JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 175-182

Vol. 116, No. 1
Printed in U.S.A.

Evidence for the Control by *exrA* and *polA* Genes of Two Branches of the *uvr* Gene-Dependent Excision Repair Pathway in *Escherichia coli* K-12

DAVID A. YOUNGS AND KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

Received for publication 2 April 1973

A *polA1 exrA* strain of *Escherichia coli* K-12 was found to be more sensitive to ultraviolet radiation than the closely related *polA1* or *exrA* strains, but not as sensitive as either the *exrA uvrB* or *recA* strains. The *exrA* and *polA1* mutations both resulted in a deficiency in the repair of single-strand breaks arising in the deoxyribonucleic acid as a result of the excision repair process (incision breaks). These deficiencies were at least partially independent since the double mutant, *polA1 exrA*, was more deficient than a strain containing either the *polA1* or *exrA* mutation alone. These results suggest that the *polA1* and *exrA* mutations result in defects in two different branches of the *uvr* gene-dependent excision repair process. The repair of incision breaks was still observed in the *polA1 exrA* strain after low exposures of ultraviolet radiation, suggesting the existence of a third branch of the excision repair process which is dependent on neither the *polA* nor *exrA* genes. The *polA1* and *polA1 uvrA* strains were not deficient in post-replicational repair. The *exrA* strain was partially deficient in post-replicational repair, but the *polA1 exrA* strain was no more deficient than the *exrA* strain in this repair process. Thus, the increased ultraviolet irradiation sensitivity of the *polA1 exrA* strain relative to the *polA1* and *exrA* strains appears to be related to the effect of the *polA1* and *exrA* mutations on different branches of the *uvr* gene-dependent excision repair process as well as to the effect of the *exrA* mutation on the post-replicational repair process.

Two general systems for the dark repair of ultraviolet (UV) radiation damage in the deoxyribonucleic acid (DNA) of *Escherichia coli* have been described (10, 29, 31): (i) the excision repair of UV-induced lesions, and (ii) the post-replicational repair of gaps in daughter-strand DNA arising from replication past photochemical lesions in the parental strands.

The excision repair process is dependent on the *uvr* gene products (11). The *uvrA* and *uvrB* gene products appear to be necessary for the initial DNA incision steps (9, 10, 15), while the *uvrC* gene product may be involved in a reaction intermediate between the incision and excision steps (15). The excision of the UV-induced lesions may involve DNA polymerase I (2, 16). In vitro experiments have shown that proteolytic fragments of DNA polymerase I which retain the 5'-3'-exonuclease activity are able to excise thymine dimers (28).

The rate of closing of incision breaks in *polA1* cells is slower than in *pol*$^+$ cells (13, 26). Since the *polA1* strain is deficient in DNA polymerase I activity (5), this finding suggests that the DNA repair resynthesis step of excision repair is partially dependent on DNA polymerase I activity. The repair resynthesis which occurs in *polA1* strains could be due either to residual DNA polymerase I activity (18) or to some other DNA polymerase.

Monk et al. (22) reported that a *polA1 uvrA* strain was only slightly more sensitive to UV irradiation than the related *uvrA* strain, thus indicating that the involvement of DNA polymerase I in the repair of UV-induced damage is mainly in the excision repair process. Their finding was confirmed by Witkin and George (35) who also observed that a *polA1* mutation sensitized a *uvrA* strain only when the cells were plated on complex medium. Under these condi-

tions, DNA polymerase I played a role in minimizing the sensitizing effect of the complex medium.

The results of Cooper and Hanawalt (3, 4) indicate that the resynthesis step of the excision repair process sometimes produces very large patches of resynthesized DNA. The large patches were not produced in a *recA recB* strain. On the basis of this observation and their finding that a *polA1* strain performed an increased amount of repair resynthesis, they have postulated that excision repair involves DNA polymerase I performing efficient, short patch repair and a *recA*- and/or *recB*-dependent system performing large patch repair. Unfortunately the *polA1 rec* strains, which might be expected to lack both of the postulated repair resynthesis pathways, are not viable (8, 21).

The post-replicational repair process is deficient in *recA* strains (32) and is somewhat deficient in the strain B_{s-1} (30), which carries the *exrA* and *uvrB* mutations (20). The finding that a *polA1* mutation sensitizes a *uvr* strain only slightly (22, 35) suggests that DNA polymerase I is not normally involved in the post replicational repair process.

It has been shown that the *exrA* and *lex* strains are quite similar (9, 24, 25) and have many of the characteristics of *recA* strains (6, 9, 23), with the notable exception of differences in recombinational ability. The *recA* (14) and *exrA* (19, 27, 36) strains are both deficient in the type III (growth medium dependent) repair of X-ray-induced DNA single-strand breaks, and, in contrast to the inviability of the *polA1 rec* combinations (8, 21), the *polA1 exrA* strain is viable (36).

In this report, the effects of the *exrA* mutation on the excision and post-replicational repair processes are examined. The relationship of the defects in repair processes caused by an *exrA* mutation to those produced by the *polA1* and *uvrB* mutations is explored by examining the repair capacities of the double mutants, *polA1 exrA* and *exrA uvrB5*.

MATERIALS AND METHODS

Bacterial strains. The bacteria used, their genotypes, and the sources from which they were obtained are listed in Table 1.

Media. A minimal medium (MM) (7) supplemented with thiamine hydrochloride (0.5 μg/ml) was used. In addition, this was supplemented, as necessary, with L-methionine at 40 μg/ml, 10^{-3} M leucine, and thymine at 10 μg/ml for overnight growth or at 2 μg/ml for exponentially growing cells.

Solidified minimal medium (MM-agar) contained 1.6% Noble agar (Difco).

TABLE 1. *List of strains*[a]

Number	Genotype	Source or reference
DY98	W3110 *lacZ thy metE str*	36
DY99	W3110 *lacZ thy metE str exrA*	36
DY100	W3110 *lacZ thy metE str polA1*	36
DY101	W3110 *lacZ thy metE str polA1 exrA*	36
JG136	W3110 *lacZ thy rha str polA1 uvrA6*	J. D. Gross
DY145	W3110 *lacZ thy rha str metE leuB uvrB5*	This paper
DY146	W3110 *lacZ thy rha str metE leuB uvrB5 exrA*	This paper
AB2499	*uvrB5 leu thi thr arg pro his thy lac ara gal mtl xyl str tsx*	R. P. Boyce
MM450	W3110 *lacZ rha str recA56*	M. Monk
KH21	W3110 *leuB bio rha lacZ str thy metE malB*	R. B. Helling

[a] Abbreviations are as used by Taylor (33). The mating type of all the strains is F^-.

Survival after UV irradiation. An overnight culture grown in minimal medium was diluted 1:50 into fresh medium and grown about three generations to exponential phase (10^8 to 2×10^8 cells/ml) at 37 C. The cells were collected on 0.45-μm membrane filters (Millipore Corp.), washed with DTM buffer (minimal medium without glucose or supplements), and resuspended in DTM buffer at 10^8 to 2×10^8 cells/ml.

Samples (10 ml) in 90-mm diameter glass petri dishes on a rotary shaker were irradiated at room temperature with an 8-W General Electric germicidal lamp emitting primarily at 254 nm. The exposure rate was determined with an International Light, Inc., germicidal photometer (no. Il-254) and was varied as desired between 1 and 10 ergs per mm^2 per s by the insertion of wire mesh grids. To prevent photoreactivation, the irradiations and subsequent handling of the cells were carried out under General Electric "Gold" fluorescent lamps.

Samples were diluted in buffer (11.7 g of Na_2HPO_4 and 7.1 g of KH_2PO_4 per liter, pH 7.0) and plated on MM-agar supplemented as necessary for each strain.

Alkaline sucrose gradients. For the measurement of the repair of incision breaks, cells were grown and irradiated as indicated above except that thymine-*methyl*-^3H (New England Nuclear Corp.; ~18 μCi/mmol) at 100 or 200 μCi/ml was present in the medium for exponentially growing cells. After irradiation at room temperature in DTM buffer, the cells were added to an equal volume of MM with twice the normal concentrations of glucose and supplements and incubated for the desired time. Approximately 10^6 cells were layered onto a 0.1-ml cap of 0.5% Sarkosyl (Geigy NL30) and 0.01 M ethylenediaminetetraacetic acid (EDTA) in 0.5 N NaOH (C. D. Town, K. C. Smith, and H. S. Kaplan,

Radiat. Res., in press) on top of 4.8-ml linear gradients of 5 to 20% (wt/vol) sucrose in 0.1 N NaOH. After standing for 40 min, the gradients were centrifuged for 105 min at 30,000 rpm at 20 C in an SW50.1 rotor. The techniques for processing the gradients and analysing the data have been described (34).

For the measurement of post-replicational repair, a pulse-labeling procedure was used (32). Exponentially growing cells were resuspended in DTM buffer at 3×10^8 to 4×10^8 cells/ml. Immediately after irradiation, a sample of cells was added to an equal volume of warm MM with twice the usual concentration of glucose, thiamine, amino acids, and thymine (thymine-*methyl*-^3H at 600 μCi/ml). The cells were incubated at 37 C for 10 min, collected on 0.45-μm membrane filters, (Millipore Corp.), washed with warm MM, and resuspended in warm MM. After incubation at 37 C for the desired time, samples were layered onto alkaline sucrose gradients and processed as described above. The number of cells layered per gradient was 10^6 to 5×10^6, depending on the level of radioactivity incorporated into the cells. For any given experiment the same number of cells in the same volume (0.05 ml) was layered onto each gradient.

UV-induced DNA degradation. The cells were labeled with ^{3}H-thymine, irradiated, and incubated as described in the previous section. The only exception was that for cells labeled before UV irradiation, ^{3}H-thymine was present at 50 μCi/ml.

At desired times during the post-irradiation incubation period, triplicate samples were placed on filter paper disks which had been previously soaked in 10% trichloroacetic acid and dried. The techniques for processing the disks have been described (34).

Strain construction. The techniques used for transduction with phage P1kc have been described (36). The *uvrB5* (DY145) and *exrA uvrB5* (DY146) strains were derived from the *bio malB* strain, KH21. First the *uvrB5* mutation was introduced by transduction of *bio*$^+$ *uvrB5* using phage P1kc grown on strain AB2499. The *exrA* mutation was then introduced into a resulting *uvrB5* isolate by transduction of *mal*$^+$ *exrA* with phage P1 grown on strain DY99. In each case the initial selection was for the nutritional marker (*bio*$^+$ or *mal*$^+$), and the presence of the *uvrB5* or *exrA* mutation was determined by checking UV or X-ray sensitivity, respectively.

RESULTS

The UV survival curves for the wild-type, *exrA*, *polA1*, *polA1 exrA*, *uvrB5*, *exrA uvrB5*, and *recA56* strains growing exponentially in MM and plated on MM-agar are shown in Fig. 1. The double mutant, *polA1 exrA*, was more sensitive than either the *polA1* or *exrA* single mutants but was not as sensitive as the *exrA uvrB5* or *recA56* strains.

The repair of single-strand breaks (incision breaks) produced in DNA by the excision repair process after UV irradiation was measured (Fig. 2–5). The wild-type strain repaired essentially

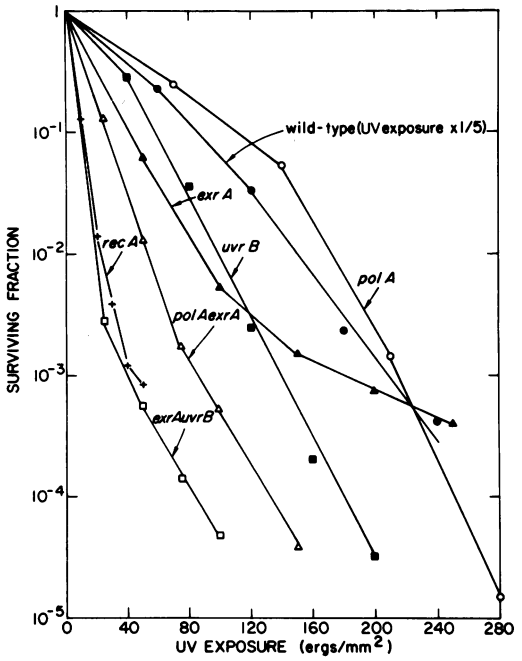

FIG. 1. *Survival after UV irradiation. Log-phase cells grown in MM were irradiated in DTM buffer and plated on MM-agar to determine survival. Strains are:* (●) *wild-type (DY98);* (▲) *exrA (DY99);* (○) *polA1 (DY100);* (△) *polA1 exrA (DY101);* (■) *uvrB5 (DY145);* (□) *exrA uvrB5 (DY146); and* (+) *recA56 (MM450). Note different UV exposure scale for the wild-type strain.*

all incision breaks within 60 min after a UV exposure of 400 ergs/mm^2 (Fig. 2A), but only a slight shift in the DNA profile was observed 60 min after a UV exposure of 700 ergs/mm^2 (Fig. 2B). In the *exrA* strain, after a UV exposure of 200 ergs/mm^2 there were few incision breaks remaining after incubation for 60 min (Fig. 3A), but after 400 ergs/mm^2 repair was not complete after 60 min (Fig. 3B) or 180 min (data not shown) of incubation. The *polA1* strain repaired the incision breaks completely after an exposure of 100 ergs/mm^2 (Fig. 4A), but showed only a slight shift in the DNA profile during 70 or 180 min of incubation after an exposure of 200 ergs/mm^2 (Fig. 4B). Thus, the data show that both the *exrA* and *polA1* mutations result in a partial deficiency in the repair of incision breaks.

The *polA1 exrA* strain was more deficient in the repair of incision breaks than either the *polA1* or *exrA* strains. After 200 ergs/mm^2 there was no shift in the DNA profile for the *polA1 exrA* strain even after incubation for 120 min (Fig. 5C). After an exposure of 100 ergs/mm^2

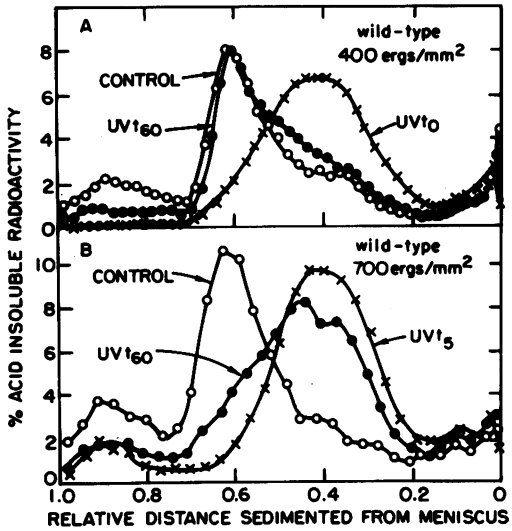

FIG. 2. *Repair of incision breaks in the wild-type strain (DY98) after UV irradiation. Log-phase cells grown in MM containing ^{3}H-thymine were irradiated in DTM buffer and incubated in MM at 37 C. The amount of strand breakage was determined by alkaline sucrose gradient techniques. The UV exposures and incubation times are shown in the figure.*

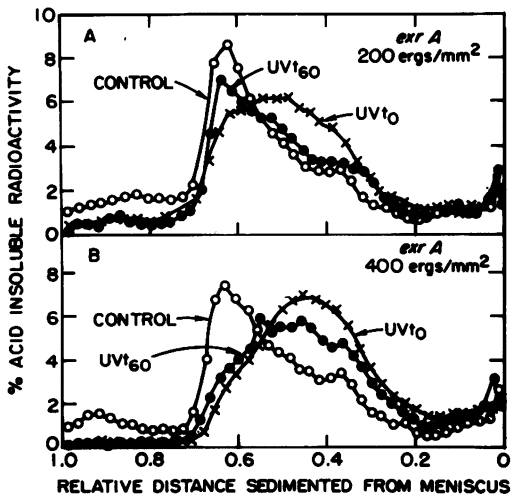

FIG. 3. *Repair of incision breaks after UV irradiation in the exrA strain (DY99). For procedures, see legend for Fig. 2. The UV exposures and incubation times are indicated on the gradient profiles.*

there was a shift in the DNA profile towards higher molecular weights after 60 min, but no additional repair was observed after 120 min of incubation (Fig. 5B). Complete repair was observed with the *polA1 exrA* strain within 60 min after an exposure of 50 ergs/mm² (Fig. 5A).

The effect of the *exrA* and *polA1* mutations

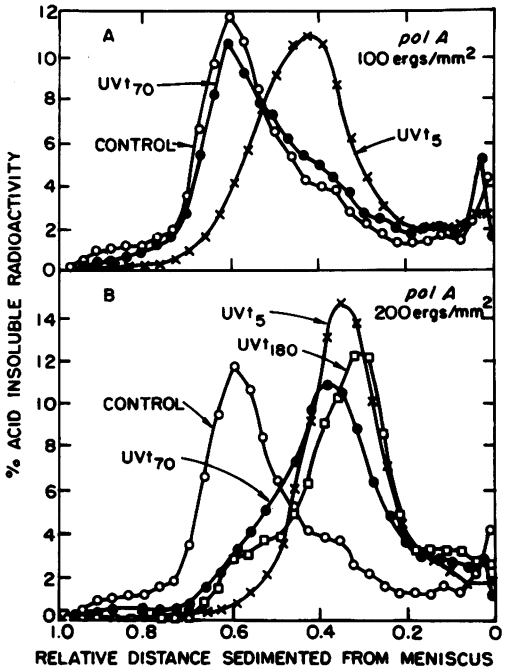

FIG. 4. *Repair of incision breaks after UV irradiation in the polA1 strain (DY100). For procedures, see legend for Fig. 2. The UV exposures and incubation times are indicated on the gradient profiles.*

on the post-replicational repair process was also examined (Fig. 6 and 7). After an exposure of 63 ergs/mm² the wild-type (Fig. 6A), *polA1* (not shown), and *polA1 uvrA* (Fig. 6B) strains completely repaired the gaps in their daughter-strand DNA during a 60-min incubation period in MM medium. The *exrA* and *polA1 exrA* strains both showed only partial repair under these conditions (Fig. 7A and B).

In an effort to avoid the problem of competition between excision repair and gap production, we examined post-replicational repair in an *exrA uvrB5* strain. This strain was somewhat more deficient in post-replicational repair than the *exrA* strain, showing partial repair after 60 min of incubation (Fig. 8B) and no further repair after 120 min of incubation (Fig. 8C) after an exposure of 63 ergs/mm². The isogenic *uvrB5* strain showed complete repair after 60 min (Fig. 8A), indicating that the deficiency of the *exrA uvrB5* strain was due to the *exrA* mutation.

The extent of UV-induced degradation of DNA in cells labeled for several generations before (prelabeled) or for 10 min after (pulse labeled) UV irradiation was determined. The results for prelabeled DNA (Table 2) indicate

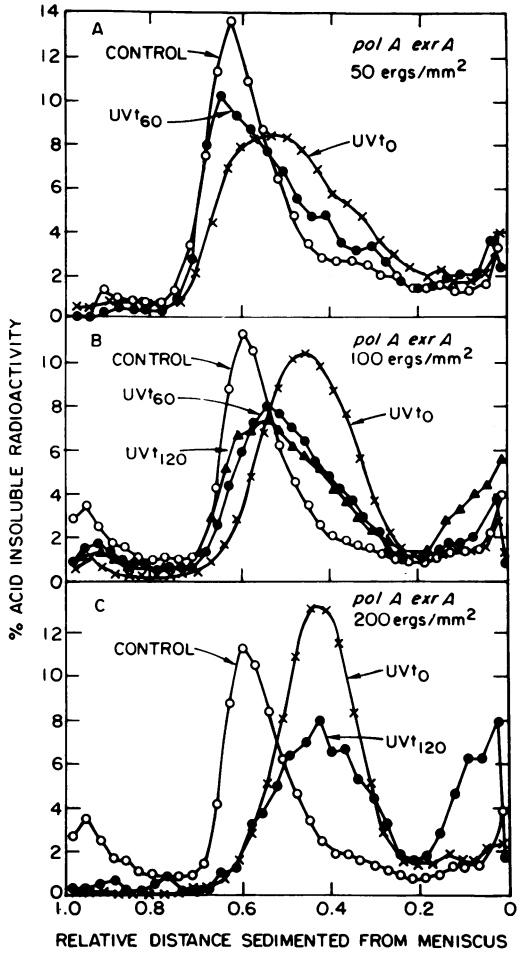

FIG. 5. *Repair of incision breaks after UV irradiation in the polA1 exrA strain (DY101). For procedures, see legend for Fig. 2. The UV exposures and incubation times are indicated on the gradient profiles.*

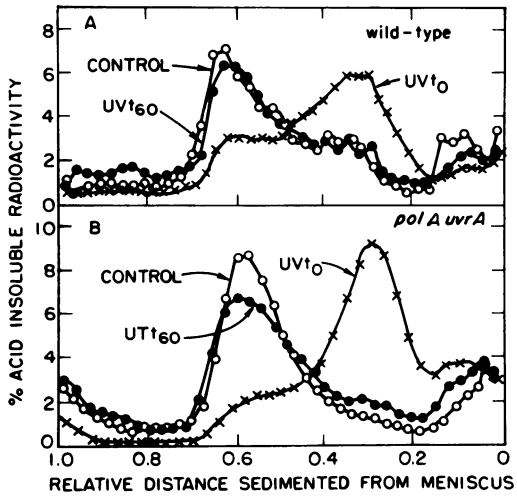

FIG. 6. *Post-replicational repair after UV irradiation in the wild-type (DY98) and polA1 uvrA6 (JG136) strains. The cells were pulse-labeled with ^{3}H-thymine after UV irradiation and analyzed by alkaline sucrose gradient techniques. The UV exposure was 63 ergs/mm^2. The strains and incubation times (in MM) are noted on the gradient profiles.*

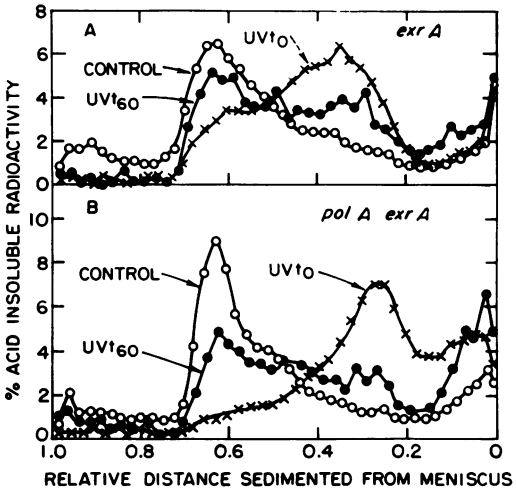

FIG. 7. *Post-replicational repair in the exrA (DY99) and polA1 exrA (DY101) strains. Procedures are indicated in Fig. 6.*

that the extent of UV-induced degradation correlates inversely with the capability of a strain to repair incision breaks in DNA. For example, the *polA1 exrA* strain was the most deficient of the strains examined in the repair of incision breaks (Fig. 5) and also degraded prelabeled DNA to the greatest extent.

Similarly, the *exrA uvrB5* strain was the most deficient in post-replicational repair (Fig. 8) and also degraded pulse-labeled DNA most extensively (Table 3). Some of the results for degradation of pulse-labeled material are not readily explained. For example, the *exrA* strain degraded pulse-labeled DNA more extensively than the *polA exrA* strain and yet seemed to be no more deficient in post-replicational repair (Fig. 7).

DISCUSSION

The *polA1 exrA* strain of *E. coli* K-12 was more UV sensitive than either the *exrA* or *polA1* strains (Fig. 1). This result suggests that the *polA1* and *exrA* mutations result in defects in different repair pathways. The *exrA uvrB5* strain was more UV sensitive than was the *uvrB5* strain, in agreement with the results of

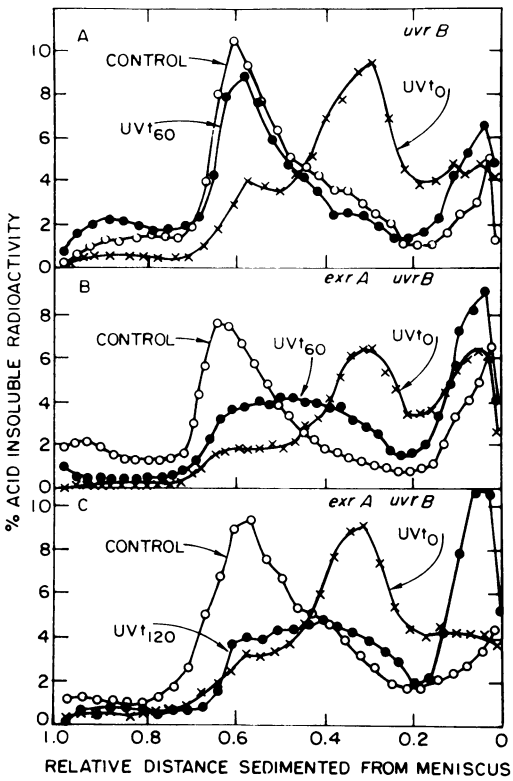

FIG. 8. *Post-replicational repair in the uvrB5 (DY145) and exrA uvrB5 (DY146) strains. Procedures are indicated in Fig. 6.*

TABLE 2. *UV-induced degradation of prelabeled DNA*[a]

Strain	Percent labeled DNA remaining trichloroacetic acid-insoluble after a UV exposure (ergs/mm²) of							
	50	100	200	300	400	500	600	800
Wild-type (DY98)			87.5		72.7		61.9	46.4
exrA (DY99)		71.3	51.5	37.7	31.5	27.9		
polA1 (DY100)	89.4	73.6	26.7	16.1	12.2		10.0	
polA1 exrA (DY101)	64.2	40.5	16.0	11.8	10.4	9.7		

[a] The values listed indicate the percentage of radioactivity remaining trichloroacetic acid-insoluble after incubation for 120 min in MM medium at 37 C after the indicated UV exposure. The values are expressed relative to an unirradiated sample incubated for the same period.

Mattern et al. (20), suggesting that at least part of the sensitizing effect of the *exrA* mutation is due to an effect on some repair process other than excision repair.

TABLE 3. *UV-induced degradation of pulse-labeled DNA*[a]

Strain	Percent labeled DNA remaining trichloroacetic acid-insoluble after	
	60 min	120 min
Wild-type (DY98)	107.0	106.1
exrA (DY99)	60.3	49.5
polA1 exrA (DY101)	83.2	79.4
polA1 uvrA (JG136)	70.2	61.5
uvrB5 (DY145)	63.0	55.8
exrA uvrB5 (DY146)	41.8	28.5

[a] The values listed indicate the percentage of radioactivity remaining trichloroacetic acid-insoluble relative to a zero time sample. The cells were pulse labeled for 10 min after a UV exposure of 63 ergs/mm². The labeled cells were filtered, washed, resuspended in MM medium, and incubated at 37 C for the indicated period.

The *polA1* strain was partially deficient in the repair of incision breaks (Fig. 4), in agreement with previous results (13, 26), but the *polA1 uvrA* strain was not deficient in post-replicational repair after an exposure of 63 ergs/mm² (Fig. 6B). This finding is consistent with survival results (22, 35) which showed that a *polA1* mutation only slightly sensitized a *uvrA* strain, thus indicating that the *polA* gene product, DNA polymerase I (5, 17), is involved mainly in the excision repair process.

The *exrA* mutation resulted in partial deficiencies in both the repair of incision breaks (Fig. 3) and in post-replicational (Fig. 7A and 8B) repair. The deficiency in the repair of incision breaks resulting from an *exrA* mutation was at least partially independent of that caused by a *polA1* mutation since the presence of both mutations (i.e., in the *polA1 exrA* strain) resulted in a greater deficiency than when only one of these mutations was present (Fig. 3-5). The *polA exrA* strain was no more deficient in post-replicational repair than was the *exrA* strain (Fig. 7). Thus, the increased UV sensitivity of the *polA1 exrA* strain relative to the *polA1* and *exrA* single mutants appears to be related to the effect of the *polA1* and *exrA* mutations on different branches of the *uvr* gene-dependent excision repair process as well as to the effect of the *exrA* mutation on the post-replicational repair process.

Van der Schueren and Smith (submitted to Photochem. Photobiol.) have found that 2,4-dinitrophenol (DNP), sensitizes wild-type and *polA1* cells but not *uvrB* or *exrA* strains to UV irradiation, suggesting that DNP inhibits an *exrA* gene-dependent branch of the excision

repair process which is not dependent on DNA polymerase I. These data support our suggestion that the *exrA* and *polA* genes control separate branches of the excision repair process.

Thus, the *uvr* gene-dependent excision repair process appears to consist of at least three different branches: (i) a branch dependent on the *polA* gene, (ii) an *exrA* gene-dependent branch, and (iii) a branch which is not dependent on either the *polA* or *exrA* genes and which is responsible for the repair observed in the *polA1 exrA* strain.

The residual repair of incision breaks which was observed in the *polA1 exrA* strain does not appear to be due to incomplete inactivation of the *polA* gene-dependent and *exrA* gene-dependent branches of excision repair because of the following reasons. (i) Lehman and Chien (18) have observed only about 0.2% of the wild-type level of DNA polymerase I in extracts of a *polA1* strain similar to those used in the experiments reported here. The wild-type strain repaired the incision breaks completely up to a UV exposure of between 400 and 700 ergs/mm^2 (Fig. 2). Thus, the residual DNA polymerase I activity present in the *polA1* strains could account for the complete repair of incision breaks only after UV exposures of less than 1 to 2 ergs/mm^2, whereas complete repair was observed in these strains after 50 to 100 ergs/mm^2 (Fig. 4, 5). (ii) The *recA* strain appears to be no more deficient in the repair of incision breaks than the *exrA* strain (D. A. Youngs, E. Van der Schueren, and K. C. Smith, in preparation). Thus, the *recA* and *exrA* mutations appear to be equally efficient in blocking the repair of incision breaks, although the *recA* mutation causes a more pronounced deficiency in post-replicational repair (see below). (iii) Our results with a *polA1 dnaE* strain (Nature [London], in press) indicate that the repair of incision breaks which occurs in *polA1* strains is largely dependent on DNA polymerase III activity. This suggests that both the *exrA*-dependent branch and the *exrA*- and *polA1*-independent branch of excision repair may require DNA polymerase III.

Cooper and Hanawalt (3, 4) have hypothesized that excision repair results in the formation, by repair resynthesis, of large DNA patches by a *recA* and/or *recB* dependent process and of small DNA patches by a DNA polymerase I dependent process. The relationship between the pathways of excision repair controlled by the *rec* and *exrA* genes has not yet been determined. However, results obtained with *recA* and *recB* strains (D. A. Youngs, E. Van der Schueren, and K. C. Smith, in preparation) indicate that these strains are deficient in the repair of incision breaks to about the same extent as the *exrA* strain. This suggests that the *recA*, *recB*, and *exrA* genes may control a single branch of the *uvr* gene-dependent excision repair process.

During the course of the experiments involving the repair of incision breaks, it was observed that each of the strains, wild-type, *exrA*, *polA1*, and *polA1 exrA*, completed the repair of incision breaks within about 60 min up to a certain critical UV exposure, which was strain dependent and correlated with the UV sensitivity of the strain. Above this exposure, repair was completed much more slowly, if at all. This result is similar to the finding of Achey and Billen (1) that DNA repair synthesis was complete within 45 min after UV irradiation. The basis of this phenomenon is not clear, but the implication is that repair occurs in each strain for 45 to 60 min until some event occurs which greatly reduces the rate of repair of at least a portion of the remaining incision breaks.

The UV-induced degradation of prelabeled DNA (Table 2) seems to correlate inversely with the ability of the strains examined to repair incision breaks (Fig. 2-5). The *recB21* strain shows only very limited degradation of prelabeled DNA after UV irradiation (12) but appears to be deficient in the repair of incision breaks (D. A. Youngs, E. Van der Schueren, and K. C. Smith, in preparation) similar to the *exrA* strain. Thus, the UV-induced DNA degradation response appears to be a result of, rather than the cause of, the deficiency in the repair of incision breaks.

The *exrA* strain was partially deficient in post-replicational repair. This was most apparent if a *uvr* mutation was also present (compare Fig. 7A and 8B). A possible explanation for this result is that the presence of the *uvr* mutation would block excision repair and thereby eliminate the competition between the excision repair process and DNA replication (the gap production process). This would result in a greater yield of gaps in the DNA of the *uvr* strain for a given UV exposure.

The *recA* strain (32) was markedly more deficient in post replicational repair than the *exrA* strain (Fig. 7A). This observation correlates with the survival data, which show that a *recA* strain is more sensitive to UV radiation than is the *exrA* strain (Fig. 1). The difference in post-replicational repair deficiencies observed in the *exrA* and *recA* strains may be a reflection of defects in different steps of the post-replication repair process. The defect caused by the *exrA* mutation is apparently specific for repair rather than normal recombi-

nation since *exrA* strains are not markedly deficient in genetic recombinational ability (6).

ACKNOWLEDGMENTS

We thank Patricia Pent and Leroy J. Pinto for excellent technical assistance.

The work was supported by Public Health Service grant CA-02896 and research project grant CA-10372 from the National Cancer Institute.

LITERATURE CITED

1. Achey, P., D. Billen. 1969. Saturation of dark repair synthesis: accumulation of strand breaks. Biophys. J. **9**:647-653.
2. Boyle, J. M., M. C. Paterson, and R. B. Setlow. 1970. Excision-repair properties of an *Escherichia coli* mutant deficient in DNA polymerase. Nature (London) **226**:708-710.
3. Cooper, P. K., and P. C. Hanawalt. 1972. Heterogeneity of patch size in repair replicated DNA in *Escherichia coli*. J. Mol. Biol. **67**:1-10.
4. Cooper, P. K., and P. C. Hanawalt. 1972. Role of DNA polymerase I and the *rec* system in excision-repair in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **69**:1156-1160.
5. de Lucia, P., and J. Cairns. 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164-1166.
6. Donch, J., J. Greenberg, and M. H. L. Green. 1970. Repression of induction by u.v. of λ phage by *exrA* mutations in *Escherichia coli*. Genet. Res. **15**:87-97.
7. Ganesan, A. K., and K. C. Smith. 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. **96**:365-373.
8. Gross, J. D., J. Grunstein, and E. M. Witkin. 1971. Inviability of *recA*$^-$ derivatives of the DNA polymerase mutant of de Lucia and Cairns. J. Mol. Biol. **58**:631-634.
9. Howard-Flanders, P. 1968. Genes that control DNA repair and genetic recombination in *Escherichia coli*. Advan. Biol. Med. Phys. **12**:299-317.
10. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175-200.
11. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119-1136.
12. Howard-Flanders, P., and R. P. Boyce. 1966. DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. Radiat. Res. Suppl. **6**:156-184.
13. Kanner, L., and P. Hanawalt. 1970. Repair deficiency in a bacterial mutant defective in DNA polymerase. Biochem. Biophys. Res. Commun. **39**:149-155.
14. Kapp, D. S., and K. C. Smith. 1970. Repair of radiation-induced damage in *Escherichia coli*. II. Effect of *rec* and *uvr* mutations on radiosensitivity, and repair of X-ray-induced single-strand breaks in deoxyribonucleic acid. J. Bacteriol. **103**:49-54.
15. Kato, T. 1972. Excision repair characteristics of *recB*$^-$ *res*$^-$ and *uvrC*$^-$ strains of *Escherichia coli*. J. Bacteriol. **112**:1237-1246.
16. Kelly, R. B., M. R. Atkinson, J. A. Huberman, and A. Kornberg. 1969. Excision of thymine dimers and other mismatched sequences by DNA polymerase of *Escherichia coli*. Nature (London) **224**:495-501.
17. Kelly, W. S., and H. J. Whitfield, Jr. 1971. Purification of an altered DNA polymerase from an *E. coli* strain with a *pol* mutation. Nature (London) **230**:33-36.
18. Lehman, I. R., and J. R. Chien. 1973. DNA polymerase I activity in polymerase I mutants of *Escherichia coli*. *In* R. D. Wells and R. B. Inman (ed.), DNA synthesis in vitro. University Park Press, Baltimore, Md.
19. McGrath, R. A., and R. W. Williams. 1966. Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; the rejoining of broken pieces. Nature (London) **212**:532-535.
20. Mattern, I. E., H. Zwenk, and A. Rörsch. 1966. The genetic constitution of the radiation-sensitive mutant *Escherichia coli* B_{s-1}. Mutat. Res. **3**:374-380.
21. Monk, M., and J. Kinross. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase. I. J. Bacteriol. **109**:971-978.
22. Monk, M., M. Peacey, and J. D. Gross. 1971. Repair of damage induced by ultraviolet light in DNA polymerase-defective *Escherichia coli* cells. J. Mol. Biol. **58**:623-630.
23. Moody, E. E. M., K. B. Low, and D. W. Mount. 1973. Properties of strains of *Escherichia coli* K12 carrying mutant *lex* and *rec* alleles. Mol. Gen. Genet. **121**:197-205.
24. Mount, D. W., and C. Kosel. 1973. Properties of strains of *Escherichia coli* K12 carrying mutant *lex-1* and *uvrA6* alleles. Mol. Gen. Genet. **120**:291-299.
25. Mount, D. W., K. C. Low, and S. J. Edmiston. 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**:886-893.
26. Paterson, M. C., J. M. Boyle, and R. B. Setlow. 1971. Ultraviolet- and X-ray-induced responses of a deoxyribonucleic acid polymerase-deficient mutant of *Escherichia coli*. J. Bacteriol. **107**:61-67.
27. Sedgwick, S. G., and B. A. Bridges. 1972. Survival, mutation and capacity to repair single-strand DNA breaks after gamma irradiation in different Exr$^-$ strains of *Escherichia coli*. Mol. Gen. Genet. **119**:93-102.
28. Setlow, P., and A. Kornberg. 1972. Deoxyribonucleic acid polymerase: two distinct enzymes in one polypeptide. II. A proteolytic fragment containing the 5′ → 3′ exonuclease function. Restoration of intact enzyme functions from the two proteolytic fragments. J. Biol. Chem. **247**:232-240.
29. Setlow, R. B., and J. K. Setlow. 1972. Effects of radiation on polynucleotides. Annu. Rev. Biophys. Bioengr. **1**:293-346.
30. Smith, K. C. 1971. The roles of genetic recombination and DNA polymerase in the repair of damaged DNA, p. 209-278. *In* A. C. Giese (ed.), Photophysiology, vol. 6. Academic Press Inc., New York.
31. Smith, K. C., and P. Hanawalt. 1969. Molecular photobiology. Academic Press Inc., New York.
32. Smith, K. C., and D. H. C. Meun. 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on postreplication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**:459-472.
33. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
34. Town, C. D., K. C. Smith, and H. S. Kaplan, 1970. Production and repair of radiochemical damage in *Escherichia coli* deoxyribonucleic acid; its modification by culture conditions and relation to survival. J. Bacteriol. **105**:127-135.
35. Witkin, E. M., and D. L. George, 1973. Ultraviolet mutagenesis in *polA* and *uvrA polA* derivatives of *Escherichia coli* B/r: evidence for an inducible error-prone repair system. Genet. **73**(Suppl.):91-108.
36. Youngs, D. A., and K. C. Smith. 1973. X-ray sensitivity and repair capacity of a *polA1 exrA* strain of *Escherichia coli* K-12. J. Bacteriol. **114**:121-127.