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Rat DNA polymerase β gene can join in excision repair of *Escherichia coli*

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ABSTRACT

Though DNA polymerase I (polI) of *Escherchia (E.) coli* is understood to play a role in repair synthesis of excision repair, it is still obscure whether DNA polymeraseβ (polβ) plays a similar role in eukaryotic cells. To estimate the role of polβ in excision repair processes, we inserted the rat *polβ* gene into several mutant *E. coli* defective in a diverse set of enzymatic activities of polI. UV resistance was seen only when the 5′→3′exonuclease (exo) activity of polI molecules remained. Therefore it is suggested that 5′→3′exo activity as well as polβ activity are essential for repair synthesis of excision repair in eukaryotic cells.

INTRODUCTION

Polβ is known to be the simplest DNA polymerase which is involved only in DNA synthesis activity, but not in other activities such as nuclease. The enzyme is purified from eukaryotic cells as a holoenzyme composed of a single polypeptide chain ca. 40 KD (1). The exact cellular role of polβ is still obscure, since a repair deficient mutant in polβ such as xeroderma pigmentosum (XP) in UV-specific endonuclease has not been isolated in eukaryotic cells. However, levels of the enzyme do not correlate with replication of genomic DNA in mammalian cells, and the enzyme has been described as being constitutive in different organs by immunological methods against polβ (2). Recently the induction of *polβ* mRNA by DNA damage has been reported (3), even though DNA damage induced SOS responses have not been established in mammalian cells. In addition, the inhibitors of polβ enzymic activity inhibit DNA repair synthesis (4, 5) and enhance the sensitivity to DNA damaging agents. Therefore polβ is believed to play a part in excision repair. On the other hand, the cellular role of bacterial polI is better known. Repair synthesis in excision repair in *E. coli* is due to this enzyme. *E. coli* mutants defective in the DNA polymerase activity (*polA1*) are sensitive to UV, and the repair synthesis has not been observed in the strain after UV irradiation (6). PolI possesses different enzymic activities which are 5′→3′polymerase activity and two kinds of 5′→ 3′exo and 3′→5′exo activities, and several kinds of mutants partialy defective in those activities have been isolated. Therefore it is very useful to use these mutants for investigation of the roles of their enzymic activities in excision repair. Recently two laboratories (7, 8) succeeded in a full gene expression of *polβ* gene of rat or human in *E. coli*. To clearly establish the biological role of polβ, therefore, we have inserted the cloned rat *polβ* gene (9) into several mutants of *E. coli* having different capacities of the enzymic activities of polI, and investigated the role of polβ in excision repair processes.

MATERIALS AND METHODS

Bacteria strains and their culture. Bacterial cells were cultured in Luria (L) broth with or without ampicillin at 30°C, since all of the strains have the $recA^{ts}$ gene. Four strains used here were pol^{ts} (pol^+ and exo^+, wild-type for repair at 30°C), *polA107* (pol^+, 5′→3′exo^- and 3′→5′ exo^+), *polA1* (pol^-, 5′→ 3′exo^+ and 3′→5′exo^-), *resA1* (pol^-, 5′→3′exo^- and 3′→5′exo^-). These strains were provided by Dr. T. Kato (Osaka University, Japan). Another *E. coli* strain, *KY116* (*uvrA6*), characteristics of which have been described in detail in a previous paper (10), was cultured at 37°C.

$NaDodSO_4$-polyacrylamide gel electrophoresis (SDS-PAGE). Protein was analyzed by electrophoresis in a 11% polyacrylamide gel containing $NaDodSO_4$. Protein in gel was stained with coomassie brilliant blue. Other details were as described in Laemmli (11).

Purification of polβ. Bacterial cells were cultured at 30°C in 250 ml of 2×YT medium supplemented with 50 μg/ml ampicillin. When cells were grown to 0.5 of an optical density (600 nm), IPTG was added to a final concentration of 0.5 mM, and culturing was continued for an additional 6 hrs before cell harvest. The wet weight of obtained *E. coli* was about 1 g. The cells were resuspended in 3 ml solution containing 50 mM Tris-HCl (pH 7.8) and 25% sucrose, and then 8.0 mg of egg white lysozyme was added for digestion of cell wall. Then 300 μl of 0.5 M EDTA was added to the bacterial suspension, and the mixture was

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incubated for an additional 5 min. Cells were lysed by mixing the cell suspension with 3 ml of lysing solution containing 0.5% Nonidet P-40, 0.8 M KCl, 2 mM phenylmethylsulfonyl fluoride, and 50 mM Tris-HCl (pH 7.8), and the suspension was sonicated for 1 min. The extract was clarified by centrifugation for 30 min at 12000 rpm. After 30 min of the batchwise process with DEAE-sephadex, the extract was diluted by PC buffer (50 mM Tris-HCl at pH 7.6, 0.1 mM EDTA, 1mM dithiothreitol and 10% glycerol) to adjust KCl concentration to 0.4 M and loaded onto a DEAE-sephadex column (1 by 7 cm) previously equilibrated with 0.4 M KCl-PC buffer. The eluent (18.5 ml) was diluted by an equal volume of PC buffer to reduce KCl concentration, and applied to a phosphocellulose column (1 by 10 cm) equilibrated by 0.2 M KCl-PC buffer. After extensive washing, the column was stepwise eluted by 20 ml of 0.7 M KCl-PC buffer. Each fraction (2.0 ml) was assayed for DNA polymerase activity (9). The two fractions (4 ml) at peak of activity were pooled and diluted 3-fold with PC buffer and loaded onto a denatured calf thymus DNA-cellulose column (0.6 by 5 cm) equilibrated with 0.15 M KCl-PC buffer. After extensive washing, DNA polymerase was eluted with 0.7 M KCl-PC buffer from the column.

Assay for DNA polymerase. DNA polymerase assays were carried out using the following reaction mixture. Each reaction mixture contained, in a final reac tion volume of 20 μl, 50 mM Tris-HCl (pH 8.8), 1 mM dithiothreitol, 0.5 mM $MnCl_2$, 40 μg/ml (rA)n, 40 μg/ml $(dT)_{12-18}$, 0.1 mM [^{3}H]dTTP (60 cpm/pmol), 12% (v/v) glycerol, 400 μg/ml bovine serum albumin, 100 mM KCl, and 5 μl of enzyme solution. After incubation at 37°C for 10 min, radioactive DNA product was collected on a DEAE-cellulose paper (DE81) disc as described by Lindel (9). Radioactivity was measured by liquid scintillation counting. One unit of DNA polymerase activity is defined as the amount of enzyme which catalyzes the incorporation of 1 nmol of dTTP into polymer DNA for 60 min at 37°C.

UV-irradiation and survival assay. After washing with phosphate buffer containing 0.9% NaCl (PBS), the bacterial cells in the growing phase were resuspended with PBS. Then the cells were irradiated by a germicidal lamp (254 nm) at 5 J/m^2/sec at room temperature, diluted and spread on L-plates. After incubation for about 48 hrs at 30°C or for 24 hrs at 37°C, the colonies were counted. All experiments were done under yellow light to prevent photoreactivation.

RESULTS AND DISCUSSION

Since plasmid DNA is unable to propagate in DNA polymerase-defective strains (*polA1* and *resA1*) in *E. coli*, we aim to recombine the plasmid DNA into chromosomal DNA at the position of leader peptidase gene of pTD101 (13). Therefore we made a new plasmid DNA (pLPOL 15) from pTD101 and JMp5 (7). Physical maps of the plasmid are shown in Fig. 1. Stable transformants were made depending on the homologous recombination at a leader peptidase gene sequence in their strains. In wild-type and *polA107* strains, the recombinant *E. coli* cells contained episomal plasmid DNA at 30°C. Therefore high amounts of 40 kD polypeptide in both strains was yielded in the analysis for bacterial extracts of the transformants, though the 40 KD polypeptide was not detected in the non- transformed cells (Fig. 2). In both the strains, more efficient gene expression of *polβ* was detected when IPTG was present in the culture medium. In *polA1* and *resA1* strains, on the other hand, even in the presence of IPTG little expression of *polβ* gene was observed by analysis of SDS-PAGE for whole proteins of *E. coli*. The different efficiency of polβ production among them may depend on the existing form of *polβ* gene in either episomal DNA or chromosomal DNA, namely copy number of *polβ* gene. Therefore, we estimated the formation of polβ in the recombinant in both strains by the purification of polβ from bacterial cells (Fig. 3). The DNA polymerase activity was recovered in a single peak from a DNA-cellulose column. Apparently high activity in the fractions eluted from a DNA-cellulose column was detected in the transformants of *polA1* and *resA1* strains, but not in the non-transformants. In addition, the exsistence of polβ only in their transformants was also estimated by analysis of SDS-PAGE (inserted figure in Fig. 3).

In the wild-type strain, the UV sensitivity of the transformant decreased by a factor of two as compared with that of the non-transformant (Fig. 4A). These results suggest to us that the *polβ* gene can be expressed in bacterial cells, and that the polβ enzyme probably joins in the excision repair. On the other hand, the *polA107* mutant carries a point mutation in the 5'→3'*exo* position of the *polI* gene. This enzyme has little 5'→3'exo activity and full 3'→5'exo activity (14). In this mutant, the *polβ* gene was non-effective in decreasing the UV sensitivity (Fig. 4B). The *polA1* mutant is an amber mutant which actively synthesizes the 5'→3'exonuclease fragment (15), and is defective in DNA polymerase activity and 3'→5'exo activity. This enzyme has the excision activity of pyrimidine dimers from DNA at a rate of only 10% of that of the intact enzyme *in vitro* (16). In addition, the rate of excision of pyrimidine dimers *in vivo* in this mutant is lower than the wild-type strain (17). The experimental results indicate the possibility of a role of polβ in excision repair (Fig. 4C). In the presence of *polβ* , pyrimidine dimers may be easily removed from DNA, because *polA1* mutant still has 5'→3'exo activity. A *resA1* mutant lacks all of these enzymatic activities (18, 19). Even when the *polβ* gene was inserted into this mutant, the UV sensitivity was not changed (Fig. 4D). All of these results suggest to us that the limited excision of pyrimidine dimers is due to the 5'→3'exo, and that polβ requires the 5'→3'exo activity

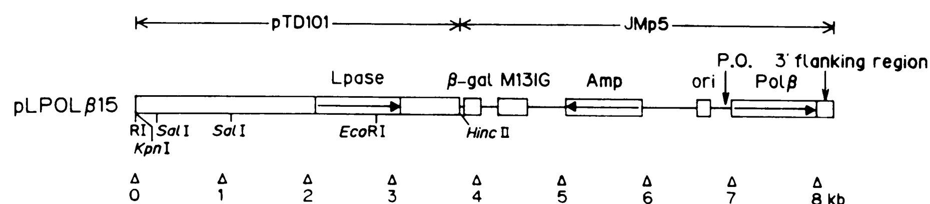

Fig. 1. Physical maps of pLPOL 15 opened at *Eco*RI site.

to play a part in excision repair. In *uvrA6* strain defective in incision step, the UV sensitivity of the transformant was almost the same as that of the non-transformant even in the presence of IPTG (data not shown).

In excision repair in *E. coli*, one possible mechanism of the incision step is due to the direct endonucleotic cleavage of the phophodiester backbone at both sides of pyrimidine dimers with 12−13 nucleotide-long by UV-specific endonuclease activity of UvrABC complex (20). At that position, the single strand gap appears after the removal of pyrimidine dimers. Though the excision gap has been currently understood to be filled only by polβ activity of polI, the 5′→3′exo must play an important role in excision repair, on the grounds that the mutants themselves (non-transformants) deficient in the exo were more sensitive to UV than the wild-type strain, and that the *polβ* gene in the transformants approximately halved their sensitivity to UV only in the presence of the 5′→3′exo activity. Therefore, the excision repair gap may be enlarged by the 5′→3′exo activity and, then the enlarged gap is presumably subjected to long-patch excision repair (21). As shown in the present paper, polβ instead of polI also must play a significant role to seal the gap by synthesis of complementary nucleotide sequences in the bacterial cells, since 3′-OH produced by UvrABC is throughly supplied to polI or polβ as the primer. When the cleavage on damaged DNA molecules does not occur because of defect in UV specific endonuclease, the *polβ* gene is supported to possess hardly any role in excision repair in *E. coli* cells. In conclusion, we assume that the *polβ* gene can play a part in excision repair and the 5′→3′exo is necessary for nucleotide polymerization of repair synthesis in eukaryote. These assumptions are not in conflict with reports that polβ could form a special complex at a 1:1 stoichiometry with DNase V (12KD) carrying both 3′→5′exo and 5′→3′exo activities, which has been proposed to act in excision repair in cooperation with polβ in animal cells (22, 23).

The gene product of *polβ* was also apparently shown to not fully complement to polI, when the UV sensitivity of the transformant of *polA1* was compared with that of the non-transformant of the wild-type strain (Fig. 4A and 4C). Therefore, we speculate that characteristics of polβ in repair synthesis are not completely similar to those of polI, or that polβ may require the help of other factors such as polα for excision repair synthesis (4, 5). Otherwise the complemental efficiency by *polβ* gene may depend on the yield of polβ, because the efficiency of polβ production in the transformant of *polA1* was quite low.

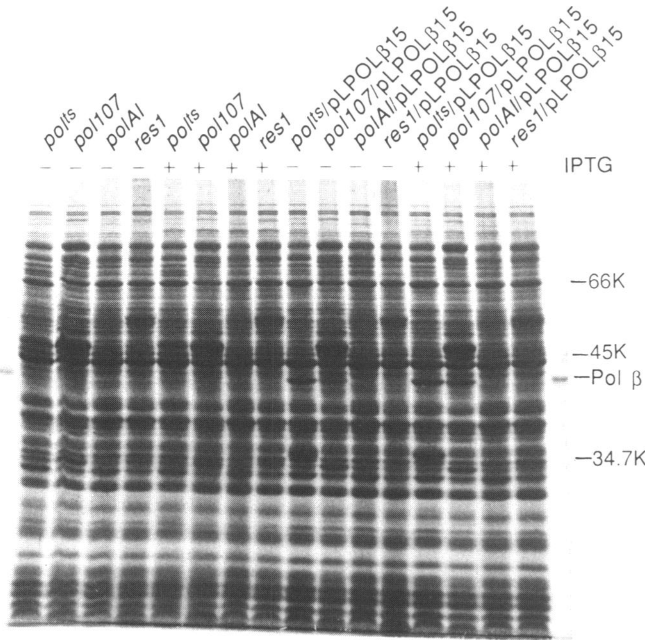

Fig. 2. Gene expression of *polβ* in bacterial cells. The lanes on right and left sides are polβ as makers. The other lanes are indicated at top. IPTG is absent (−) or present (+) in culture medium.

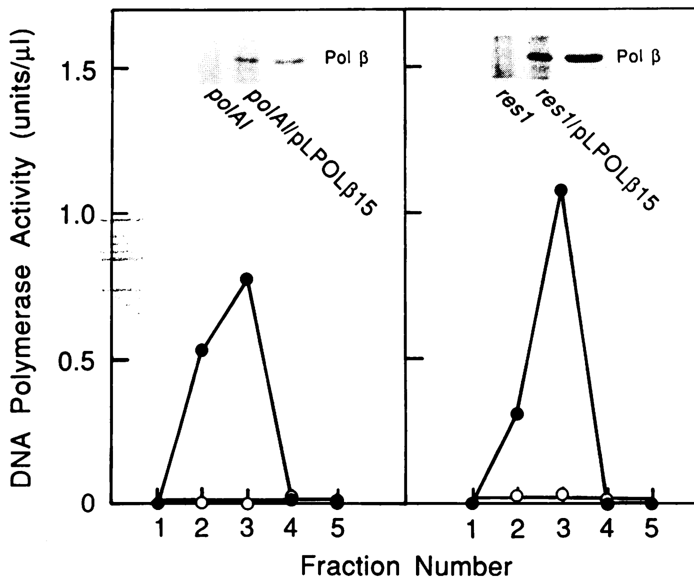

Fig. 3. Purification of polβ from *polA1* and *resA1*. Non-transformants (○) and transformants (●) were cultured with IPTG. Enzy mic activities of each fraction (2 ml) eluted from a DNA-cellulose column were measured. The samples of No. 2 and No. 3 fractions were analyzed by SDS-PAGE gel (inserted figure). Right column is marker enzyme of purified polβ.

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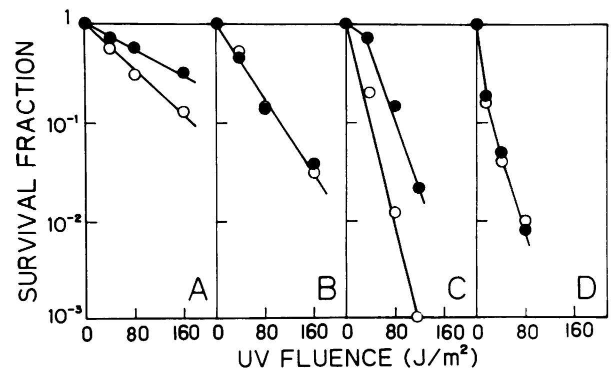

Fig. 4. UV sensitivities of transformants and non-transformants of *polI* mutants. ○, non-transformants; ●, transformants. A, *pol^ts* (*pol*$^+$ and *exo*$^+$ at 30°C, wild-type for repair); B, *polA107* (*pol*$^+$, 5′→3′*exo*$^-$ and 3′→5′*exo*$^+$); C, *polA1* (*pol*$^-$, 5′→3′*exo*$^+$ and 3′→5′*exo*$^-$); D, *resA1* (*pol*$^-$, 5′→3′*exo*$^-$ and 3′→5′*exo*$^-$).

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