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Impaired Incision of Ultraviolet-Irradiated Deoxyribonucleic Acid in *uvrC* Mutants of *Escherichia coli*

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The production of single-strand breaks in the deoxyribonucleic acid of irradiated *uvrC* mutants of *Escherichia coli* K-12 was studied both in vivo and in vitro. In vivo, *uvrC* mutants displayed a slow accumulation of breaks after irradiation, and in this respect appeared different from *uvrA* mutants, in which very few breaks could be detected. The breakage observed in *uvrC* mutants differed from that observed in wild-type strains in both the slow rate of break accumulation and the very limited dose response. The behavior of the *uvrC lig-7*(Ts) double mutant was shown not to be consistent with the suggestion of ligase reversal as the explanation for the lower rate and limited dose response of break formation observed in ultraviolet-irradiated *uvrC* mutants in vivo. Rather, there appeared to be a real defect in incision. In toluene-treated cells, we studied the effect of the ligase inhibitor nicotinomide mononucleotide on strand incision. Whereas *uvrC* mutants displayed more strand breakage in the presence of this inhibitor, the same amount of breakage was seen in *uvrA* mutants, and as such the breakage could be judged as not due to the main excision repair pathway. Experiments using a cell-free system comprising the partially purified uvr^+ gene products demonstrated clearly that there is a requirement for the $uvrC^+$ gene product for strand incision. We suggest that in vivo in the absence of the $uvrC^+$ gene product, a partial analog of this protein may allow some abnormal incision.

The *uvrA*, *uvrB*, and *uvrC* genes of *Escherichia coli* were originally identified as loci involved in the excision of thymine dimers from UV-damaged DNA (9). A single mutation in any one of these genes is sufficient to prevent efficient removal of thymine dimers from UV-irradiated DNA, and these gene functions have therefore been assigned to early steps in DNA excision repair (for review, see reference 8). Although studies of UV-induced DNA strand breakage in the mutants have consistently shown that $uvrA^+$ and $uvrB^+$ gene functions are essential for the endonucleolytic event initiating repair (10, 17, 22), conflicting reports have been published with respect to the role of the $uvrC^+$ gene in the same process. Some reports indicate that the $uvrC^+$ gene function is involved in the actual incision reaction (2, 17, 18, 22), whereas others point to a role for the $uvrC^+$ function in a succeeding preexcision step (10, 19, 20). In view of this confusion, we reexamined the role of the *uvrC* function in the incision of UV-irradiated DNA, both in vitro and in vivo. For the in vitro studies, we used both nucleotide-permeable toluene-treated cells and a completely cell-free system comprising partially purified uvr^+ gene products.

MATERIALS AND METHODS

Bacterial strains. The strains used and their relevant genotype are listed in Table 1. The *E. coli uvr* mutants carrying *polA12* and *lig-7*(Ts) have been used in previous work (19, 20, 24), but their construction has not previously been described.

E. coli uvr mutants carrying *polA12* were constructed by conjugation using AB2433 (HfrH *uvrC34 his* Str^r) and BW114 HfrP4X (*uvrA6* Str^s) as donors and MM383 (*polA12 thyA* Str^r) as recipient, selecting for his^+ thy^+ and thy^+ Str^r, respectively. Strain MM383 contains a temperature-dependent *polA* mutation isolated by Monk and Kinross (12), showing a partial *polA* phenotype at 30°C and a mutant *polA* phenotype at 42°C. Recombinants were tested for methyl methane sulfonate (MMS) sensitivity on MMS plates and UV sensitivity on LB plates, using a liquid culture drop-replica technique allowing precise survival determinations. Recombinants showing high MMS sensitivity at 42°C, moderate MMS sensitivity at 30°C, and high UV sensitivity at 30°C were scored as double mutants. Strains designated AB5025 (*uvrC34 polA12*) and AB5026 (*uvrA6 polA12*) were further tested by the rapid mapping technique described by Low (11) and confirmed to have mutations conferring UV and MMS sensitivity in loci corresponding to the known map positions of (i) *uvrA* or *uvrC* and (ii) *polA*, respectively (1).

Surprisingly, the *uvrA6 polA12* strain was found to

TABLE 1. *Bacterial strains used*

Strain	Description	Origin	Source and/or reference
AB1157	*thr leu proA his thi argE lac gal ara xyl mtl* Str^r *sup-37*		P. Howard-Flanders
AB2497	AB1157 *thyA deo*		P. Howard-Flanders
AB1886	AB1157 *uvrA6*	Mutagenized derivative of AB1157	P. Howard-Flanders (9)
AB1885	AB1157 *uvrB5*	Mutagenized derivative of AB1157	P. Howard-Flanders (9)
AB1884	AB1157 *uvrC34*	Mutagenized derivative of AB1157	P. Howard-Flanders (9)
AB6000	AB1884 *thyA deo*	Derived from AB1884 by trimethoprim selection	This work
AB2433	Hfr H *uvrC34 his lac thi*		P. Howard-Flanders
BW114	Hfr P4x *uvrA6 thi* Str^s		K. B. Low (B. Wilkins strain)
MM383	*polA12 thy lac rha* Str^r		Monk et al. (13) via K. B. Low
AB5025	*uvrC34 polA12*	AB2433 × MM383	This work
AB5026	*uvrA6 polA12*	BW113 × MM383	This work
N2672	Hfr KL16 *lig-7*(Ts) *thi* Str^s		Gottesman et al. (16) via B. Bachmann
AB6006	*lig-7*(Ts) *thyA deo*	N2672 × AB2497	This work
AB6003	*uvrC34 lig-7*(Ts) *thyA deo*	N2672 × AB6000	This work
AB6004	*uvrA6 lig-7*(Ts)	N2672 × AB1886	This work
AB6005	*uvrA6 lig-7*(Ts) *thyA deo*	Derived from AB6004 by trimethoprim selection	This work
JC4583	*endA his gal thi* Str^s		A. J. Clark
BK101	JC4583 *his*$^+$	His$^+$ transductant of JC4583	This work
BK100	*uvrC34 endA*	AB2433 × BK101	This work

plate with low efficiency at 42°C in spite of the previous isolation of *uvrA6 polA1* which is viable (13). The reason for this is unclear and may be due to differences between the *polA1* and *polA12* mutation. Similar differences in viability have been reported for *polA recB* double mutants (5), and it should be noted that *polA12 ΔuvrB* cells have also been reported to be nonviable at 42°C (23).

E. coli uvr mutants carrying *lig-7*(Ts) were constructed by conjugation using N2672 [HfrKL16 *lig-7*(Ts) Str^s] (6) as donor and AB2497 (*his* Str^r), AB1886 (*uvrA6 his* Str^r), and AB6000 (*uvrC34 his* Str^r) as recipients, selecting for *his*$^+$ Str^r recombinants. Strains carrying the *lig-7*(Ts) mutation are nonviable at 42°C but grow normally at temperatures below 36°C. Matings were done at 33°C and interrupted after 45 min of incubation. Recombinants showing comparable UV sensitivity to the parent recipient at 30°C and nonviability at 42°C were scored as double mutants. The genotype of the strains designated AB6006 [*lig-7*(Ts)], AB6004 [*uvrA6 lig-7*(Ts)], and AB6003 [*uvrC34 lig-7*(Ts)] were further confirmed by rapid mapping of the loci determining UV sensitivity and conditional lethality.

Strain BK100 (*uvrC34 endA*) was constructed by conjugation between AB2433 (HfrH *uvrC34 his lac thi*) and a *his*$^+$ transductant of JC4583, selecting for *his*$^+$ *gal*$^+$ recombinants and scoring for the UV-sensitive phenotype. The inheritance of the *uvrC* mutation was verified in crosses with appropriate Hfr strains and by in vitro complementation analysis for the *uvr*$^+$ gene products as previously described (16).

Treatment of cells with toluene. Toluene-treated cells were prepared either by the modification of the method of Moses and Richardson described previously (24) or by a modification of the method of Halegoua et al. (7) described below.

Log-phase cells at ice temperature were washed twice with equal volumes of 0.04 M Tris-hydrochloride, pH 8.0, and were suspended at a concentration of 10^{10} cells/ml in 10 mM Tris-hydrochloride, pH 7.8–50 mM NH_4Cl–10 mM magnesium acetate–1 mM dithiothreitol. An equal volume of a 2% emulsion of toluene (Eastman Kodak, spectrophotometric grade) in the same buffer was added, and the mixture was shaken on ice for 10 min. A cushion of 17% sucrose in the same buffer was introduced beneath the treated cells with a syringe, and the cells were sedimented through this layer by centrifugation at 6,000 rpm for 5 min. The cells were finally resuspended in the sucrose-containing buffer at a concentration of 10^{10} cells/ml and either used immediately or frozen in liquid nitrogen for later use.

Partial purification of the products of the *uvrA*$^+$, *uvrB*$^+$, and *uvrC*$^+$ genes. The *uvr*$^+$ and the *uvrB*$^+$/*uvrC*$^+$ gene products were isolated by means of an in vitro complementation assay for the individual *uvr*$^+$ gene products by a combination of DEAE-cellulose and phosphocellulose chromatography as previously described (16). Briefly, proteins were extracted from 1.8-liter batches of log-phase cells (2×10^8 cells/ml) of strain JC4583 by a combination of sucrose plasmolysis and lysozyme treatment. The extract (approximately 200 mg of protein) was applied to a

DEAE-cellulose column (1 by 10 cm) equilibrated with 100 mM KCl-50 mM morpholinepropanesulfonate (MOPS), pH 7.5-1 mM EDTA-10 mM mercaptoethanol. The nonadsorbed fraction was applied to a phosphocellulose column (1 by 8 cm) equilibrated with the same buffer. Both columns were eluted with a linear salt gradient (0.1 to 0.4 M KCl) in buffer as defined above, also containing 25% glycerol. The presence of mercaptoethanol and high concentrations of glycerol is essential for stabilization of the enzyme preparation. The *uvrA*$^+$ product was recovered from the phosphocellulose column at approximately 0.25 M KCl. The *uvrB*$^+$ and *uvrC*$^+$ gene products have cochromatographic properties and elute together from the DEAE-cellulose column at approximately 0.3 M KCl (16). These preparations are essentially free of endonuclease activity on nonirradiated DNA. To isolate the *uvrB*$^+$ product separated from functional *uvrC*$^+$ activity, the purification procedure as described above was carried out with a cell extract from strain BK100 (*uvrC34*). The chromatographic properties of the *uvrA*$^+$ and *uvrB*$^+$ products from *uvrC* mutant cells were indistinguishable from those denoted above for the isolation from *uvr*$^+$ cells, although the *uvrB*$^+$ fraction was, as expected, devoid of *uvrC* complementing activity (see Fig. 5).

Measurements of strand breaks introduced by partially purified *uvr*$^+$ gene products in vitro. Standard reaction mixtures contained 0.05 μg of ^{3}H-labeled covalently closed circular ColE1 DNA (about 800 cpm), 5 μmol of MOPS (pH 7.7), 10 μmol of KCl, 2 μmol of $MgSO_4$, 0.1 μmol of EDTA, 0.1 μmol of dithiothreitol, 10 μl of *uvrA*$^+$ fraction (2 to 3 μg of protein), and various amounts of the *uvrB*$^+$/*uvrC*$^+$ fraction from strain JC4583 or the *uvrB*$^+$ fraction from strain BK100 as indicated in a total volume of 140 μl. Incubation was for 20 min at 37°C. Introduction of breaks in the covalently closed DNA substrate was measured by the filter-binding method of Center et al. (4) as later modified (3, 16). This involves brief exposure of the DNA to pH 11.9 followed by neutralization and filtration through nitrocellulose filters. Nicked molecules are denatured and retained on the filter under these conditions, whereas closed molecules are renatured and will pass through. The average number of breaks per molecule was calculated as described (4), assuming a Poisson distribution of breaks in the DNA circle preparation. Assays were always performed in duplicate or triplicate and with both UV-irradiated and nonirradiated DNA. When irradiated, the DNA was exposed to UV light (18 J/m^2) from a low-pressure mercury lamp emitting light predominantly at 254 nm. This dose of UV light induces on average 2.5 pyrimidine dimers per DNA molecule as determined by incubation of DNA samples exposed to even lower doses with purified pyrimidine dimer-specific endonuclease from *Micrococcus luteus*.

Other methods. Procedures for growth and labeling of bacteria, UV irradiation of cell suspensions, cell lysis, and analysis of chromosomal DNA strand breaks were those described by Seeberg and Strike (20).

RESULTS

UV-induced DNA strand break formation in vivo.

Kato (10) demonstrated that the behavior of *uvrC* mutants could be distinguished from that of *uvrA* mutant strains since, in the former, single-strand breaks accumulated during incubation after UV irradiation. In *uvrA* mutant strains, no breaks appeared. However, a closer examination of the kinetics of UV-induced strand breakage, using superinfecting lambda DNA as a probe, revealed that breaks in the *uvrC* mutant arose much more slowly than in *uvr*$^+$ cells and that the dose-dependent increase in strand break formation terminated at a much lower dose (17). Results similar to those observed with superinfected lysogens were obtained when the incision of *E. coli* chromosomal DNA was studied (Fig. 1A). When a *polA* mutation was introduced into the strains under study to prevent rapid break closure by short-patch repair, breaks accumulated rapidly in the *uvr*$^+$ *polA* strain, slowly in the *uvrC polA* strain, and only to a very limited extent in the *uvrA polA* strain. In the presence of the wild-type *pol* allele, rapid rejoining occurred in the *uvr*$^+$ strain (Fig. 1B), but the presence of DNA polymerase I had little effect on the extent of break formation in the *uvrC* strain, suggesting that these breaks are not subjected to rapid DNA polymerase I-dependent repair.

A comparison of the dose response for incision

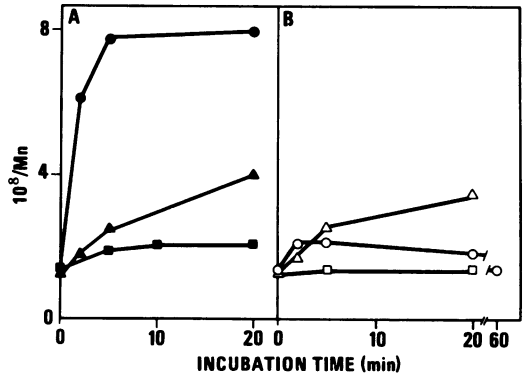

FIG. 1. *Kinetics of chromosomal DNA strand breakage of UV-irradiated E. coli cells in vivo. Cells prelabeled with [^{3}H]thymidine were exposed to a UV dose of 12 J/m^2 at 0°C in phosphate buffer and incubated in complete medium at 42°C (A) or 37°C (B). At intervals, cell samples were lysed on alkaline sucrose gradients and centrifuged for 90 min at 30,000 rpm in a Spinco SW50.1 rotor. ^{32}P-labeled λ DNA was included as a molecular weight reference in each gradient. The reciprocal value of the number average molecular weight was calculated from the sedimentation data and plotted as a function of incubation time. Strains used were AB1157 (○), AB1884 (uvrC34; △), AB1886 (uvrA6; □), MM383 (polA12; ●), AB5025 (uvrC34 polA12; ▲), and AB5026 (uvrA6 polA12; ■).*

of chromosomal DNA in *uvr*$^+$, *uvrA*, and *uvrC* mutant strains is shown in Fig. 2. Again, the *polA* mutation was present to prevent break reclosure. The results parallel those obtained for superinfecting lambda DNA (17). After 3 min of incubation, a linear dose-dependent increase was observed for strand incision in *uvr*$^+$ cells, whereas *uvrC* and *uvrA* cells showed very few single-strand breaks. After 20 min of incubation, however, breaks had accumulated in the *uvrC* cells, although the dose dependence of this break formation declined at a lower dose than in *uvr*$^+$ cells.

Figure 3 shows the dose response for the formation of breaks in chromosomal DNA of cells where the *lig*-7(Ts) mutation had been introduced to prevent break rejoining. When cells were grown at 36°C, irradiated at ice temperature, and incubated at 42°C after irradiation, results were similar to those obtained with the *polA* mutant strains (Fig. 3A). The *lig*-7(Ts) mutation caused rapid accumulation of breaks in the *uvr*$^+$ strain, but had little effect on break formation in *uvrA* or *uvrC*. After 3 min of incubation, the response of the *uvrC lig*-7(Ts) strain was similar to that of the *uvrA lig*-7(Ts) strain. Seeberg and Rupp (19) have previously reported that the *lig*-7(Ts) mutation causes rapid accumulation of breaks in *uvrC* mutants, in contrast to the data presented in Fig. 3A. However, in

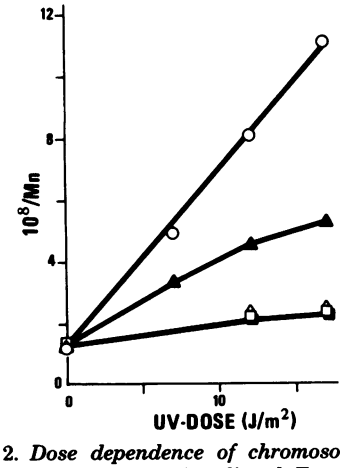

FIG. 2. *Dose dependence of chromosomal DNA strand breakage in UV-irradiated E. coli polA12 cells in vivo. Cells prelabeled with [^{3}H]thymidine were UV irradiated at 0°C in phosphate buffer and incubated for 3 min (open symbols) or 20 min (closed symbols) at 42°C before strand break analysis as described in legend to Fig. 1. Irradiated cell samples from strain MM383 were centrifuged for 120 min; the others were centrifuged for 90 min at 30,000 rpm. Strains used were MM383 (polA12; ○), AB5025 (uvrC34 polA12; △, ▲), and AB5026 (uvrA6 polA12; □, ■).*

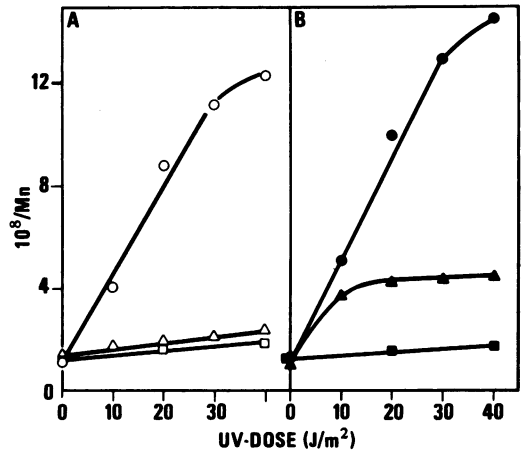

FIG. 3. *Dose dependence of chromosomal DNA strand breakage in UV-irradiated E. coli lig-7(Ts) cells in vivo. Cells were grown for several generations at 36°C in medium containing [^{3}H]thymidine (2 μg/ml, 50 μCi/ml) to a cell density of 10^8 cells/ml. The cells were washed twice by centrifugation and resuspended in growth medium with unlabeled thymidine (50 μg/ml). Each culture was then split in two parts; one portion was placed at 36°C (A; open symbols), the other was placed at 42°C (B; closed symbols), and both were incubated for 60 min with aeration. Cells from each culture were then collected by centrifugation, UV irradiated in phosphate buffer at 0°C, and incubated for 3 min at 42°C in complete medium before lysis and strand break analysis as described in legend to Fig. 1. Irradiated cell samples of strain AB6006 exposed to 20 J/m^2 or more were centrifuged for 135 min; the others were centrifuged for 90 min at 30,000 rpm. Strains used were AB6006 [lig-7 (Ts); circles], AB6003 [uvrC34 lig-7 (Ts); triangles), and AB6005 [uvrA6 lig-7 (Ts); squares].*

those experiments the cells had been preincubated at 42°C for 80 min before irradiation in order to maximize the efficiency of incisions in the *uvrC* mutant. This period of incubation has previously been interpreted as being required for effective inactivation of all ligase molecules. This interpretation seems open to doubt however, since no preincubation was necessary for rapid accumulation of breaks in the *uvr*$^+$ strain (Fig. 3A). Nevertheless, preincubation for 60 min or longer promoted the accumulation of UV-induced strand breaks in *uvrC lig*-7(Ts) cells, although the dose response of this break formation became zero order after UV irradiation at 10 J/m^2 (Fig. 3). Thus, even under conditions of cell preincubation at the nonpermissive temperature for a long period, there was a distinct difference between the number of UV-induced strand breaks formed in *uvr*$^+$ and *uvrC* cells, particularly for higher doses of UV.

UV-induced strand break formation in

toluene-treated cells. Sharma and Moses (21) have recently reported results from experiments with toluene-treated permeable cells indicating that the number of UV-induced strand breaks formed in *uvrC* mutant cells is similar to that observed in *uvr*$^+$ cells, provided that ligase is inhibited by nicotinamide mononucleotide (NMN). These results therefore support the hypothesis suggested previously that the lower rate and extent of UV-induced break formation observed in *uvrC* mutants relative to *uvr*$^+$ is due to ligase reversal of the incision reaction (3, 17). However, other investigators using cells made permeable by plasmolysis (20) or by toluene treatment (2) have failed to observe UV-induced strand breakage in *uvrC* mutant cells to any greater degree than that seen in *uvrA* cells. We therefore reexamined the question of strand incision in toluene-treated cells, looking closely at the dose dependence of any strand breakage and the effects of ligase activation and inhibition (Fig. 4).

In the presence of NMN, it is clear that wild-type *uvr*$^+$ cells produced many more breaks per unit dose than did *uvrA* or *uvrC* mutant cells. This effect was most apparent for UV doses that caused values of 10^8/Mn to reach 4 or more. Below such a dose, a considerable amount of breakage was observed in *uvrA*, *uvrC*, and *uvr*$^+$ strains. In the mutant strains, however, the ability to produce breaks appeared to be rapidly saturated and became zero order for higher doses. The UV-dependent production of single-strand breaks in *uvr* mutants has been previously observed in vivo by Youngs and Smith (25), who suggest that these breaks may be the initial events in a *uvrA*$^+$,*uvrB*$^+$-independent excision repair process acting on UV photoproducts other than thymine dimers. This explanation seems plausible in the context of breaks observed in toluene-treated *uvrA* and *uvrC* mutant strains. Our interpretation of the breakage observed in toluene-treated *uvrC* mutant cells differs from that of Sharma and Moses (21), who reported similar experiments with *uvrC56* cells. However, the main difference between these two pieces of work lies not in the behavior of the *uvrC* mutant, but in the number of breaks observed in the wild type. We observed approximately one incision break for every three dimers in the DNA of intact cells (20) and approximately one break for every seven dimers in toluene-treated cells. A similar efficiency of incision has been observed by other workers using toluene-treated cells, and the reasons for this decreased efficiency have been discussed previously (20). The efficiency of incision observed by Sharma and Moses in wild-type cells is two- to threefold lower than that which we observed,

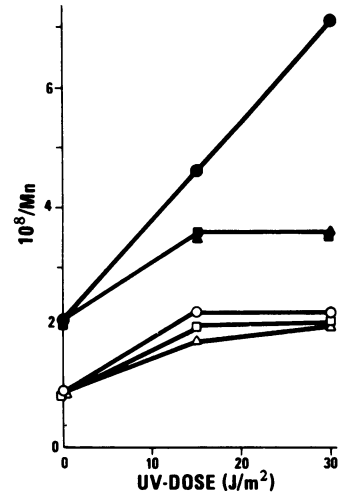

FIG. 4. *Efects of NAD and NMN on UV-induced single-strand breakage of chromosomal DNA in toluene-treated cells. Cells of each strain prelabeled with [^{3}H]thymidine were UV irradiated at 0°C and toluene treated by a slight variation of the method of Halegoua et al. (7). The treated cells were added to prewarmed reaction buffer containing 50 mM MOPS, pH 7.5–0.1 M KCl–10^{-3} M EDTA–10^{-3} M dithiothreitol–12.5 mM $MgSO_4$–2 mM ATP as described previously (24). NAD and NMN were present at 0.1 and 1 mM, respectively, as appropriate. Open symbols refer to incubations with NAD, and closed symbols refer to incubations with NMN. Incubation was for 15 min at 37°C, when samples were lysed on alkaline sucrose gradients and centrifuged for 90 min at 30,000 rpm, 20°C, in an SW50.1 rotor. The reciprocal value of the number average molecular weight was calculated from the sedimentation data and plotted versus UV dose. Strains used were AB1157 (uvr*$^+$*; circles), AB1886 (uvrA6; squares), and AB1884 (uvrC34; triangles). Very similar results to those presented were obtained when unirradiated cells were treated with toluene as described by Moses and Richardson (15) and then UV irradiated.*

and this makes comparisons difficult, especially since Sharma and Moses do not present *uvrA* controls and dose-response curves.

The effect of exogenous NAD added to any of the toluene-treated strains is clearly seen in Fig. 4. Without irradiation, the presence of NAD markedly reduced the number of breaks present in the DNA of incubated cells, and a similar reduction was seen in irradiated cells. Some UV-induced strand breakage was still observed in *uvrA*, *uvrC*, and *uvr*$^+$ strains, but it occurred to almost the same extent in all three strains and did not appear to be susceptible to rapid reclosure by ligase. The efficiency of ligase in rapidly rejoining the breaks made in *uvr*$^+$ strain is very apparent from Fig. 4 and presumably corresponds to the rapid completion of short-patch

repair. It is clear that in toluene-treated cells, *uvrC* mutants are capable of making no more UV-induced strand breaks than *uvrA* mutants, and although some of the breaks observed in *uvrC* strain can be rejoined by ligase, this rejoining is also observed in *uvrA* and *uvr*$^+$ strains. It may represent rejoining of gaps created by some process independent of the *uvr* pathway of excision repair.

Incision of UV-irradiated DNA by partially purified *uvr*$^+$ gene products in vitro. A method has recently been established for the study of *uvr*$^+$ gene functions in a soluble in vitro system (18). This involves purification of the *uvr*$^+$ gene products by means of an in vitro complementation assay, and it has been shown that the *uvrA*$^+$ and *uvrB*$^+$ gene products can be separated from each other by simple ion-exchange chromatography, whereas the *uvrC*$^+$ product cochromatographs with the *uvrB*$^+$ product (16).

When the *uvrA*$^+$ and *uvrB*$^+$/*uvrC*$^+$ fractions are combined, an endonuclease activity is generated which is ATP dependent and acts at pyrimidine dimers in UV-irradiated DNA (16; E. Seeberg, Mutat. Res., in press). Because one of these fractions contains both the *uvrB*$^+$ and the *uvrC*$^+$ product, it is unclear from these results whether the complementation is caused by the combination of the *uvrA*$^+$ and *uvrB*$^+$ products alone, or whether the *uvrC*$^+$ product is also required for the endonuclease activity. Therefore, to investigate the role of the *uvrC*$^+$ product in the endonuclease activity, we isolated the *uvrB*$^+$ product in parallel from wild-type and *uvrC* mutant cells. The *uvrB*$^+$ fraction from wild-type cells contained functional *uvrC*$^+$ activity, whereas the *uvrB*$^+$ fraction from the *uvrC* mutant was, as expected, devoid of such activity (Fig. 5A, B). However, the chromatographic properties and specific activity of the *uvrB*$^+$ product were similar in both strains. When various amounts of these fractions were added to equal portions of the *uvrA*$^+$ fraction, efficient complementation was observed in mixtures containing *uvrB*$^+$/*uvrC*$^+$ products from wild-type cells (Fig. 5C). No complementation was observed, however, in mixtures with the *uvrB*$^+$ product from the *uvrC* mutant, irrespective of the presence of the ligase inhibitor NMN. Separate control experiments with mixed *uvrB*$^+$ fractions from strains JC4583 and BK100 (*uvrC34*) gave results similar to those presented for JC4583 alone (data not shown). We therefore conclude that the *uvrC*$^+$ product is an essential component of the *uvr*$^+$-coded endonuclease in vitro.

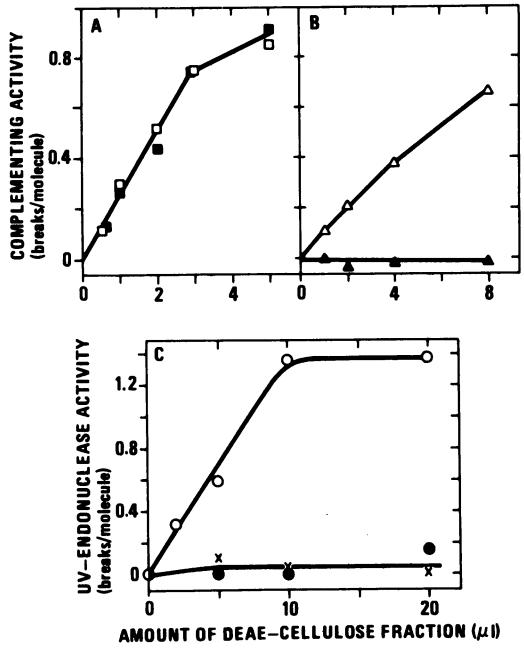

FIG. 5. *Role of the uvrC*$^+$ *product for strand incision of UV-irradiated DNA by semipurified E. coli extracts in vitro. Protein extracts from strains JC4583 (endA) and BK100 (uvrC34 endA) were chromatographed on DEAE-cellulose, and protein fractions eluting from the column by a salt gradient were assayed for the uvrB gene product by an in vitro complementation assay as previously described (16). Active fractions were pooled and assayed for protein, which amounted to 0.95 mg/ml from strain JC4583 and 0.90 mg/ml from strain BK100. (A and B) Various amounts of these fractions as indicated were tested for uvrB*$^+$*-complementing (squares) or uvrC*$^+$*-complementing activity (triangles), using receptor extracts from strains AB1885 or AB1884, respectively. Conditions for the complementation assay were as previously described (16), and results are plotted as increase in strand breakage of UV-irradiated DNA (ColE1 DNA exposed to UV at 180 J/m*^{2}*) caused by the addition of the DEAE-cellulose fraction relative to that observed with receptor extract alone. Open symbols refer to results obtained with the uvrB*$^+$ *fraction from JC4583, and closed symbols refer to results obtained with the uvrB*$^+$ *fraction from BK100. (C) Portions of the uvrB*$^+$ *fractions from JC4583* (○) *or BK100* (●, ×) *were mixed with a fixed amount of uvrA protein (2.3 μg of protein of the phosphocellulose fraction described in reference 16), and the mixtures were tested for endonuclease activity on covalently closed ColE1 DNA exposed to UV at 18 J/m*^{2}*. Results are corrected for the breakage observed with nonirradiated DNA incubated in parallel, which in all cases amounted to less than 0.2 breaks/molecule. Crosses show the results obtained when 3.5 mM NMN was included in the reaction mixtures.*

DISCUSSION

The in vivo data show that *uvrC* mutant cells, in contrast to *uvrA* mutant cells, accumulate single-strand breaks during incubation after UV irradiation. These breaks are, however, formed less rapidly and less extensively than in the corresponding *uvr*$^+$ cells, and it therefore apears that the *uvrC* function is required for fully efficient incision. This conclusion is supported by the results observed with the ligase-defective strains, in particular when higher UV doses are used. The dose-response relationship observed in ligase-deficient strains is not compatible with the hypothesis of ligase reversal of strand incision as the explanation of the lower rate and extent of break formation in *uvrC* cells (19, 21). It is possible that some residual ligase activity is present in the *lig-7*(Ts) strains at the nonpermissive temperature, and that it masks some break formation. If this were the only explanation for the reduced strand incision observed in the *uvrC* mutant, one would simply expect strand breakage per unit dose to be proportionally reduced, compared with wild type, across the full dose range. This is clearly not the case. Although a dose-dependent increase in the number of strand incisions was observed within the whole dose range tested for the *uvr*$^+$ *lig-7*(Ts) mutant, the dose dependence in the *uvrC lig-7*(Ts) mutant terminated after 10 to 15 J/m^2, quite the opposite of what would be expected if a limited amount of ligase becomes saturated by extensive incision.

In toluene-treated permeable cells, it is clear that more breaks were observed in the *uvrC* mutant strain in the presence of the ligase inhibitor NMN than in the presence of the ligase cofactor NAD. However, some of this breakage appeared to be unrelated to the dose of UV irradiation; moreover, the amount of breakage which can be ascribed to irradiation of the cells was seen to the same extent in both *uvrA* and *uvrC* mutant cells. On this criterion, it can be judged as not being part of the main excision repair pathway. To observe a difference between the number of breaks observed in *uvr*$^+$ and *uvr* toluene-treated cells, doses of radiation must be sufficient to give values of $10^8/M_n$ greater than 4. At low doses, the amount of breakage appeared similar in *uvr*$^+$, *uvrA*, and *uvrC* strains. In the mutant strains, however, the dose response rapidly became zero order, whereas in the *uvr*$^+$ strain it remained first order for doses up to 30 J/m^2.

The data obtained with the semipurified in vitro system provide the most clear-cut evidence for the requirement of the *uvrC*$^+$ function for strand incision of UV-irradiated DNA. Whereas the combination of *uvrA*$^+$, *uvrB*$^+$, and *uvrC*$^+$ gene products efficiently complemented each other to produce strand breaks in a lightly irradiated DNA substrate, no strand breaks were introduced by the combination of *uvrA*$^+$ and *uvrB*$^+$ products alone. The failure to observe breaks in the absence of an active *uvrC*$^+$ product was not caused by rapid ligase rejoining, since addition of the ligase inhibitor was without effect. Moreover, we also failed to detect any ligase activity in these fractions when a DNA substrate was included which contained a limited amount of single-strand breaks induced by DNase I (data not shown).

Although the experiments with all three systems investigated consistently indicate that the *uvrC* function is required for efficient incision break formation, it is clear that the in vivo situation differs somewhat from the in vitro situation, since the *uvrC* mutant in vivo accumulated single-strand breaks after UV irradiation. The nature of the strand breaks produced in *uvrC* cells may be anomalous, since these breaks do not provide an efficient substrate for subsequent dimer excision (10, 21) and their appearance is hardly affected by the presence or absence of DNA polymerase I (Fig. 1). However, their formation does seem to be *uvrA*$^+$,*uvrB*$^+$-dependent since no accumulation of breaks was observed in the double mutant *uvrA uvrC* or *uvrB uvrC* (data not shown). It is possible that the *uvrC* response depends on the induction of a *uvrC* protein analog which could, to a limited extent, replace *uvrC*$^+$ for incision. Such a protein would not be present in toluene-treated cells or in semipurified extracts, which originate from cells which have not been preirradiated or induced in any other way. This idea could also explain the more rapid accumulation of strand breaks occurring in *uvrC lig-7*(Ts) cells after preincubation, since incubation of the *lig-7*(Ts) strains at the nonpermissive temperature is known to induce the so-called error-prone repair functions (14) and may well induce other proteins as well. We are currently investigating the possible inducible nature of strand incision in *uvrC* mutant cells.

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LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976.

Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.

2. **Ben-Ishai, R., and R. Sharon.** 1978. Patch size and base composition of ultraviolet light-induced repair synthesis in toluenized *Escherichia coli*. J. Mol. Biol. **120**:423–432.
3. **Braun, A., and L. Grossman.** 1974. An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from *uvrA* and *uvrB* mutants. Proc. Natl. Acad. Sci. U.S.A. **71**:1838–1842.
4. **Center, M. S., F. W. Studier, and C. C. Richardson.** 1970. The structural gene for a T7 endonuclease essential for phage DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **65**:242–248.
5. **Emmerson, P. T., P. McAthey, and P. Strike.** 1974. Some properties of mutants of *Escherichia coli* K12 defective in DNA polymerase I and exonuclease V. Mol. Gen. Genet. **130**:29–38.
6. **Gottesman, M. M., M. Hicks, and M. Gellert.** 1973. Genetics and function of DNA ligase in *Escherichia coli*. J. Mol. Biol. **77**:531–547.
7. **Halegoua, S., A. Hirashina, and M. Inouye.** 1976. Puromycin-resistant biosynthesis of a specific outer-membrane lipoprotein of *Escherichia coli*. J. Bacteriol. **126**:183–191.
8. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Ann. Rev. Biochem. **48**:783–836.
9. **Howard-Flanders, P., R. B. 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.
10. **Kato, T.** 1972. Excision repair characteristics of *recB res* and *uvrC* strains of *Escherichia coli*. J. Bacteriol. **112**: 1237–1246.
11. **Low, K. B.** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. **113**:798–812.
12. **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.
13. **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.
14. **Morse, L. S., and C. Pauling.** 1975. Induction of error-prone repair as a consequence of DNA ligase deficiency in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**: 4645–4649.
15. **Moses, R. E., and C. C. Richardson.** 1970. Replication and repair of DNA in cells of *Escherichia coli* treated with toluene. Proc. Natl. Acad. Sci. U.S.A. **67**:674–681.
16. **Seeberg, E.** 1978. Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated *uvrA*$^+$ and *uvrB*$^+$/*uvrC*$^+$ gene products. Proc. Natl. Acad. Sci. U.S.A. **75**:2569–2573.
17. **Seeberg, E., and I. Johansen.** 1973. Incision in ultraviolet-irradiated circular bacteriophage λ DNA molecules in excision proficient and deficient lysogens of *Escherichia coli*. Mol. Gen. Genet. **101**:227–244.
18. **Seeberg, E., J. Nissen-Meyer, and P. Strike.** 1976. Incision of ultraviolet irradiated DNA by extracts of *E. coli* requires three different gene products. Nature (London) **263**:524–526.
19. **Seeberg, E., and W. D. Rupp.** 1975. Effects of mutations in *lig* and *polA* on UV-induced strand cutting in a *uvrC* mutant of *Escherichia coli*, p. 439–441. *In* P. C. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms in DNA repair. Plenum Publishing Corp., New York.
20. **Seeberg, E., and P. Strike.** 1976. Excision repair of ultraviolet-irradiated deoxyribonucleic acid in plasmolyzed cells of *Escherichia coli*. J. Bacteriol. **125**:787–795.
21. **Sharma, S., and R. E. Moses.** 1979. *uvrC* gene function in excision repair in toluene-treated *Escherichia coli*. J. Bacteriol. **137**:397–408.
22. **Shimada, K., H. Ogawa, and J. I. Tomizawa.** 1968. Studies on radiation sensitive mutants of *Escherichia coli*. II. Breakage and repair of ultraviolet irradiated intracellular DNA of phage lambda. Mol. Gen. Genet. **101**:245–246.
23. **Shizuya, H., and D. Dykhuizen.** 1972. Conditional lethality of deletions which include *uvrB* in strains of *Escherichia coli* lacking deoxyribonucleic acid polymerase I. J. Bacteriol. **112**:676–681.
24. **Strike, P.** 1978. DNA synthesis and degradation in UV-irradiated toluene treated cells of *E. coli* K12: the role of polynucleotide ligase. Mol. Gen. Genet. **157**:99–107.
25. **Youngs, D. A., and K. C. Smith.** 1976. Single strand breaks in the DNA of the *uvrA* and *uvrB* strains of *Escherichia coli* K-12 after ultraviolet irradiation. Photochem. Photobiol. **24**:533–541.