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The Involvement of DNA Polymerase I in the Postreplication Repair of Ultraviolet Radiation-Induced Damage in *Escherichia coli* K-12

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Summary. A deficiency in DNA polymerase I increased the ultraviolet (UV) radiation sensitivity of a *uvrA* strain of *Escherichia coli* K-12 when plated on minimal growth medium. The slope of the survival curve for the *uvrA polA* strain was 2.0-times greater than that for the *uvrA* strain. The fluence-dependent yield of unrepaired deoxyribonucleic acid (DNA) parental-strand breaks following UV irradiation and incubation in minimal growth medium was similar in both strains. However, the fluence-dependent yield of *unrepaired* DNA daughter-strand gaps observed following UV irradiation was 1.8-fold greater in the *uvrA polA* strain than in the *uvrA* strain. These results suggest that DNA polymerase I is involved in the filling of at least some daughter-strand gaps during postreplication repair. Also, the *uvrA polA* strain was sensitized by a post-UV treatment with chloramphenicol (CAP) to a similar extent as was the *uvrA* strain, indicating that DNA polymerase I is not involved in the CAP-inhibitable pathway of postreplication repair.

Introduction

Monk et al. (1971) demonstrated that a deficiency in deoxyribonucleic acid (DNA) polymerase I increased the ultraviolet (UV) radiation sensitivity of a *uvrA* strain of *Escherichia coli* K-12. Since *uvrA* and *uvrB* strains are unable to excise UV radiation-induced pyrimidine dimers (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964; Howard-Flanders et al., 1966), this suggests that DNA polymerase I may play a role in postreplication repair. However, a mutation in the *polA* gene that codes for DNA polymerase I (Gross and Gross, 1969) did not appear to reduce the ability of a uvr^+ or a *uvrA* strain of *E. coli* to complete the postreplicational repair of DNA daughter-strand gaps (Youngs and Smith, 1973; Sedgwick and Bridges, 1974; Tait et al., 1974), but did appear to reduce the rate of repair (Sedgwick and Bridges, 1974; Sedgwick, 1975a).

Most models for postreplication repair predict that repair resynthesis is required for the filling of gaps in the parental strands that are produced during the recombinational crossover to fill the daughter-strand gaps (Smith, 1971; Howard-Flanders and Rupp, 1972; Howard-Flanders, 1973). Ley (1973) has confirmed this prediction. The repair of parental-strand gaps produced during postreplication repair in *uvrA* and *uvrB* strains must occur very rapidly, since no DNA single-strand breaks were detected in *uvrA* or *uvrB* strains following UV fluences ranging from 40–90 Jm^{-2} (Achey and Billen, 1969; Kato, 1972; Seeberg and Johansen, 1973). Since DNA polymerase I plays a major role in the repair of gaps produced in parental DNA during excision repair (Youngs and Smith, 1973; Youngs et al., 1974), the *polA* gene product may also be involved in the filling of parental-strand gaps produced during post-replication repair, and such breaks might be detectable in a *uvrA polA* strain. Therefore, we have determined the UV survival (i.e., the UV fluence-dependent yield of dead cells), and the UV fluence-dependent yield of unrepaired daughter-strand gaps and of parental-strand breaks was measured after the completion of postreplication repair in a *uvrA* and a *uvrA polA* strain.

The survival results of Monk et al. (1971), and the data presented here, suggest that DNA polymerase I is involved in some step of postreplication repair. Therefore, we wished to determine if the *polA* gene product is involved in the chloramphenicol-inhibitable pathway of postreplication repair (Ganesan and Smith, 1972; Sedgwick, 1975b, Youngs and Smith, 1976a), since it appears that the chloramphenicol sensitive pathway is responsible for UV radiation mutagenesis in *uvrA* and *uvrB* strains (Witkin, 1974; Sedgwick, 1975b).

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Materials and Methods

Bacterial Strains. The bacterial strains employed were JG136 (SR142) *uvrA6 polA1 thyA deo rha lacZ rpsL* F⁻, and JG137 (SR143) *uvrA6 thyA deo rha lacZ rpsL* F⁻, which were obtained from J.D. Gross.

Media and Buffers. The glucose-salts minimal medium (MM) described by Ganesan and Smith (1968) was supplemented with 0.5 μg/ml thiamine·HCl and 10 μg/ml thymine for overnight liquid cultures (or 2 μg/ml thymine for exponential phase cultures). The MM was solidified with 1.6% Difco Noble agar (Van der Schueren et al., 1974) when required for plating.

DTM buffer, which is MM lacking glucose, thymine, and thiamine, was used for washing and resuspending the cell cultures. A 0.067 M phosphate buffer, pH 7.0, was used for the dilution of cells for survival determinations (Ganesan and Smith, 1968).

Growth Conditions. Exponential phase cultures were obtained by diluting overnight stationary phase cultures 50-fold into fresh MM, followed by growth at 37° C until a cell density of $\sim 2 \times 18^8$ cells/ml was reached. Cells were harvested by filtration using sterile Millipore filters (0.45 μm pore size), washed with 0.5 to 1 original volume of 37° C DTM, and resuspended in DTM to a density of 1 to 4×10^8 cells/ml, depending on the experiment.

Labeling and Alkaline Sucrose Gradient Techniques. Labeling of parental-strand DNA was carried out by growing exponential phase cultures in MM supplemented with 100 μCi/ml [methyl-^{3}H]-thymidine (Amersham/Searle, 46 Ci/mmol) with a final thymine concentration of 2 μg/ml. Cells were grown to a density of $\sim 2 \times 10^8$ cells/ml, harvested, washed and resuspended in an equal volume of DTM buffer. Following UV irradiation, 1 ml samples of cells were collected by filtration, washed with 5 ml of MM, resuspended in 1 ml of MM, and incubated with shaking at 37° C for 90 min to allow the completion of postreplication repair. Unirradiated cells were treated in a similar manner.

The DNA of cells was pulse labeled by adding 0.5 ml of cells in DTM at a concentration of 4×10^8 cells/ml to 0.25 ml of MM containing four times the normal concentration of glucose and thiamine, and 0.25 ml of [methyl-^{3}H]-thymidine (250 μCi/ml, 1 μg/ml final concentration of thymine). Irradiated cells were pulse labeled for periods of 15 to 30 min, depending on the UV fluence, in order to equalize the uptake of [methyl-^{3}H]-thymidine between the control (10 min pulse) and irradiated cells (Sedgwick and Bridges, 1974). Following the pulse labeling period, the cells were collected by filtration, washed with 3 ml of MM, resuspended in 1 ml of MM, and incubated at 37° C with shaking for 90 min to allow the completion of postreplication repair.

After incubation, the prelabeled or pulse-labeled cells were transformed into spheroplasts and lysed on alkaline sucrose gradients according to the method of Smith and Meun (1970), except that the final concentration of lysozyme (Worthington) was 100 μg/ml, and the number of cells layered onto the gradients was $\sim 4 \times 10^6$.

Gradients were centrifuged in SW 50.1 rotors at 20° C in either a Beckman L2 or L2-65B ultracentrifuge. Prelabeled DNA samples were centrifuged at 10,000 rpm for 16 h, while pulse-labeled DNA samples were centrifuged at 10,000 or 12,000 rpm for 16 h, depending on the UV fluence given to the cells. Bacteriophage T2 with [2-^{14}C]-thymine-labeled DNA was used in each centrifugation run as a molecular weight marker. The procedures for processing the gradients and calculating the number average molecular weights (M_n) have been described (Hamelin et al., 1976; Youngs and Smith, 1976a, b).

Survival Determination. Cells at a density of $\sim 2 \times 10^8$ cells/ml in DTM buffer were UV irradiated, the appropriate dilutions were prepared in phosphate buffer, and 0.05 or 0.1 ml samples were spread on MM plates. Incubation was at 37° C for 48–60 h.

The effect of post-UV irradiation treatment with chloramphenicol (CAP) was determined by adding 1 ml samples of cells at 2 to 4×10^8 cells/ml to 1 ml of MM containing twice the normal concentration of glucose, thymine, thiamine, and 100 μg/ml CAP (Sigma). The cells were incubated for 90 min at 37° C, and then 1 ml samples were collected by filtration, washed with 3 ml of DTM, and resuspended in 1 ml of DTM. Samples were then diluted and plated. Unirradiated cells were treated in a similar manner.

UV Irradiation. The method, source, and dosimetry of UV irradiation were as described (Smith, 1976). Three ml samples of cells (2 to 4×10^8/ml) suspended in DTM buffer were irradiated in 60 mm Pyrex Petri dishes. When necessary, the fluence rate was reduced by placing wire grids between the source and cell sample. The UV radiation fluence was corrected for sample absorption according to the method of Youngs and Smith (1976a). All experiments were performed under General Electric "gold" fluorescent lights to prevent photoreactivation.

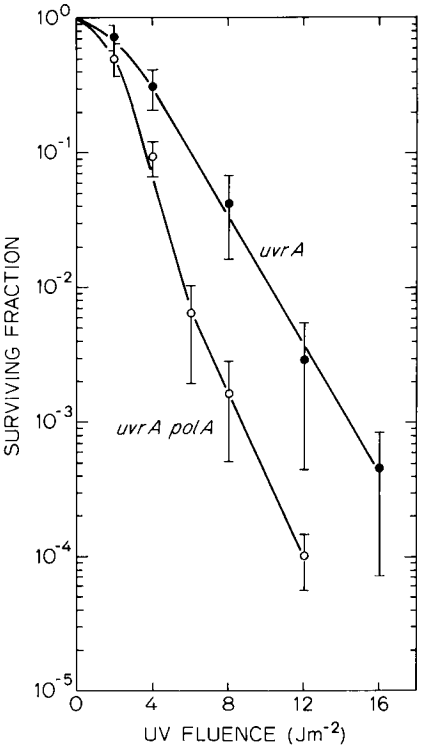

Fig. 1. UV survival of log phase *uvrA polA* and *uvrA* strains of *E. coli* K-12 on minimal growth medium. Symbols are: *uvrA polA* (○), *uvrA* (●). Each point represents the average of at least three experiments. The vertical lines represent the standard deviations

Results

The survival curves of the *uvrA* and *uvrA polA* strains on minimal growth medium are shown in Figure 1. The *uvrA polA* strain was more sensitive to UV irradiation than was the *uvrA* strain. The slope of the *uvrA polA* survival curve (down to 10^{-2} survival) was 2.0 times greater than the slope of the *uvrA* survival

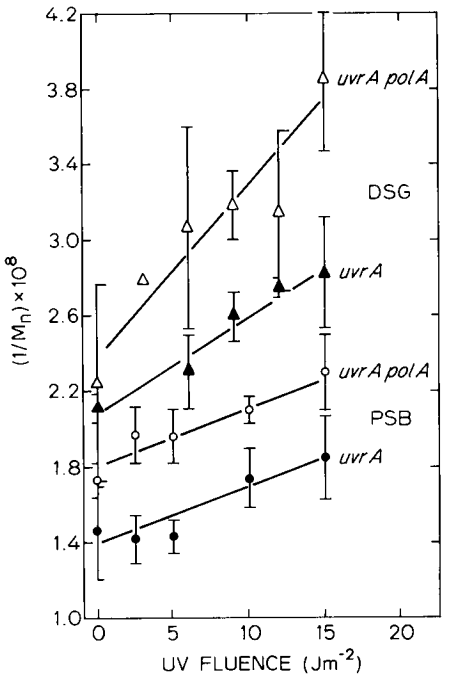

Fig. 2. The yield of unrepaired daughter-strand gaps and parental-strand breaks in the DNA of the *uvrA polA* and *uvrA* strains of *E. coli* K-12 after UV irradiation. Samples were either pulse labeled or prelabeled with [methyl-^{3}H]-thymidine, as described in Materials and Methods, and then incubated in minimal medium for 90 min at 37° C. Number average molecular weights (M_n) were calculated as described in Materials and Methods. Symbols are: *uvrA polA* daughter-strand gaps (DSG) (△), *uvrA* DSG (▲), *uvrA polA* parental-strand breaks (PSB) (○), *uvrA* PSB (●). Each point represents the average of at least three experiments. The vertical lines represent the standard deviations when they are greater than the size of the symbols. The lines were fitted to the data points by linear regression analysis

curve; below a survival of 10^{-2} the slope ratio was 1.3.

Figure 2 shows the yield of unrepaired parental-strand breaks, and unrepaired daughter-strand gaps remaining in the DNA of the *uvrA* and *uvrA polA* strains following UV irradiation and incubation in minimal growth medium. Although there was a difference in the molecular weight of the parental-strand DNA from unirradiated *uvrA polA* and *uvrA* cells, there was little or no difference in the UV radiation fluence-depend yield of *unrepaired* parental-strand breaks between the two strains (i.e., the slopes of the two lines are the same) (Fig. 2).

Only a small difference in the molecular weight of the pulse-labeled DNA was observed between the unirradiated *uvrA polA* and *uvrA* strains, but the UV radiation fluence-dependent yield of *unrepaired* daughter-strand gaps (i.e., the slopes of the lines) was 1.8 times greater for the *uvrA polA* strain than for the *uvrA* (Fig. 2).

To determine if DNA polymerase I plays a role in the CAP-inhibitable pathway of postreplication repair, the *uvrA* and *uvrA polA* strains were treated with 50 μg/ml CAP in MM for 90 min following UV irradiation. The data show that posttreatment with CAP had a small inhibitory effect on the UV survival of both the *uvrA* and *uvrA polA* strains (Fig. 3), and calculation of the dose modification factors at different survival levels demonstrates that the CAP treatment had the same effect on the survival of both strains (Table 1).

Table 1. Dose modification factors for post-UV irradiation treatment with chloramphenicol

Surviving fraction	DMF[a]		Ratio *uvrA polA*/ *uvrA*
	uvrA polA	*uvrA*	
10^{-1}	1.38	1.27	1.09
10^{-2}	1.25	1.26	0.99
10^{-3}	1.21	1.27	0.95

[a] The dose modification factors (DMF) give the amount of sensitization produced by post-UV irradiation treatment with 50 μg/ml chloramphenicol (CAP). Data shown were calculated by determining the difference in UV fluence, at the surviving fractions indicated, for the survival curve of a given strain in the absence and in the presene of CAP

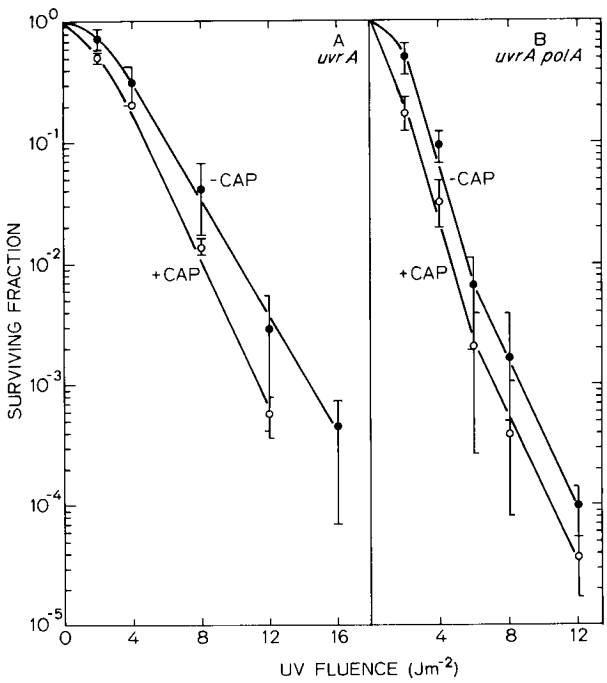

Fig. 3A and B. Effect of post-UV irradiation treatment with 50 μg/ml chloramphenicol (CAP) on the survival of log phase *uvrA polA* and *uvrA* strains of *E. coli* K-12. Cell samples were incubated in minimal medium containing 50 μg/ml CAP at 37° C for 90 min before washing and plating on minimal medium. Symbols are: Panel **A**. *uvrA* no CAP treatment (●), *uvrA* with CAP treatment (○), Panel **B**. *uvrA polA* no CAP treatment (●), *uvrA polA* with CAP treatment (○). Each point represents the average of at least three experiments. The vertical lines represent the standard deviations when they are greater than the size of the symbols

Discussion

The data of Monk et al. (1971) showed that there was a small difference between the UV sensitivities of the *uvrA* and *uvrA polA* strains. However, they used a complex plating medium, which tends to reduce the difference between the survival of these two strains in the *E. coli* K-12 genetic background (Barfknecht and Smith, unpublished data). The effect of complex medium appears to have the reverse effect on *uvrA* and *uvrA polA* strains in the *E. coli* B/r genetic background (Witkin and George, 1973), and constitutes yet another difference in response between the B and K-12 strains (Sedgwick, 1975a).

Figure 1 illustrates that there was a difference in survival between the *uvrA* and the *uvrA polA* strains when plated on minimal growth medium. The slope of the *uvrA polA* survival curve (down to 10^{-2} survival) was 2.0 times greater than the slope of the *uvrA* survival curve. Since *uvrA* and *uvrB* strains rely primarily on postreplication repair to overcome UV radiation-induced damage, our data suggest that DNA polymerase I is involved in some step of this complex repair system.

Previous data have been interpreted to suggest that an absence of DNA polymerase I has no major effect on the final extent of repair of UV radiation-induced DNA daughter-strand gaps (Youngs and Smith, 1973; Sedgwick and Bridges, 1974; Tait et al., 1974; Sedgwick, 1975a). However, the data published on *uvrA polA* strains are consistent with there being a small deficiency in repair due to the *polA*1 mutation. For example, the gradient data of Youngs and Smith (1973) show that there was a small trailing portion of the repaired peak of DNA from the *uvrA polA* strain, (60 min of incubation after 6.3 Jm^{-2}) which is due to low molecular weight DNA. These date could be interpreted to suggest that repair was not totally complete. In addition, the results in Figure 5 of Sedgwick (1975a) show that, after maximum postreplication repair (120 min of incubation) following 5 and 10 Jm^{-2} (254 nm), about twice as many breaks remain unrepaired in a *uvrA polA* strain compared to a *uvrA* strain.

After completion of postreplication repair (90 min at 37° C after pulse labeling), our results show that the slopes of the lines for the UV radiation fluence-dependent yield of unrepaired daughter-strand gaps was 1.8-fold greater in the *uvrA polA* strain compared to the *uvrA* strain (Fig. 2), which is only slightly less than the ratio of the slopes of the survival curves for these strains (i.e., 2.0), as shown in Fig. 1.

There was little difference in the UV radiation fluence-dependent yield of unrepaired parental-strand breaks between the *uvrA* and *uvrApolA* strains (i.e., the slopes of the lines in Fig. 2 are the same). At UV fluences greater than about 20 Jm^{-2}, however, there was a larger number of unrepaired parental-strand breaks in the *uvrA polA* strain compared to the *uvrA* strain (data not shown). These results confirm the data of Youngs and Smith (1976b).

The lower molecular weight (i.e., higher $1/M_n$ values) for the prelabeled and pulse-labeled unirradiated *uvrA polA* cells compared with the *uvrA* cells in Figure 2 is consistent with the observation that Okazaki fragments are joined more slowly by *polA* strains (Okazaki et al., 1971). It is more than just a rate problem, however. In experiments designed to determine of there are differences in the molecular weights of the DNA from unirradiated cells of different repair deficient strains (D.A. Youngs, unpublished observations), cells were prelabeled with ^{3}H-thymidine during logarithmic growth, and either resuspended in buffer for 10 min before lysing on the gradients, or were incubated in non-radioactive growth medium for 60 min at 37° C before lysing the cells on the gradients. Under both conditions, the molecular weight of the DNA from the *polA* cells was lower than the wild-type, *lexA*, or *recA* cells. The average $1/M_n \times 10^8$ values (2 gradients per experiment and 2 experiments in buffer) were 2.13, 1.48, 1.52, 1.62 in buffer, and 2.59, 1,57, 1.80, 1.46 in medium for the W3110 strains *polA*1, wild-type, *lexA*101 and *recA*56, respectively. These data are consistent with the higher value for $1/M_n$ shown in Figure 2 for the $polA^-$ strain vs the $polA^+$ strain.

Since the *uvrA polA* strain is no more deficient than the *uvrA* strain in the repair of parental-strand breaks over the UV fluence range used for the survival studies, but is about 1.8-fold less efficient in the repair of daughter-strand gaps, it suggests that the reduced ability of the *uvrA polA* strain to close daughter-strand gaps during postreplication repair is the major reason for the reduced survival of this strain compared to the *uvrA* strain. However, the role that DNA polymerase I plays in postreplication repair is small since the *polA* mutation only sensitizes the *uvrA* strain ~2-fold to UV radiation. For comparison, a *recA* mutation that blocks postreplication repair sensitizes a *uvrB* strain ~25-fold to UV radiation (Youngs and Smith, 1976a).

One minor pathway of postreplication repair is the CAP-inhibitable pathway (Sedgwick, 1975b; Youngs and Smith, 1976a). Our data presented in Figure 3 and Table 1 show that posttreatment with 50 μg/ml CAP had a similar effect on the UV survival of both the *uvrA* and *uvrA polA* strains. This suggests that DNA polymerase I is not involved in the CAP-inhibitable pathway of postreplication repair.

In summary, a *polA* mutation sensitizes a *uvrA*

strain ~2-fold to UV radiation-induced killing, and reduces the efficiency of the repair of daughter-strand gaps ~1.8-fold. The *polA*$^+$ gene does not appear to play a role in the chloramphenicol-inhibitable pathway of postreplication repair.

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