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# Role of DNA Polymerase I in Postreplication Repair: a Reexamination with *Escherichia coli* ΔpolA

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**Using strains of *Escherichia coli* K-12 that are deleted for the *polA* gene, we have reexamined the role of DNA polymerase I (encoded by *polA*) in postreplication repair after UV irradiation. The *polA* deletion (in contrast to the *polA1* mutation) made *uvrA* cells very sensitive to UV radiation; the UV radiation sensitivity of a *uvrA* Δ*polA* strain was about the same as that of a *uvrA recF* strain, a strain known to be grossly deficient in postreplication repair. The Δ*polA* mutation interacted synergistically with a *recF* mutation in UV radiation sensitization, suggesting that the *polA* gene functions in pathways of postreplication repair that are largely independent of the *recF* gene. When compared to a *uvrA* strain, a *uvrA* Δ*polA* strain was deficient in the repair of DNA daughter strand gaps, but not as deficient as a *uvrA recF* strain. Introduction of the Δ*polA* mutation into *uvrA recF* cells made them deficient in the repair of DNA double-strand breaks after UV irradiation. The UV radiation sensitivity of a *uvrA polA546*(Ts) strain (defective in the 5′→3′ exonuclease of DNA polymerase I) determined at the restrictive temperature was very close to that of a *uvrA* Δ*polA* strain. These results suggest a major role for the 5′→3′ exonuclease activity of DNA polymerase I in postreplication repair, in the repair of both DNA daughter strand gaps and double-strand breaks.**

Postreplication repair in UV-irradiated cells requires a functional *recA*′ gene and is the major dark repair system operating in excision repair-deficient strains (e.g., *uvrA*) of *Escherichia coli* (24). Recent studies indicate the existence of three pathways of postreplication repair (18, 26): for the repair of DNA daughter strand gaps (DSG), one pathway requires a functional *recF* gene (6, 10, 17, 27) and one pathway does not (22, 27); the repair of DNA double-strand breaks (DSB) that arise at unrepaired DSG requires a functional *recB* gene (27).

Although the precise mechanisms for the postreplicational repair of DNA DSG and DNA DSB are largely unknown, models for the recombinational repair of DSG and DSB typically presume a role for DNA polymerase (5). *E. coli* possesses three DNA polymerases, polymerase I, polymerase II, and polymerase III. We are interested in the role of DNA polymerase I, an enzyme possessing three functions: a 3′→5′ exonuclease, a polymerizing activity, and a 5′→3′ exonuclease. Most workers who have studied the role of DNA polymerase I in postreplication repair have used *polA1* (amber mutation) strains, which are known to contain about 1% polymerizing activity and almost normal levels of 5′→3′ exonuclease activity (12). Monk et al. (16) observed that a *uvrA polA1* strain is only slightly more sensitive to UV radiation than a *uvrA* strain and concluded that DNA polymerase I is involved in excision repair, but not in postreplication repair. Sedgwick and Bridges (20) and Tait et al. (25) demonstrated that UV-irradiated *E. coli* deficient in either DNA polymerase I or DNA polymerase III can efficiently and completely carry out the repair of DNA DSG. These results were confirmed by Johnson (8). However, Barfknecht and Smith (2) did observe a small effect of the *polA1* mutation on the repair of DNA DSG. These data suggest three possibilities. (i) The residual activities in *polA1* strains may be sufficient to carry out most of postreplication repair. (ii) The 5′→3′ exonuclease activity of polymerase I may be essential for postreplication repair. If true, it would explain why earlier workers, using the *polA1* strain, could not observe a major deficiency in postreplication repair. (iii) DNA polymerase II or polymerase III or both either normally fulfill the putative polymerizing step in postreplication repair or at least can partially substitute for polymerase I deficiency.

To critically test for a role of DNA polymerase I in postreplication repair, we have used Δ*polA* strains, which are deficient in all of the activities associated with DNA polymerase I, and observed, contrary to earlier observations, that DNA polymerase I plays a major role in postreplication repair (in the repair of both DNA DSG and DSB). In fact, it is the 5′→3′ exonuclease activity of DNA polymerase I that is important for carrying out postreplication repair.

## MATERIALS AND METHODS

**Bacterial strains.** The strains of *E. coli* K-12 used in this study are listed in Table 1. Strain SR1672 (CJ261), deleted for the *polA* gene, was obtained from C. M. Joyce; its construction has been described before (9). Transduction was carried out by the method described by Miller (14). The strains used were not lysogenic for bacteriophage P1.

**Growth media and culture conditions.** The salts buffer (DTM) has been described previously (22). The minimal growth medium (MM) was DTM supplemented with 0.4% glucose, 0.5 μg of thiamine hydrochloride per ml, 1 μg of biotin per ml, and, when required, each amino acid at 1 mM. Minimal plating medium was MM solidified with 1.6% Noble agar (Difco Laboratories). Phosphate buffer was $Na_2HPO_4$ (5.83 g/liter) and $KH_2PO_4$ (3.53 g/liter), pH 7.0.

Logarithmic-phase cultures were obtained by diluting (50-fold) an MM-grown fresh overnight culture into MM and shaking the culture at 37°C (or 30°C for temperature-

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TABLE 1. Strains of *E. coli* K-12[a]

| Strain | Relevant genotype | Other characteristics | Source or derivation |
|---|---|---|---|
| SR47 | *recA56* | Hfr *ilv-318 thr-300 thi-1 rel-1 rpsE* | J. Foulds (strain JC5088) |
| SR144 | *polA1* | *thyA36 deo(C2?) lacZ53 rha-5 rpsL151* | J. Gross (strain JG138) |
| SR1159 | *recB21* | *argE3 hisG4 leuB6* Δ(*gpt-proA*)*62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rfbD1 mgl-51 kdgk51 rpsL31 supE44 rac* | This laboratory |
| SR1242 | Wild type | *metE*::Tn*5 thyA* | C. M. Berg (strain CBK040) |
| SR1257 | Wild type | *cys517*::Tn*5* | A. Iida (strain TH517) |
| SR1367 | *recF332*::Tn*3* | Same as SR1159, except *tnaA*::Tn*10* HK19$^r$ φX174$^s$ S13$^s$ | A. J. Clark (strain JC10990) |
| SR1601 | *uvrA*::Tn*10* | | B. A. Bridges (strain SY55) |
| SR1672 | Δ*polA* | Δ(*gal-bio*) *thi-1 relA1 spoT1* Km$^r$ | C. M. Joyce (strain CJ261) |
| SR1728 | *uvrA*::Tn*10* Δ*polA* | Same as SR1672 | SR1672 × P1 *vir*a.SR1601, select Tc$^r$ |
| SR1758 | Wild type | Δ(*gal-bio*) *thi-1 relA1 spoT1* | C. M. Joyce (strain CM4722) |
| SR1759 | *uvrA*::Tn*10* | Same as SR1758 | SR1758 × P1 *vir*a.SR1601, select Tc$^r$ |
| SR1760 | *uvrA*::Tn*10 recF332*::Tn*3* | Same as SR1758 | SR1759 × P1 *vir*a.SR1367, select Ap$^r$ |
| SR1761 | *uvrA*::Tn*10 recF332*::Tn*3* Δ*polA* | Same as SR1672 | SR1728 × P1 *vir*a.SR1367, select Ap$^r$ |
| SR1763 | Wild type | Same as SR1758, except *metE*::Tn*5* | SR1758 × P1 *vir*a.SR1242, select Km$^r$ |
| SR1784 | *polA546*(Ts) | *trpA33* IN(*rrnD-rrnE*)*1* | *E. coli* Genetic Stock Center (strain RS5065) |
| SR1794 | *uvrA*::Tn*10* | Same as SR1758, except *cys-517*::Tn*5* | SR1759 × P1 *vir*a.SR1257, select Km$^r$ |
| SR1796 | *uvrA*::Tn*10 recA56* | Same as SR1758 | SR1794 × P1::Tn*9*cts.SR47, select Cys$^+$ |
| SR1840 | *uvrA*::Tn*10* | Same as SR1758, except *thyA* | SR1759, spontaneous Tmp$^r$ selection |
| SR1841 | *uvrA*::Tn*10 recF332*::Tn*3* | Same as SR1758, except *thyA* | SR1760, spontaneous Tmp$^r$ selection |
| SR1843 | *uvrA*::Tn*10 recB21* | Same as SR1758 | SR1840 × P1 *vir*a.SR1159, select Thy$^+$ |
| SR1845 | *uvrA*::Tn*10 recF332*::Tn*3 recB21* | Same as SR1758 | SR1841 × P1 *vir*a.SR1159, select Thy$^+$ |
| SR1861 | *polA1* | Same as SR1758 | SR1763 × P1::Tn*9*cts.SR144, select Met$^+$ |
| SR1863 | *uvrA*::Tn*10 polA1* | Same as SR1758 | SR1861 × P1 *vir*a.SR1601, select Tc$^r$ |
| SR1976 | *polA546*(Ts) | Same as SR1758 | SR1763 × P1::Tn*9*cts.SR1784, select Met$^+$ |
| SR1978 | *uvrA*::Tn*10 polA546*(Ts) | Same as SR1758 | SR1976 × P1 *vir*a.SR1601, select Tc$^r$ |

[a] Genotype symbols are those used by Bachmann (1). All strains are λ$^-$. Strains SR144, SR1159, SR1242, SR1257, SR1367, SR1601, and SR1784 are F$^-$. All othr strains are F$^+$ (or Hfr, if noted). Thy$^+$, Cys$^+$, and Met$^+$ mean that cells no longer require thymine, cysteine, and methionine, respectively. Ap$^r$, Tc$^r$, Km$^r$, and Tmp$^r$ mean that cells became resistant to ampicillin, tetracycline, kanamycin, and trimethoprim, respectively.

sensitive strains) until an optical density at 650 nm of 0.4 (Zeiss PMQ II spectrophotometer) was obtained. The cultures were diluted with MM to an optical density at 650 nm of 0.1 and UV irradiated while in MM.

**UV irradiation and viability measurements.** The source and method for UV irradiation (254 nm) have been described before (21). For survival determinations, irradiated and nonirradiated cell suspensions were diluted in phosphate buffer and plated on MM. Incubation was for 2 to 3 days at 37°C (or 30°C for temperature-sensitive strains). All experiments were done under gold fluorescent lights to prevent photoreactivation.

**Measurement of DSG repair.** The method used for alkaline sucrose gradient sedimentation and determination of DSG repair was similar to that previously described (21), except that the MM-grown cells were irradiated while in MM and were shaken at 37°C for 120 min to allow the completion of repair.

**Measurement of DSB repair.** Cells were grown and irradiated as described for alkaline sucrose gradients. The procedure described earlier for neutral sucrose gradients (4, 23) gave unsatisfactory results for the strains used here. Most of the DNA from irradiated and nonirradiated cells sedimented to the bottom of the tubes. The following modifications gave reproducible DNA sedimentation profiles.

The earlier protocol (4, 23) was modified to include an RNase treatment. Before use, RNase (Worthington Diagnostics) was dissolved in 0.07 M Tris–0.017 M EDTA (pH 7.6), heated at 60°C for 60 min, and stored at 4°C. After UV irradiation and repair incubation, 0.2 ml of the cell suspension was mixed with 0.3 ml of ice-cold spheroplasting solution containing 160 μg (final concentration) of lysozyme (Sigma Chemical Co.) and 100 μg (final concentration) of RNase per ml. After 15 min on ice, a 0.1-ml sample was layered on top of each gradient (4.8 ml of 5 to 20% sucrose containing 0.1% [vol/vol] Triton X-100 capped with 0.1 ml of 5% sucrose solution containing RNase at 100 μg/ml [final concentration]). The layered gradients were held for at least 120 min in the dark at room temperature and then were centrifuged at 3,700 rpm for 40 h at 20°C in an SW50.1 rotor in a Beckman L5-50 ultracentrifuge.

## RESULTS

**Survival after UV irradiation.** To investigate the role of DNA polymerase I in postreplication repair, we have constructed *uvrA* Δ*polA* and other relevant strains of *E. coli* K-12 (Table 1) and tested them for sensitivity to UV radiation. The UV radiation survival curves are shown in Fig. 1, and the data are summarized in Table 2. The presence of the Δ*polA* mutation sensitized *uvrA* cells ~eightfold to UV radiation, which is somewhat greater than that produced by a *recF* mutation (~sixfold) (Table 2). The *recF* gene is known to play a major role in postreplication repair (6, 10, 17, 27). Consistent with earlier observations (2, 16), the presence of a *polA1* mutation sensitized *uvrA* cells ~twofold to UV radiation; however, our data for the *uvrA* Δ*polA* strain suggest a major role of DNA polymerase I in postreplication repair.

To investigate which pathway(s) of postreplication repair DNA polymerase I is involved in, we first constructed a *uvrA recF* Δ*polA* strain. Analysis of the UV radiation

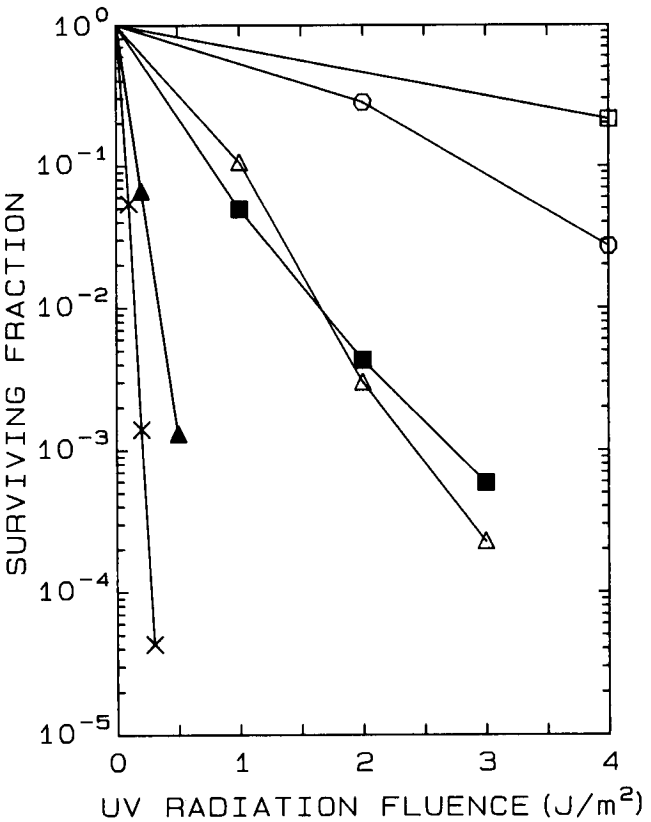


FIG. 1. UV radiation survival curves of various DNA repair-deficient strains of *E. coli*. Cells were grown to logarithmic phase in MM, UV irradiated (254 nm) in MM, and plated on MM. Symbols: Strain SR1759 (*uvrA*::Tn*10*) (□); strain SR1728 (*uvrA*::Tn*10* Δ*polA*) (■); strain SR1760 (*uvrA*::Tn*10 recF332*::Tn*3*) (△); strain SR1761 (*uvrA*::Tn*10 recF332*::Tn*3* Δ*polA*) (▲); strain SR1796 (*uvrA*::Tn*10 recA56*) (×); strain SR1863 (*uvrA*::Tn*10 polA1*) (○). Results are the average of data from two experiments.

survival data (Fig. 1) by the method of Wang and Smith (26) indicated that the Δ*polA* mutation interacts synergistically with the *recF* mutation (data not shown), suggesting that DNA polymerase I is not required for the *recF*-dependent pathway of postreplication repair and that the *polA*-dependent and *recF*-dependent pathways compete for the repair of DSG. To rule out the strain background effect, we also constructed strains *uvrA recB21* and *uvrA recB21 recF* (Table 1); consistent with earlier observations (18, 26), the

TABLE 2. Effect of a Δ*polA* mutation on UV radiation sensitivity of *uvrA*::Tn*10* strains of *E. coli* K-12

| Strain no. | Relevant genotype | $F_{10}$ (J/m²)[a] | Relative UV radiation sensitivity[b] |
|---|---|---|---|
| SR1759 | *uvrA*::Tn*10* | 5.80 | 1.0 |
| SR1728 | *uvrA*::Tn*10* Δ*polA* | 0.77 | 7.5 |
| SR1760 | *uvrA*::Tn*10 recF332*::Tn*3* | 1.01 | 5.7 |
| SR1761 | *uvrA*::Tn*10 recF332*::Tn*3* Δ*polA* | 0.17 | 34.1 |
| SR1796 | *uvrA*::Tn*10 recA56* | 0.07 | 82.9 |
| SR1863 | *uvrA*::Tn*10 polA1* | 2.97 | 2.0 |

[a] The $F_{10}$ is the UV radiation fluence required to inactivate 90% of the cell population.

[b] Calculated by dividing the $F_{10}$ value for the *uvrA*::Tn*10* strain by that for the appropriate mutant.

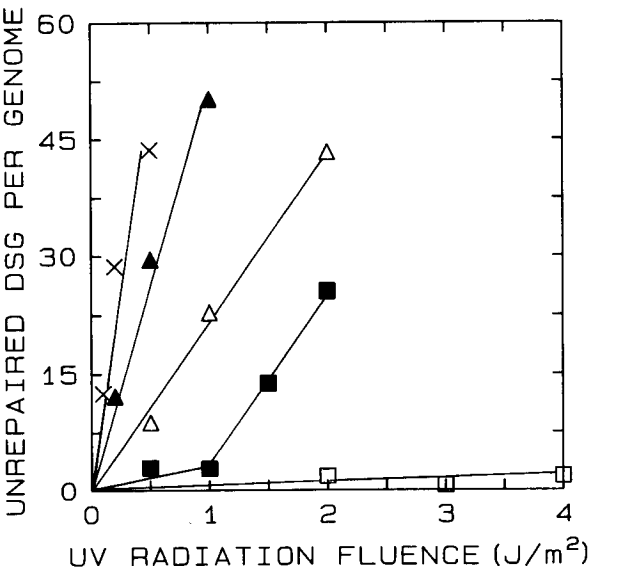


FIG. 2. Yield of unrepaired DNA DSG in the DNA of various DNA repair-deficient strains of *E. coli*. Cells were pulse-labeled with [³H]thymidine at 50 μCi/ml for 10 min in MM and shaken in MM for 120 min at 37°C before being assayed for the number of unrepaired DNA DSG as described in Materials and Methods. Symbols: Strain SR1759 (*uvrA*::Tn*10*) (□); strain SR1728 (*uvrA*::Tn*10* Δ*polA*) (■); strain SR1760 (*uvrA*::Tn*10 recF332*::Tn*3*) (△); strain SR1761 (*uvrA*::Tn*10 recF332*::Tn*3* Δ*polA*) (▲); strain SR1796 (*uvrA*::Tn*10 recA56*) (×). Results are the average of data from two experiments.

*recF* mutation was found to interact synergistically with the *recB* mutation in terms of UV radiation survival. The UV radiation survival of the *uvrA recF recB* strain was very similar to that for the *uvrA recF* Δ*polA* strain (data not shown).

**Role of DNA polymerase I in repair of DNA DSG.** To observe the role of DNA polymerase I in the repair of DNA DSG, we measured the yield of unrepaired DNA DSG following UV irradiation and 120 min of repair incubation in MM. (Note: Repair kinetics experiments showed that 120 min of postirradiation incubation is sufficient to observe the maximal repair of DNA DSG in *uvrA* Δ*polA* cells [data not shown].) When compared to *uvrA* cells, *uvrA* Δ*polA* cells were deficient in the repair of DNA DSG, but not as deficient as *uvrA recF* cells (Fig. 2). Furthermore, the presence of a Δ*polA* mutation in a *uvrA recF* strain caused an additional deficiency in the repair of DNA DSG; however, when compared to a *uvrA recA* strain, which is completely deficient in the repair of DNA DSG (24), the *uvrA recF* Δ*polA* strain was able to perform some repair of DNA DSG (Fig. 2). These data suggest that there exists a pathway for the repair of DNA DSG that does not require functional *recF* or *polA* genes. This pathway cannot be due to the leakiness of these mutations, as the strains used here have a Tn*3* insertion in the beginning of the *recF* gene (3) and are deleted for the *polA* gene; they therefore should show null phenotypes for these genes. Our earlier study demonstrated the existence of a pathway for the repair of DSG that does not require functional *recF*, *recB*, and *recC* genes (22). However, we could not thoroughly study the role of *polA* in this pathway as a mutation in the *polA* gene is incompatible with *recA* and *recB* mutations (7, 13, 15).

**Role of DNA polymerase I in repair of DNA DSB.** Since the *uvrA* Δ*polA* strain is as UV radiation sensitive as the *uvrA*

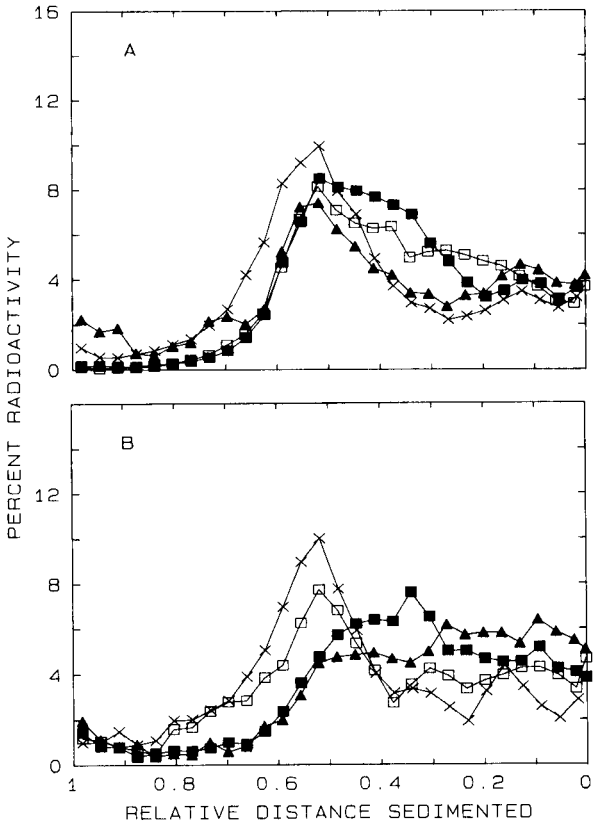


FIG. 3. Effect of a Δ*polA* mutation on formation and repair of DNA DSB in UV-irradiated *uvrA*::Tn*10* *recF332*::Tn*3* cells. UV-irradiated (0.25 J/m$^2$) cells were pulse-labeled with [$^3$H]thymidine for 10 min in MM and shaken in MM for various times at 37°C before loading onto neutral sucrose gradients. (A) Strain SR1760 (*uvrA*::Tn*10* *recF332*::Tn*3*); (B) strain SR1761 (*uvrA*::Tn*10* *recF332*::Tn*3* Δ*polA*). Symbols: Unirradiated controls (×); UV-irradiated cells after 0 (□), 2 (■), and 4 (▲) h. The data are from a representative experiment. Strain SR1760 showed no DNA degradation during the 4-h incubation; strain SR1761 degraded 24% of its DNA.

*recF* strain (Fig. 1), but is less deficient in the repair of DNA DSG (Fig. 2), the *uvrA* Δ*polA* strain may also be deficient in the repair of DNA DSB. DNA DSB have been shown to be repaired in *uvrB recF* cells, but not in *uvrB recF recB* cells, suggesting that the *recB* gene plays a major role in the repair of DNA DSB (27). To investigate the role of DNA polymerase I in the repair of DNA DSB, we determined the effect of a Δ*polA* mutation on the formation and repair of DNA DSB in UV-irradiated *uvrA recF* cells. Consistent with an earlier observation (27), DNA DSB were produced and repaired in UV-irradiated (0.25 J/m$^2$) *uvrA recF* cells (Fig. 3A). However, no repair of DNA DSB was observed in UV-irradiated (0.25 J/m$^2$) *uvrA recF* Δ*polA* cells (Fig. 3B). These results suggest that DNA polymerase I is required for the repair of DNA DSB after UV irradiation.

**Which activity of DNA polymerase I is required for postreplication repair?** DNA polymerase I from *polA1* mutants is deficient in polymerizing activity, but it has almost a normal level of 5′→3′ exonuclease activity (12). Therefore, we hypothesized that the 5′→3′ exonuclease activity of DNA polymerase I may be required for efficient postreplication repair. Consistent with this hypothesis, UV-irradiated *uvrA polA546*(Ts) cells (defective in 5′→3′ exonuclease activity [11]) showed much less survival when plated at 42°C than when plated at 30°C; however, they were still somewhat less UV radiation sensitive than the cells of a *uvrA* Δ*polA* strain (Fig. 4) but much more UV radiation sensitive than the cells of a *uvrA polA1* strain (Fig. 1).

**Role of DNA polymerase I in the CAP-inhibitable pathway of postreplication repair.** A small portion of postreplication repair is known to be inhibited by chloramphenicol (CAP) (19, 28). To find out if the *polA* gene plays a role in the CAP-inhibitable pathway of postreplication repair, the UV-irradiated *uvrA* and *uvrA* Δ*polA* cells were treated with CAP (50 μg/ml) for 2 h prior to plating. Consistent with earlier observations for *polA1* (2), the UV survival data (not shown) indicate that the CAP treatment had only a small, and about the same, effect on both strains, suggesting no significant role of DNA polymerase I in the CAP-inhibitable pathway of postreplication repair.

## DISCUSSION

Using strains deleted in the *polA* gene (9), we have reinvestigated the role of DNA polymerase I in postreplication repair and observed, contrary to earlier observations with the *polA1* mutant, that DNA polymerase I plays a major role in postreplication repair. The *uvrA* Δ*polA* strain was ~eightfold more UV radiation sensitive than the *uvrA* strain (Fig. 1, Table 2). This result is in marked contrast to the *uvrA polA1* strain, which is only ~twofold more sensitive than the *uvrA* strain (Fig. 1, Table 2). Our *uvrA polA1* survival data are consistent with the earlier observations of Monk et al. (16) and Barfknecht and Smith (2), who observed only a

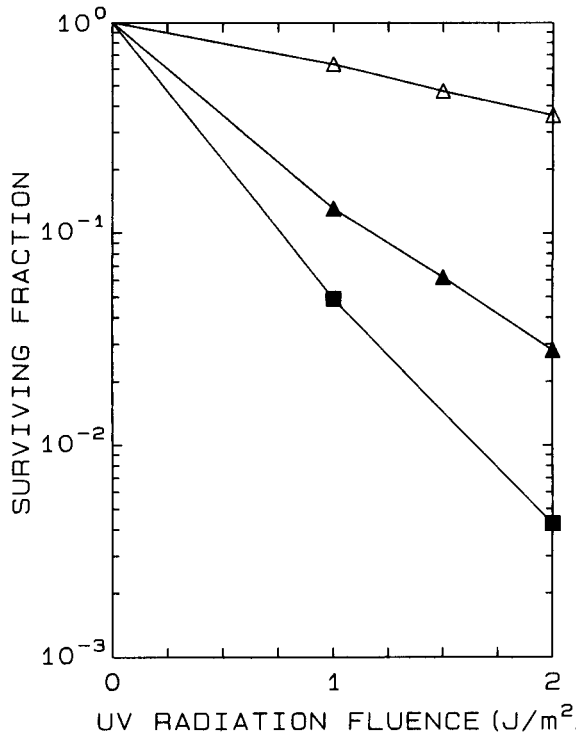


FIG. 4. UV radiation survival curves at 30 and 42°C for the *uvrA*::Tn*10* *polA546*(Ts) strain (SR1978) of *E. coli*. Cells were grown at 30°C to logarithmic phase in MM, UV irradiated in MM, and immediately plated on MM at 42°C (▲) or 30°C (△). Plates were prewarmed to 42°C and, after the cells were spread, were returned immediately to 42°C. The data for *uvrA*::Tn*10* Δ*polA* (■) are taken from Fig. 1 and are shown here for comparison. Results are the average of data from two independent experiments.

small effect of the *polA1* mutation on survival and concluded that DNA polymerase I plays only a small role in postreplication repair.

DNA polymerase I from the *polA1* strain is known to contain a small amount of polymerizing activity and almost the normal level of 5′→3′ exonuclease activity (12). The data obtained with the *polA546*(Ts) mutant defective in 5′→3′ exonuclease activity (Fig. 4) suggest that it is the 5′→3′ exonuclease activity of DNA polymerase I that is important for carrying out postreplication repair and also explains why earlier workers (2, 8, 20, 25) who used *polA1* strains did not observe a major role for DNA polymerase I in postreplication repair. To our knowledge, this is the first report that describes the role of the 5′→3′ exonuclease activity of DNA polymerase I in postreplication repair.

We wanted to know what role the *polA* gene plays in the three pathways of postreplication repair, i.e., the *recF*-dependent and *recF*-independent pathways for the repair of DNA DSG and the *recB* pathway for the repair of DNA DSB that arise at unrepaired DSG. We could not investigate the effect of the Δ*polA* mutation on a *recB* strain, since a mutation in the *polA* gene is incompatible with a *recB* mutation (15). However, our data show that *uvrA recF* Δ*polA* cells are much more UV radiation sensitive than *uvrA recF* cells (Fig. 1), suggesting that the *polA* gene plays little role in the *recF* pathway of postreplication repair. The analysis of these data by the method of Wang and Smith (26) indicated that the Δ*polA* mutation interacts synergistically with the *recF* mutation, further suggesting that DNA polymerase I is not required for the *recF*-dependent pathway of postreplication repair. These results and our results with *polA546* (Fig. 4) suggest that it is the 5′→3′ exonuclease activity of DNA polymerase I that functions in the *recF*-independent pathway of postreplication repair. Furthermore, a *uvrA recF* Δ*polA* strain is not as UV radiation sensitive as a *uvrA recA* strain (Fig. 1), suggesting that there must be yet another pathway of postreplication repair which does not require functional *recF* and *polA* genes.

Our data with Δ*polA* strains suggest that DNA polymerase I is involved in the repair of DNA DSG (Fig. 2); however, it seems to be somewhat less important than the *recF* gene product, especially at lower fluences (i.e., ≤1 J/m$^2$). Earlier workers who found no role for DNA polymerase I in the repair of DSG suggested that DNA polymerase III can efficiently substitute for a deficiency in DNA polymerase I (20, 25). This is not the case, however, at higher fluences (>1 J/m$^2$) where the deficiency in DNA polymerase I is clearly observed (Fig. 2).

Our data also demonstrate that DNA polymerase I is involved in the repair of DNA DSB. Using a similar approach, Wang and Smith (27) concluded that the *recB* gene is required for the repair of DNA DSB. However, our *uvrA recF* Δ*polA* cells are *recB*$^+$, but they were not able to repair DNA DSB (Fig. 3). These results suggest that DNA polymerase I is also essential for the repair of DNA DSB after UV irradiation.

On the basis of our results, we propose the following model for the role of DNA polymerase I in the repair of DNA DSG and DSB (Fig. 5). The gaps are formed in newly synthesized daughter strands of UV-irradiated cells (Fig. 5B). The 3′ end invades the homologous sister duplex (Fig. 5K). DNA repair synthesis primed at the 3′ end of the invading strand will fill the gap, using the sister strand as a template. We suggest that DNA polymerase I is involved in filling this gap. However, we feel that DNA polymerase III can substitute for DNA polymerase I at lower fluences (<1

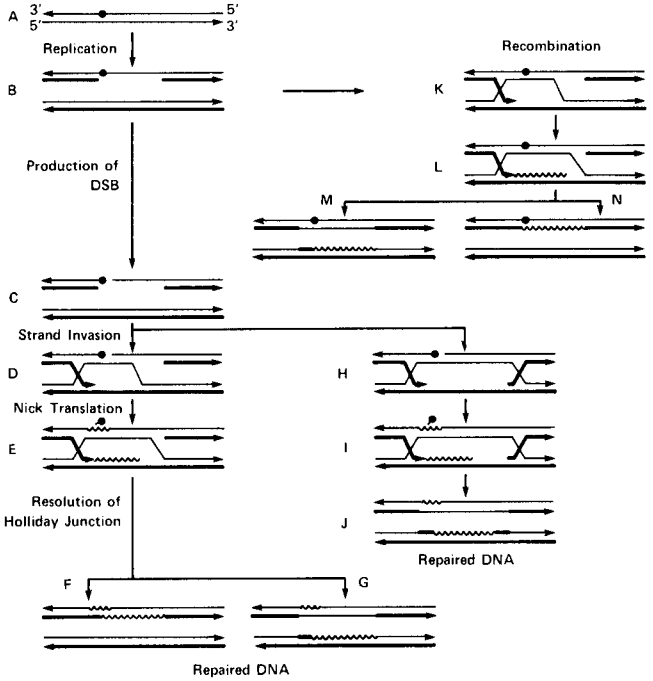


FIG. 5. Model for repair of DNA DSG and DNA DSB involving *E. coli* DNA polymerase I. Replication of chromosomal DNA containing a dimer (A) will result in the formation of a DSG (B) that can be repaired by the following mechanism. The free 3′ end invades the homologous sister duplex (K) and DNA polymerase I fills the gap (L). The repair is completed by either resolution of the Holliday junction (M or N). If not repaired, the DSG (B) can be converted to a DSB (C). The DNA DSB can be repaired by one or both of the following mechanisms. The free 3′ end invades the homologous region of the sister duplex (D), and the dimer is removed by the nick translation activity of DNA polymerase I (E). The repair of DSB is completed by either possible resolution of the Holliday junction (F or G). In the second mechanism, both 3′ and 5′ ends invade the homologous sister duplex (H), the dimer is removed by nick translation, and the gap is filled by repair synthesis (I). Resolution of the Holliday junction will result in the repair of the DSB (J).

J/m$^2$), where only a small amount of polymerase activity is required. At higher fluences (>1 J/m$^2$) DNA polymerase I becomes important for the repair of DSG. This is consistent with the fact that, while DNA polymerase I is present at 400 molecules per cell, only 10 to 20 molecules of DNA polymerase III are present per *E. coli* cell (12). After repair synthesis, the repair of the gap in DNA is completed by either of two possible resolutions of the Holliday junction (Fig. 5M or N).

The presence of lesions (pyrimidine dimers in this case) in the DNA will lead to the formation of DSG (Fig. 5B); if not repaired, these DNA DSG can be converted to DNA DSB by the action of cellular endonucleases (presently unidentified) (Fig. 5C). At this stage, the repair of DSB can be achieved by one or both of the following mechanisms. In the first mechanism, the free 3′ end invades the homologous region of the sister duplex, forming a structure similar to a D loop (Fig. 5D). The nick in the strand with the dimer is a substrate for DNA polymerase I binding, and DNA polymerase I will remove the dimer by its action of nick translation that requires 5′→3′ exonuclease activity (12). DNA polymerase III will not bind to this nicked structure (12). DNA synthesis along with nick translation will result in the removal of the dimer (Fig. 5E). The repair of DSB is completed by either of

the two possible resolutions of the Holliday junction (Fig. 5F or G). In the second mechanism, both the 3′ and 5′ ends of DSB invade the homologous sister duplex (Fig. 5H). The dimer is removed by nick translation as described above. DNA repair synthesis primed at the 3′ end of the invading strand will fill the gap (Fig. 5I), and the resolution of the Holliday junctions will result in repaired DNA (Fig. 5J).

In summary, DNA polymerase I (especially the 5′→3′ exonuclease activity) plays a major role in postreplication repair and is involved in the repair of both DNA DSG and DNA DSB.

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