, no immunoreactive band corresponding to the size of highly purified Pol β was detected in extracts from the SC18-12 strain containing the plasmid lacking Pol β . It should be noted that an extract prepared from the SC18-12 strain containing p β LN-1 also contained a protein band comigrating with Pol β .

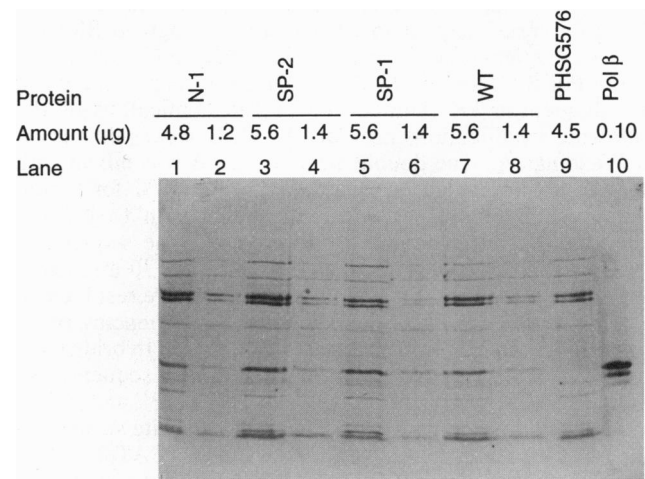


FIG. 2. Western blot of the wild-type and mutant proteins. The mutant and amounts of total protein loaded in each lane are given above each lane. The total amount of protein was determined by a Bradford assay using the dye reagent from Bio-Rad and bovine serum albumin as the protein standard.

Table 2. Incorporation of dTMP into DNA by various cell extracts

| Cell Extract | dTMP incorporated, pmol/ μ g of protein |
|-------------------|---|
| SC18-RP | 12.63 |
| SC18-12 | |
| + pHSG576 | 0.54 |
| + p β LN-1 | 0.26 |
| + p β L | 1.77 |
| + p β LSP-1 | 2.30 |
| + p β LSP-2 | 1.45 |

SC18-RP strain is *recA*⁺ *polA*⁺ and is isogenic to the SC18-12 strain.

The Mutants Express Active Protein. To determine whether active protein was expressed from each of the mutant plasmids, we determined the rate of nucleotide incorporation into activated DNA *in vitro* (Table 2). Under conditions in which the extracts from the wild-type *E. coli* parent incorporated 12.63 pmol of dTMP, an equal amount of our extract from the *recA718 polA12* double mutant incorporated 0.55 pmol. This incorporation was presumably catalyzed by *E. coli* DNA Pols II and III. The extract derived from null Pol β mutant p β LN-1 did not enhance incorporation over background, as expected. However, the null mutant extract contained only 50% of the activity of the negative control extract prepared from cells containing pHSG576; this suggests that the null Pol β protein inhibits the residual activity catalyzed by Pols II and III. Extracts prepared from cells harboring the p β L, p β LSP-1, and p β LSP-2 plasmids increased incorporation to 1.8, 2.3, and 1.5 pmol, respectively. Since the amount of incorporation catalyzed by the SC18-12 strain containing pHSG576, 0.55 pmol, likely resulted from the activity of DNA Pol II and III, the enhanced activity in extracts was directly due to the action of wild-type Pol β , p β LSP-1, and p β LSP-2 proteins in extracts and was 1.25, 1.75, and 0.95 pmol, respectively.

DNA Sequence Analysis. The DNA sequence of each of the mutants confirms that the phenotypes result from mutations within the DNA encoding Pol β . Fig. 3 displays the amino acid alterations predicted from DNA sequence analysis. The null Pol β mutant, p β LN-1, contained a Phe \rightarrow Ser mutation at position 277. The p β LSP-1 mutant plasmid possessed two alterations: Ile \rightarrow Thr at position 276 and Tyr \rightarrow Phe at position 295. The p β LSP-2 mutant plasmid contained a Glu \rightarrow Lys change at position 288. No other mutations were found in the DNA sequences of the mutants.

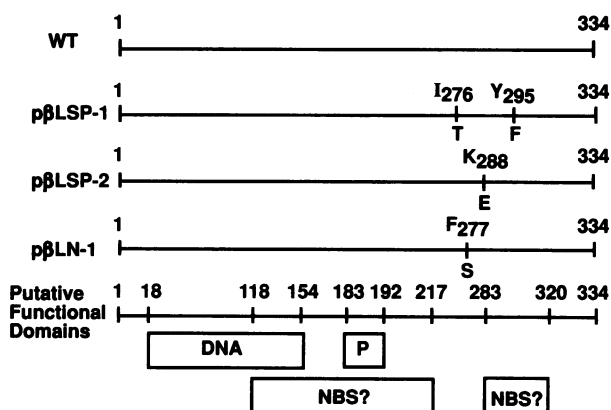


FIG. 3. Functional domains of Pol β and locations of amino acid alterations of the Pol β mutants. P, primer binding site; DNA, DNA binding site; NBS, nucleotide binding site. Amino acid substitutions have been inferred from the DNA sequence.

DISCUSSION

We have used a genetic screen in *E. coli* to isolate 207 catalytically active mutants of mammalian DNA Pol β . The screen relies on the ability of wild-type Pol β to complement a growth defect of a *recA718 polA12* double mutant and to confer MMS resistance on this strain. Pol β mutants defective in either or both of these complementation functions have been identified and 3 of them have been characterized. The null Pol β mutant in plasmid p β LN-1 is unable to complement the growth defect or to confer MMS resistance. Two of the active Pol β mutants, p β LSP-1 and p β LSP-2, displayed a split phenotype in the initial screen: they conferred MMS resistance but did not promote growth on rich medium at 42°C. Further *in vivo* characterization of these mutants revealed that they confer intermediate levels of MMS resistance and growth ability in the presence of low amounts of Casamino acids. One of them, p β LSP-1, permits growth on higher levels of Casamino acids and confers greater resistance to MMS than the other mutant, p β LSP-2. These two mutants and wild type are expressed at similar levels and also produce similarly active proteins as measured in extract incorporation assays. In contrast, both of these mutants exhibit a 10- to 100-fold decrease in efficiency of plating at the highest concentration of Casamino acids tested and a >10-fold increase in sensitivity to 0.08% MMS compared to the wild type. Therefore, the amino acid alterations of each of the mutant enzymes and not the amount of enzyme most likely accounts for the phenotypes we have detected. We expect that highly purified fractions of these mutant proteins will be differentiated *in vitro* when assayed in different conditions.

Growth on Rich Medium. The cause of the growth deficiency of the double mutant on rich medium has not been established. Maaløe and coworkers (19) observed that the growth rate of bacteria increases when they are transferred from minimal to rich medium. This is followed by an increase in the number of replication forks per genome. In cells possessing a defective DNA Pol I, the increased number of forks could lead to an increase in the amount of single-stranded DNA, because these cells are unable to complete lagging-strand synthesis before the next round of replication initiates. The single-stranded DNA would be prone to damage by endogenous cellular components, including oxygen free radicals, and to cleavage by endonucleases, ultimately resulting in cell death.

Strains carrying only the *polA12* mutation can grow as single colonies on rich medium at 42°C whereas strains carrying both the *polA12* mutation and the *recA718* mutation cannot (11). The growth defect in the double mutant could be due to the deleterious interaction of the two mutations, the *polA12* mutation resulting in persistent gaps in lagging-strand DNA synthesis (1) and the *recA718* mutation causing a defect in recombinational repair (11), an important mechanism used in repairing these single-stranded gaps.

The accumulation of single-stranded DNA in the double mutant upon growth on rich medium can be circumvented by the presence of another DNA Pol such as Pol β or large amounts of the α subunit of DNA Pol III holoenzyme (8, 11). We have demonstrated that Okazaki fragments produced during replication of the lagging strand are joined at a slower rate in the SC18-12 double mutant than in a wild-type isogenic strain (8). Expression of DNA Pol β in the double mutant increases the rate of joining of Okazaki fragments, presumably by substituting for *E. coli* DNA Pol I. By analogy we believe that the phenotype of the Pol β mutants that allows only intermediate levels of growth of the bacterial double mutant on rich medium is the result of a defect in the ability of the mutant enzymes to fill-in gaps.

MMS Sensitivity. Grindley and Kelley (20) have reported that *E. coli polA* mutants are sensitive to the methylating

agents such as MMS. This sensitivity is presumably caused by the inability of DNA Pols II and III, the remaining DNA Pols, to fill in the small gap resulting from removal of methylated bases by a DNA glycosylase and an apurinic endonuclease (1). *In vitro*, mammalian DNA Pol β can carry out repair synthesis on small gaps in DNA (9); we believe that this ability renders the SC18-12 double mutant resistant to MMS when Pol β is expressed *in vivo*. The Pol β mutants that are partially defective in conferring MMS resistance could have either a decreased affinity for short gaps or a decreased ability to fill them. The differences in the phenotypes of p β LSP-1 and p β LSP-2 could reflect differences in these properties.

Functional Domains of Pol β (Fig. 3). Thus far, Pol β has been found to share homology only with terminal deoxynucleotidyltransferase, a non-template-directed and nonprocessive DNA polymerase; to our knowledge, no Pol β -like enzyme has been found in yeast or in filamentous fungi. Date *et al.* (6, 7) have made mutants at sites in the Pol β rat cDNA that encode amino acid residues conserved in terminal deoxynucleotidyltransferase and, after kinetic analysis of the resulting mutant proteins, have suggested that aa 183–192 interact with the primer. DNA binding studies of tryptic peptides of Pol β (21) have shown that a peptide consisting of residues 18–154 has both single-stranded and double-stranded DNA binding capacity. Recently, Recupero *et al.* (22) have obtained monoclonal antibodies to Pol β that recognize the nucleotide binding site of DNA Pols in general and concluded from their epitope mapping studies of Pol β that the deoxynucleoside triphosphate binding site of Pol β lies between aa 283 and 320. Evans *et al.* (23) have proposed that the region of Pol β encompassing aa 188–217 may be the nucleotide binding domain of the protein, based on photoaffinity-labeling studies.

Pol β Mutants and Functional Domains of the Protein. The phenotypes of the mutants we have identified most likely result from the sequence changes we have detected. The mutants possessing a split phenotype, p β LSP-1 and p β LSP-2, contain amino acid sequence alterations residing in one of the proposed nucleotide binding domains of the protein. The p β LSP-1 mutant contains Phe \rightarrow Tyr change at position 295 of the polypeptide. This change results in the replacement of a polar amino acid with a hydrophobic one. The tyrosine could interact with a nucleotide base and this interaction could be destroyed by its replacement with phenylalanine. This same mutant also contains another alteration in an area that has not been proposed to constitute a catalytic domain of the protein. This Ile \rightarrow Thr change at residue 276 results in the substitution of a polar residue for a nonpolar one and could lead to a change in the tertiary structure of the protein or weaken the interaction of Pol β with DNA. Each of these changes must be examined separately to make any conclusions regarding the function of these residues. The amino acid alteration of the p β LSP-2 mutant is also in a proposed nucleotide binding domain. Lys 288 could interact with the phosphate moiety of the nucleotide; a change to glutamic acid would disrupt electrostatic interaction. However, since this enzyme functions *in vivo*, the residues at this site might not be necessary for catalysis or to maintain the tertiary structure of the protein. The null mutant contains a

serine residue in place of phenylalanine that results in a more polar character and a greatly decreased size of the side chain.

We have taken an approach for the isolation and evaluation of mutants of a mammalian DNA Pol, employing the power of bacterial genetics. A library of partially functional Pol β mutants will allow the identification of important catalytic residues in the active site of the enzyme and will ultimately lead to a better understanding of the mechanism of action of this enzyme and to precisely defining its role in vertebrate cells.

This paper is dedicated to the memory of Hatch Echols; he will be sorely missed. We thank Ann Blank and Michael Fry for helpful discussions, Dr. Akio Matsukage for providing us with Pol β antiserum and protein, and Thanh T. B. Nguyen and Jason Timmons for technical assistance. This work was supported by a grant from the National Cancer Institute (OIG-R35-CA-39903); J.B.S. was also supported by a postdoctoral fellowship from the National Institutes of Health (CA08923).

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