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Separate Branches of the *uvr* Gene-Dependent Excision Repair Process in Ultraviolet-Irradiated *Escherichia coli* K-12 Cells; Their Dependence upon Growth Medium and the *polA*, *recA*, *recB*, and *exrA* Genes

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The extent of repair of single-strand breaks (incision breaks) induced in the deoxyribonucleic acid (DNA) of *Escherichia coli* K-12 cells by the *uvr* gene-dependent excision repair process after ultraviolet (UV) radiation was determined in the wild-type, *polA1*, *recA56*, *recB21*, and *exrA* strains. The wild-type strain repaired all incision breaks after incident doses of UV radiation (254 nm) of ~60 J m^{-2} or less when incubated in growth medium, or ~15 J m^{-2} or less when incubated in buffer. The *polA1* strain repaired the incision breaks completely after incident doses of ~12 J m^{-2} or less when incubated in growth medium, or after ~4 J m^{-2} when incubated in buffer. The *recA13*, *recB21*, and *exrA* strains showed essentially complete repair after incident doses of 10 to 15 J m^{-2} whether the cells were incubated in buffer or growth medium. These results suggest that the *uvr* gene-dependent excision repair process may be divided into two branches, one which is dependent on the presence of growth medium and also the rec^+ exr^+ genotype, and a second which can occur in buffer (growth medium-independent) and is largely dependent on DNA polymerase I. The presence of chloramphenicol in the growth medium resulted in an inhibition of the growth medium-dependent repair occurring in wild-type and *polA1* cells and had little or no effect on the extent of repair observed in *recA56*, *recB21*, or *exrA* cells. The similarities between the growth medium-dependent and -independent branches of excision repair and two known processes for the repair of X-ray-induced single-strand breaks are discussed.

The excision repair of ultraviolet (UV)-induced lesions is dependent on the *uvr* gene products (10). The *uvrA* and *uvrB* gene products appear to be necessary for the initial deoxyribonucleic acid (DNA) incision steps (9, 10, 14, 19), whereas the *uvrC* gene product may be involved in the control of the incision and excision steps (19).

Strains of *Escherichia coli* K-12 carrying a *polA1* mutation are deficient in DNA polymerase I activity (5) and also show a slower rate of excision of UV-induced thymine dimers than the related wild-type strain (1). The *polA1* strain is partially deficient in repairing single-strand breaks in DNA (incision breaks) produced by the excision repair process (11, 17). Most of the repair of incision breaks which occurs in the *polA1* strain appears to require DNA polymerase III, since this repair is much reduced in a *polA1 polC* strain at the restrictive temperature (29). Thus, both DNA polymerases I and III appear to be involved in the DNA repair resynthesis step of the excision repair process.

Masker et al. (15) have shown that DNA polymerase II (deficient in *polB* mutants [2]) is involved in UV-induced repair replication in toluene-treated cells, which lack both DNA polymerase I and normal DNA replication. However, no role for DNA polymerase II in vivo has yet been demonstrated. DNA polymerase II is probably not involved in a major way in repair processes in vivo, since wild-type or *polA1* cells which also contain a *polB* mutation are no more sensitive to UV or X radiation than the related $polB^+$ strains (2, 30).

Cooper and Hanawalt (3, 4) have shown that a *recA recB* strain is deficient in the production of "large patches" of DNA by repair resynthesis, thus implicating the *recA* and/or *recB* gene products in the excision repair process. The *exrA* strain is also partially deficient in the

excision repair process as shown by its deficiency in the repair of incision breaks (28) and by the finding that 2,4-dinitrophenol sensitizes wild-type cells, but not *exrA* or *uvr* strains, to killing by UV radiation (E. Van der Schueren and K. C. Smith, Photochem. Photobiol., in press). In addition, the *recA*, *recB*, and *exrA* strains lack a branch of the *uvr* gene-dependent excision repair process which can be inhibited by an impurity present in certain brands of agar (Photochem. Photobiol., in press). This suggests that the *recA*, *recB*, and *exrA* strains are all deficient in the same branch of the excision repair process.

In this paper we have examined the extent of repair of incision breaks in wild-type, *recA56*, *recB21*, *exrA*, and *polA1* cells incubated in buffer or growth medium. The results indicate that the *uvr* gene-dependent excision repair process may be divided into two branches, one which is dependent on growth medium and on the rec^+ exr^+ genotype and can be inhibited by chloramphenicol, and a second which can occur in buffer and is mainly dependent on DNA polymerase I.

MATERIALS AND METHODS

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

Media. The cells were grown in a minimal medium (MM) (7) supplemented with thiamine hydrochloride at 0.5 μg/ml and, when necessary, 10^{-3} M L-methionine and thymine at 10 μg/ml for overnight cultures or 2 μg/ml for exponentially growing cells. DTM buffer (12) was MM medium without glucose, thiamine, or supplements.

Chloramphenicol (chloromycetin) was obtained from Parke, Davis and Co. and was freshly prepared for each experiment.

Alkaline sucrose gradients. Overnight cultures were diluted 1:50 into MM medium containing 100 μCi of [*methyl*-^{3}H]thymine (New England Nuclear Corp.; 12 to 18 Ci/mmol) per ml and grown for about 3 generations at 37 C to exponential phase (10^8 to 2×10^8 cells/ml). Deoxyguanosine (100 μg/ml) was present in the MM medium for exponentially growing cells which did not require thymine. Cells were collected on 0.45-μm membrane filters (Millipore Corp.) and resuspended in 10 ml of DTM buffer (5×10^7 to 1×10^8 cells/ml). UV irradiation was done with a General Electric germicidal lamp (8 W) emitting primarily at 254 nm, with the sample in a 90-mm-diameter glass Petri dish on a rotary shaker. The incident dose rate was ~1 J m^{-2} s^{-1} and was accurately determined with an International Light, Inc., germicidal photometer (no. IL-254) before each experiment. The dose rate was reduced to ~0.2 J m^{-2} s^{-1} for the more sensitive mutants by insertion of wire mesh grids. Irradiation and subsequent handling of the cells was carried out under General Electric "gold" fluorescent lights to prevent photoreactivation. After irradiation, samples were diluted 1:1 either in DTM buffer or in MM medium containing twice the normal concentrations of glucose, thiamine, and supplements, and incubated for 80 min at 37 C. When desired, chloramphenicol was added to the post-irradiation incubation medium to give a final concentration of 50 μg/ml.

Approximately 10^6 cells (in 0.05 ml) were layered onto a 0.1-ml cap of 0.5% Sarkosyl (Geigy NL30) and 0.01 M ethylenediaminetetraacetic acid in 0.5 N NaOH on top of a 4.8-ml linear alkaline sucrose gradient (5 to 20% [wt/vol] in 0.1 N NaOH) (27). After a lysis time of 40 to 60 min at room temperature, the gradients were centrifuged at 30,000 rpm at 20 C in an SW50.1 rotor for 105 min. The procedures for fractionating and processing the gradients have been described (13). The number of single-strand breaks was calculated as described by Town et al. (24, 26).

UV-induced DNA degradation. The cells were labeled with [*methyl*-^{3}H]thymine (25 μCi/ml), irradiated, and incubated as described above. After incubation for 80 min at 37 C, duplicate samples were placed on filter paper disks which had been soaked in 10% trichloroacetic acid and dried. The techniques for processing the disks have been described (24).

Strain construction. Strain DY130 was formed by transduction of the *recB21* and thy^+ markers from strain AB2470 into strain DY98, using P1kc (31). The initial selection was for thy^+ transductants with subsequent screening for X-ray sensitivity to detect transductants which also received the *recB21* marker.

TABLE 1. *List of strains*[a]

No.	Genotype	Source or reference
DY98	*lacZ thy str metE*	31
DY99	*lacZ thy str metE exrA*	31
DY100	*lacZ thy str metE polA1*	31
DY130	*lacZ str metE recB21*	See text
MM450	*rha lacZ str recA56*	M. Monk
AB2470	*leu ara pro lac tsx gal his str xyl mtl arg thi thr recB21*	A. J. Clark

[a] Symbols are as used by Taylor and Trotter (22). All strains are derivatives of *E. coli* K-12 and are of the F^- mating type. All strains, except AB2470, were derived from the W3110 strain.

RESULTS

E. coli K-12 wild-type cells introduce single-strand breaks (incision breaks) in their DNA during the excision repair of UV damage. As the single-strand breaks are repaired, new breaks are produced by the incision endonuclease until the nuclease-sensitive sites have been removed (9). Excision repair is normally completed in ~60 min, and any incision breaks remaining at this time are repaired only at a very slow rate, if at all (28). After high incident doses of UV radiation, complete repair does not occur even

after 2 to 3 h of incubation, and the number of single-strand breaks remaining unrepaired increases with the dose (28).

Figure 1 shows the number of single-strand breaks remaining in the DNA of wild-type cells which were incubated for 80 min at 37 C in MM medium, DTM buffer, or MM medium containing chloramphenicol (CAP) at 50 μg/ml. The dashed lines represent the pooled *recA*, *recB*, and *exrA* results (from Fig. 3 to 5) for the indicated incubation condition. After incubation in MM medium (Fig. 1B), essentially complete repair was observed after incident doses of UV radiation of ~60 J m^{-2} or less. As the incident dose of UV radiation increased above 60 J m^{-2}, the number of unrepaired single-strand breaks increased at a rate of 0.31 per single-strand genome per J m^{-2}. By using a value of 60 pyrimidine dimers per *E. coli* genome per J m^{-2} (9), about one single-strand break remained unrepaired for every 100 dimers produced after doses greater than 60 J m^{-2}.

If the wild-type cells were incubated in DTM buffer (Fig. 1A) or in MM medium containing chloramphenicol (Fig. 1C), complete repair during the 80-min incubation period occurred only after incident doses of 15 to 20 J m^{-2} or less. As the incident dose increased above this threshold value, an increasing number of breaks remained unrepaired.

The *polA1* cells, similar to the wild-type strain, repaired incision breaks completely after a higher incident dose of UV radiation in MM medium than in DTM buffer or in MM medium containing chloramphenicol (Fig. 2). Essentially complete repair was observed after 10 to 12 J m^{-2} or less in MM medium (Fig. 2B) but only after ~5 J m^{-2} or less in DTM buffer or MM medium with chloramphenicol (Fig. 2A, C). The yield of unrepaired single-strand breaks per single-strand genome in MM medium was 1.4 per J m^{-2}, or about 4.7 single-strand breaks for every 100 dimers produced after doses greater than 12 J m^{-2}. Both the wild-type and *polA1* strains appeared to repair incision breaks completely after slightly higher incident doses of UV radiation in MM medium with chloramphenicol than in DTM buffer.

The *recA56*, *recB21*, and *exrA* strains all appeared to be deficient in the growth medium-dependent repair of incision breaks which was observed in the wild-type and *polA1* strains. Essentially complete repair of incision breaks occurred after incident doses of UV radiation of 11.7 J m^{-2} (average threshold value) or less with the *recA56* (Fig. 3), *recB21* (Fig. 4), or *exrA* (Fig. 5) strains, whether the cells were incubated in DTM buffer, MM medium, or MM

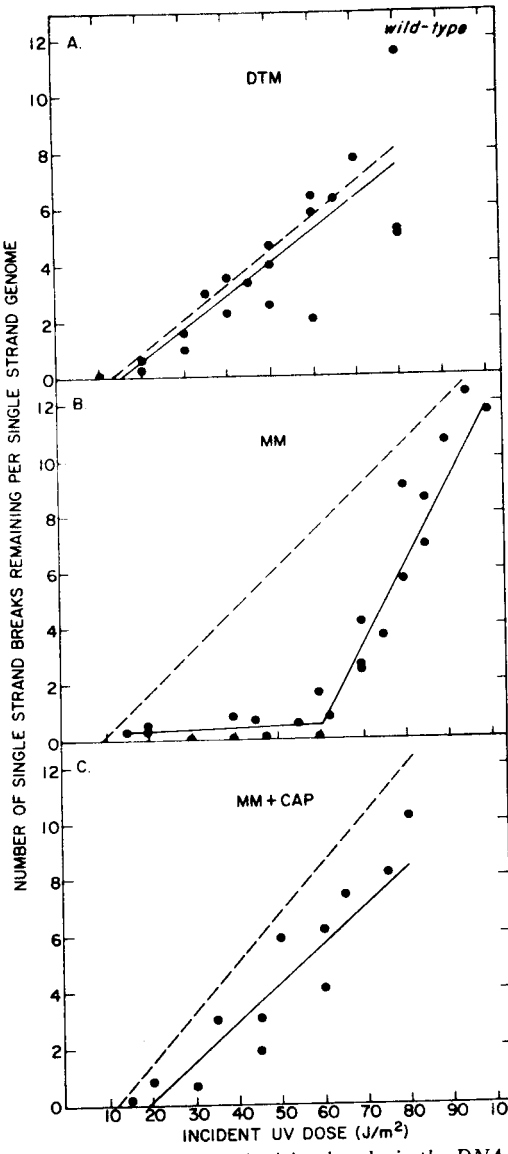

FIG. 1. *The repair of incision breaks in the DNA of E. coli K-12 wild-type (DY98) cells. The cells were labeled with [³H]thymine during several generations of exponential growth in MM medium. Samples were irradiated in DTM buffer and then incubated for 80 min at 37 C in DTM buffer (A), MM medium (B), or MM medium containing chloramphenicol (CAP) at 50 μg/ml (C). The yield of single-strand breaks was determined by alkaline sucrose gradient centrifugation techniques. The solid lines were fitted to the data shown in each figure by linear regression analysis. For Fig. 1B two lines were fitted, one for incident doses of UV radiation less than 60 J m^{-2} and a second for incident doses of 60 J m^{-2} or greater. The dashed lines were drawn from linear regression analyses of data pooled from Fig. 3 to 5 (strains exrA, recB, and recA) for the indicated incubation condition.*

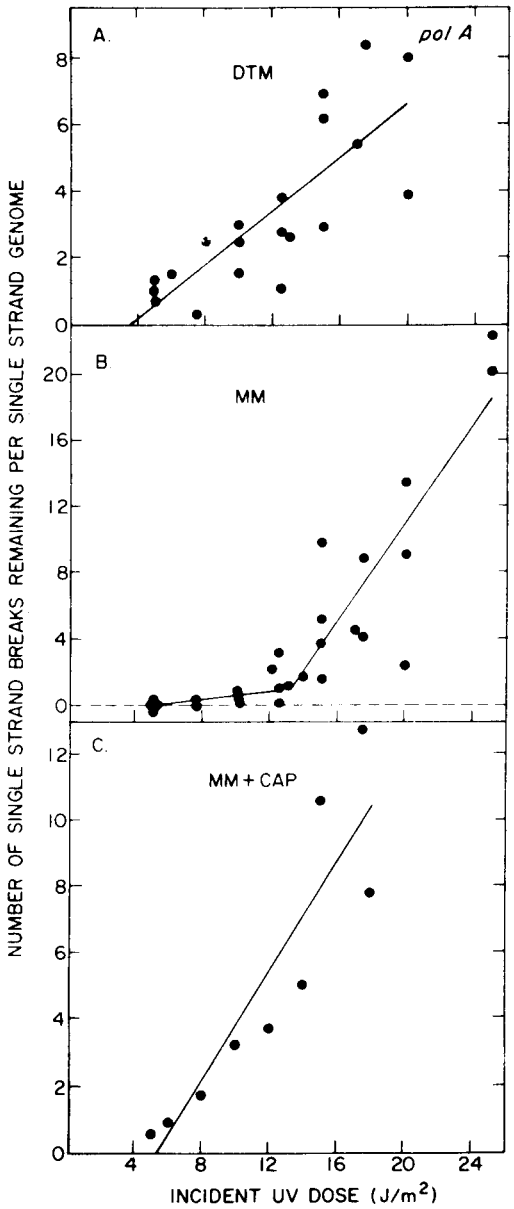

FIG. 2. *Repair of incision breaks in the DNA of the polA1 (DY100) strain of E. coli K-12. See Fig. 1 for procedures. For Fig. 2B two lines were fitted, one for incident doses of 10 J m⁻² or less and a second for incident doses greater than 10 J m⁻². Note the different scale for the y-axis of Fig. 2B.*

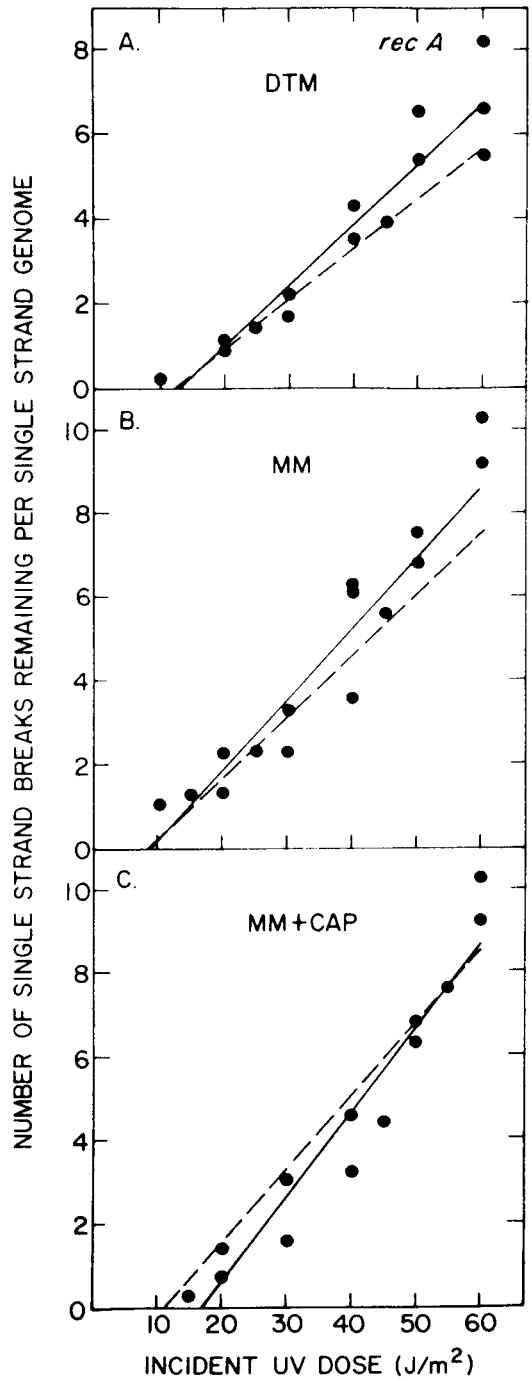

FIG. 3. *Repair of incision breaks in the DNA of the recA56 (MM450) strain of E. coli K-12. See Fig. 1 for procedures. The incubation conditions are indicated on the graphs. The dashed lines represent the pooled data from Fig. 3 to 5 for the indicated incubation conditions.*

medium containing chloramphenicol. Small differences in the threshold values were observed, but these were not significant (Student's *t* test).

At incident doses of UV radiation greater than the threshold value, the number of un-

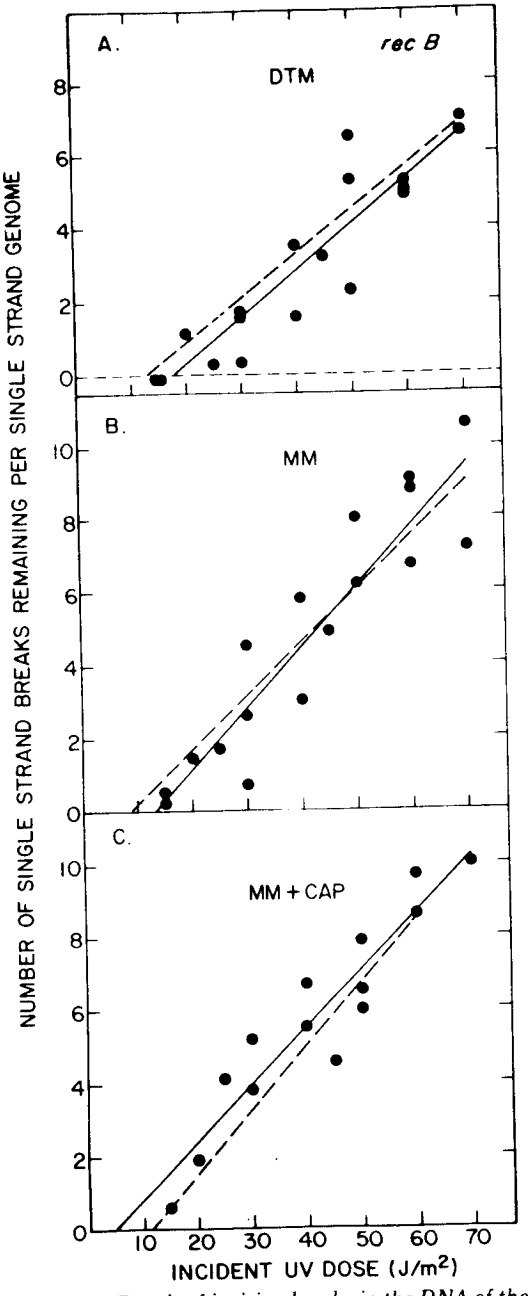

FIG. 4. *Repair of incision breaks in the DNA of the recB21 (DY130) strain of E. coli K-12. See Fig. 1 for procedures. The incubation conditions are indicated on the graphs. The dashed lines represent the pooled data from Fig. 3 to 5 for the indicated incubation conditions.*

repaired single-strand breaks increased more rapidly in MM medium or MM medium containing chloramphenicol than in DTM buffer. The difference was greatest with the *polA1* (Fig. 2) and wild-type (Fig. 1) strains but was also noticeable with the *recA*, *recB*, and *exrA* strains (Fig. 3 to 5).

The extent of UV-induced DNA degradation in each of the strains is shown in Table 2. The

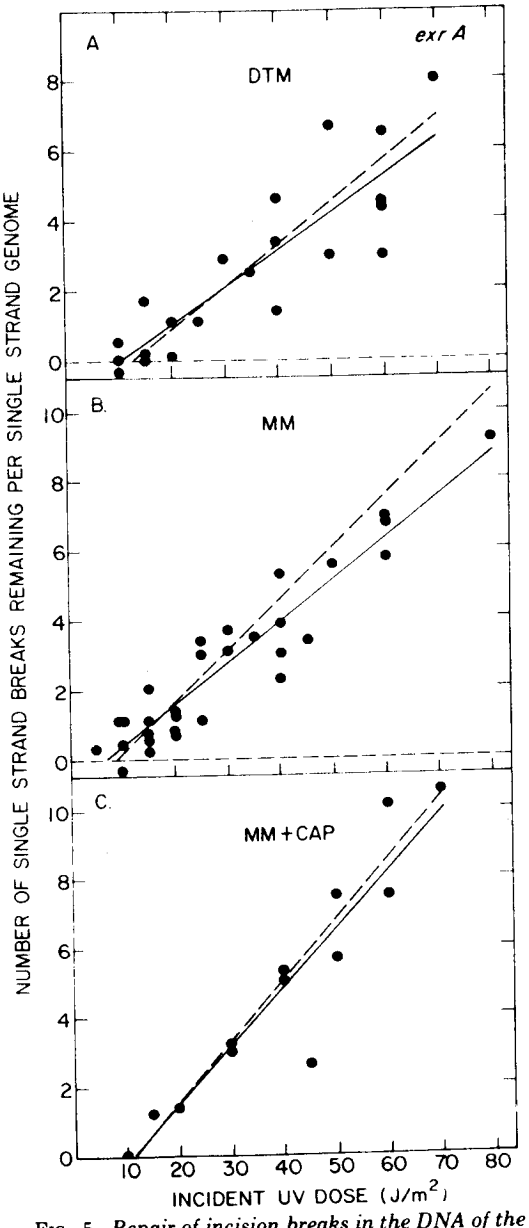

FIG. 5. *Repair of incision breaks in the DNA of the exrA (DY99) strain of E. coli K-12. See Fig. 1 for procedures. The incubation conditions are indicated on the graphs. The dashed lines represent the pooled data from Fig. 3 to 5 for the indicated incubation conditions.*

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

Strain	Incubated for 80 min in:	Labeled DNA (%) remaining acid insoluble after indicated UV dose (J m^{-2})							
		5	10	20	40	60	80	100	120
Wild type (DY98)	DTM		90.8	91.0	83.6	74.0	69.9	65.8	64.9
	MM		95.6	91.0	87.3	66.9	40.9	39.2	37.9
	MM + CAP		86.6	71.8	55.7	44.7	36.8	34.5	18.1
polA1 (DY100)	DTM	99.3	97.6	72.7	31.2	27.1			
	MM	98.3	74.6	30.9	16.6	13.3			
	MM + CAP	75.7	49.2	20.8	12.2	10.2			
exrA (DY99)	DTM		90.8	79.1	71.4	66.0	61.7		
	MM		70.0	58.7	40.5	31.4	26.3		
	MM + CAP		74.1	60.2	44.1	34.3	31.2		
recA56 (MM450)	DTM		86.5	83.1	74.0	75.5	71.0		
	MM		63.1	52.3	38.5	30.3	28.5		
	MM + CAP		70.1	57.3	43.5	34.0	33.0		
recB21 (DY130)	DTM		96.6	88.4	91.0	87.9	85.8		
	MM		90.1	84.8	85.0	80.3	76.9		
	MM + CAP		90.9	83.1	80.6	81.3	72.6		

[a] The values listed indicate the percentage of radioactivity remaining trichloroacetic acid-insoluble relative to an unirradiated sample incubated under the same conditions. The data shown are the average of two experiments.

conditions used were the same as for the gradient experiments (Fig. 1 to 5). The *recB21* strain showed only a small amount of DNA degradation, in agreement with previous results (6). The wild-type, *polA1*, *exrA*, and *recA* strains degraded their DNA more extensively in MM medium than in DTM buffer. Chloramphenicol increased DNA degradation in the wild-type and *polA1* strains but had little or no effect on degradation in the *recA*, *recB*, or *exrA* strains.

DISCUSSION

Incision breaks are induced in the DNA of *E. coli* K-12 cells by the excision repair process after UV irradiation. In the wild-type and *polA1* strains, more extensive repair of incision breaks occurred when the cells were incubated in growth medium than in buffer. These results suggest that the excision repair process can be operationally divided into two branches, one which requires growth medium and a second which does not.

DNA polymerase I does not seem to be involved in the growth medium-dependent branch of excision repair, since both wild-type and *polA1* cells repaired the incision breaks more extensively in growth medium than in buffer (Fig. 1, 2). Rather, DNA polymerase I appears to act mainly in the growth medium-independent branch of excision repair. This is indicated by the observation that wild-type cells repaired the incision breaks completely in buffer after ~15 J m^{-2} or less (Fig. 1A), whereas, under the same conditions, the *polA1* strain showed complete repair only after ~4 J m^{-2} or less. Thus, the *polA1* strain was only about 25% as efficient as the wild-type strain in performing the growth medium-independent repair of incision breaks.

The *recA56*, *recB21*, and *exrA* strains appear to lack the growth medium-dependent branch of excision repair. Complete repair was observed with these strains after incident doses of UV radiation of 10 to 15 J m^{-2} or less whether the cells were incubated in buffer or growth medium. The threshold values obtained for the *recA56*, *recB21*, and *exrA* strains in buffer or growth medium do not differ significantly (Student's *t* test). Thus, the growth medium-dependent repair seems to require the *rec*$^+$ *exr*$^+$ genotype.

The results obtained with wild-type cells incubated in buffer agree well with the pooled results (the dashed line) for the *recA56*, *recB21*, and *exrA* strains incubated in buffer (Fig. 1A). This indicates that the *rec* and *exrA* strains are not significantly deficient in the growth medium-independent repair of incision breaks.

Chloramphenicol inhibited the growth medium-dependent repair of incision breaks in wild-

type (Fig. 1) and *polA1* (Fig. 2) cells but had no significant effect on the extent of repair in the *recA56*, *recB21*, or *exrA* strains (Fig. 3 to 5).

A schematic presentation of the postulated branches of the *uvr* gene-dependent excision repair process is given in Fig. 6. The growth medium-dependent branch of excision repair is inactivated by the *exrA*, *recA*, and *recB* mutations, and by chloramphenicol treatment, as shown by the present results. The *recC* mutation should have an effect similar to that of *recB*, since these two genes appear to be the structural genes for a particular adenosine 5'-triphosphate-dependent nuclease (23). About 75% of the growth medium-independent branch is dependent on DNA polymerase I, as discussed above. Since the repair of incision breaks which occurs in *polA1* cells appears to be largely dependent on DNA polymerase III (29), we have indicated the probable involvement of the *polC* gene product in the growth medium-dependent branch and also in the residual growth medium-independent repair which occurs in *polA1* cells.

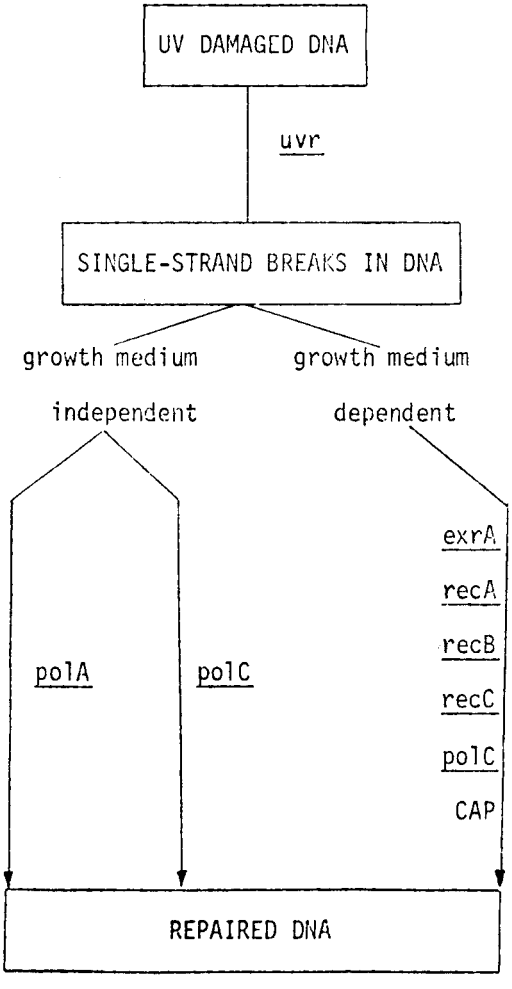

FIG. 6. *Separate branches of the excision repair process.*

The growth medium-dependent branch of the excision repair process may be the same as the *recA* and/or *recB* gene-dependent "large patch" repair described by Cooper and Hanawalt (3, 4). If this hypothesis is correct, then each of the single mutants, *recA*, *recB*, *recC*, *exrA*, and *polC*, should be deficient in "large patch" repair. In addition, this type of repair should require the presence of growth medium.

The relative abilities of strains to repair incision breaks in DNA should correlate with their survival after UV irradiation if the strains differ only in the excision repair process. The *polA1* strain appears to be deficient only in the *uvr* gene-dependent excision repair process (16). The *recA* strain (21) and, to a lesser extent, the *recB* (the *recB21* strain [DY130] completely repaired gaps in newly synthesized DNA only after ~10 J m^{-2} or less, whereas the wild-type strain [DY98] repaired the gaps completely after 20 to 30 J m^{-2} or less [unpublished results]) and *exrA* (29) strains are deficient in the post-replicational repair process as well as excision repair.

The *polA1* strain repaired incision breaks completely in MM medium after incident doses of UV radiation of 10 to 12 J m^{-2} (Fig. 2) compared to ~60 J m^{-2} for the wild-type strain (Fig. 1). Thus, the *polA1* strain had a five- to sixfold deficiency in the repair of incision breaks relative to the wild-type strain. This is in good agreement with survival data which indicate that the *polA1* strain is four to five times more sensitive than the wild-type strain (1, 28).

The nature of the breaks in the DNA which remain unrepaired after incident doses of UV radiation greater than the threshold values is not completely clear. These breaks are repaired either very slowly or not at all (28). Preliminary results indicate that some of the breaks are in fact double-strand breaks, possibly occurring as a result of excision on both DNA strands (T. Bonura, unpublished results). The characteristics of the unrepaired breaks are being investigated further.

The extent of UV-induced DNA degradation in MM medium in the wild-type, *polA1*, *recA56*, and *exrA* strains correlated inversely with their ability to repair UV-induced single-strand breaks in DNA. The *polA1*, *recA*, and *exrA* strains were deficient in the repair of incision breaks and also degraded their DNA more extensively than the wild-type strain.

The *recB* strain was also deficient in the repair of incision breaks (Fig. 4) but degraded its DNA only to a limited extent (Table 2). This is in agreement with previous observations (6) that the *recB-recC* nuclease (exonuclease V) is necessary for expression of UV-induced DNA degradation and also indicates that extensive DNA degradation is not necessary to demonstrate a deficiency in the repair of incision breaks.

Chloramphenicol inhibited the growth medium-dependent repair of incision breaks in the wild-type and *polA1* strains and also increased the extent of DNA degradation in these strains. Chloramphenicol had little effect on the extent of DNA degradation in the *recA*, *recB*, or *exrA* strains which lack the growth medium-dependent repair process.

The repair of X-ray-induced single-strand breaks in DNA has been divided into three processes (27): (i) type I, a very rapid process which appears to act preferentially on breaks produced under anoxic conditions; (ii) type II, a fast process which can occur in buffer and which requires DNA polymerase I (25) or III (31; manuscript in preparation); and (iii) type III, a slower, growth medium-dependent repair process which is deficient in *recA*, *recB*, *recC* (13; Youngs and Smith, manuscript in preparation), *exrA* (18, 31), and *polC* (Youngs and Smith, manuscript in preparation) strains.

The growth medium-dependent repair of incision breaks, which we have reported in this paper, is deficient in the *recA56*, *recB21*, and *exrA* strains (Fig. 3 to 5). This process seems analogous to the type III repair of X-ray-induced single-strand breaks. In addition, chloramphenicol inhibits the type III repair of X-ray-induced single-strand breaks (8) and also the growth medium-dependent repair of UV-induced incision breaks (Fig. 1).

The repair of incision breaks which occurs in buffer appears to be quite similar to the type II repair of X-ray-induced single-strand breaks, since each seems to require DNA polymerase I or III, or both.

Thus, the repair of single-strand breaks in DNA requires similar incubation conditions and gene products whether the breaks are induced by X radiation or by the *uvr* gene-dependent excision repair process after UV irradiation. In each case the *recA*, *recB*, and *exrA* mutations result in a deficiency in growth medium-dependent repair, whereas the *polA1* mutation affects mainly the repair which can occur in buffer.

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ADDENDUM IN PROOF

The formula used for calculating the number of strand breaks (24, 26) has been found to be in error by a factor of 4.8. When deriving this method of calculation, Town et al. (24, 26) used a phage preparation which was purchased as T2 but has subsequently been shown to be T7. Thus, the number of breaks given in this paper and in previous papers (24, 26, 27, 31) should be increased by a factor of 4.8 (assuming a molecular weight for T7 DNA of 25.2×10^6).

We thank E. Witkin for bringing to our attention two papers, concerning UV mutagenesis, by H. Nishioka and C. O. Doudney (Mutat. Res. **8**:215–228 [1969] and **9**:349–358 [1970]). They discuss the probable existance of an inaccurate, mutagenic branch of the excision repair process which may be controlled by the *exr* and *rec* genes. In addition, alkaline sucrose gradient (S. Yonei and K. Nozu, J. Mol. Biol. **65**:213–225 [1972]) and survival data (G. B. Smirnov, E. V. Filkova, and A. G. Skavronskaya, J. Gen Microbiol. **76**:407–416 [1973]) implicate the *recA* gene product in the excision repair process. These papers support the conclusions made in the present report.

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