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Involvement of the *recB-recC* Nuclease (Exonuclease V) in the Process of X-Ray-Induced Deoxyribonucleic Acid Degradation in Radiosensitive Strains of *Escherichia coli* K-12[1]

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The *ras*, *polA*, *exrA*, *recA*, and *uvrD3* strains of *Escherichia coli* K-12 degrade their deoxyribonucleic acid more extensively than wild-type strains after X irradiation. The relationship of the *recB-recC* nuclease (exonuclease V) to the degradation process in these strains was determined by comparing the degradation response of the original strains with that of strains containing an additional *recB21* or *recC22* mutation. The initial rate of degradation in *ras*, *polA12*, *exrA*, and *recA13* strains after an exposure of 20 to 30 kR was reduced more than 10-fold by the presence of an additional *recB21* or *recC22* mutation. The extent of degradation in these irradiated strains after 90 to 120 min of incubation was reduced two- to fivefold. In the *uvrD3* strain, a *recC22* mutation caused a fourfold decrease in initial degradation rate and reduced the extent of degradation after 90 min of incubation by a factor of 1.6. The results are consistent with the statement that the degradation process is normally dependent on exonuclease V activity. However, the observation that 10 to 30% degradation always occurred even in *recB* or *recC* strains, which lack this enzyme, suggests that alternative degradation mechanisms exist.

The *exrA*, *recA*, and *polA* radiosensitive mutants of *Escherichia coli* K-12 degrade their deoxyribonucleic acid (DNA) much more extensively than the related wild-type strains after X irradiation (15, 17, 19). These strains are also four to six times more sensitive to the lethal effects of X irradiation than wild-type strains (9, 11, 22). The *recA* strain (of K-12) is deficient in the slow (Rec) repair of X-ray-induced single-strand breaks in DNA (12), as is the *exrA* strain, B_{s-1} (14). The *polA* strain lacks detectable DNA polymerase I activity (6) and is deficient in the fast (Pol) repair of X-ray-induced DNA chain breaks (22).

Two other strains, *uvrD3* and *ras* also degrade their DNA extensively after irradiation (17, 23). However, these strains are only 1.5 to 2.0 times more sensitive than wild-type strains to X radiation (17, 23). The *uvrD3* and *ras* strains have been postulated to be deficient in excision repair of ultraviolet (UV) damage (13, 17), but their deficiency in terms of repair of X-ray damage is unknown.

The radiosensitive mutants *recB21* and *recC22* have been termed "cautious" with regard to their degradation response since they degrade less DNA than wild-type strains after UV irradiation (10). Emmerson (7) has shown that these strains also degrade less DNA then their *rec*$^+$ counterparts after X irradiation. In addition, the *recA13 recB21* strain degrades less of its DNA than the *recA13* strain after either UV or X irradiation but is as sensitive as *recA13* to UV or X-ray killing (12, 24). Since *recB* and *recC* strains have been shown to lack

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a nuclease activity (1, 4) of a complex nature (8, 18), these findings suggest that this nuclease (designated exonuclease V [25]) is involved in X-ray-induced degradation of DNA in wild-type and *recA13* strains. The *recB* and *recC* strains show the same X-ray sensitivity (7) and are deficient in the slow (Rec) repair of X-ray-induced single-strand breaks in DNA (12), implicating exonuclease V in the Rec repair process.

This paper reports a comparison of the degradation response of *E. coli* K-12 strains *exrA*, *recA13*, *polA12*, *uvrD3*, *ras*, and wild type with their counterparts containing an additional *recB21* or *recC22* mutation. The results confirm the earlier observations and allow further generalization as to the involvement of exonuclease V in the X-ray-induced DNA degradation process in strains which normally demonstrate more extensive DNA degradation than wild-type strains.

MATERIALS AND METHODS

Bacteria and bacteriophage. A list of the bacterial strains used, their genotypes, and the sources from which they were obtained is presented in Table 1. Phage P1kc was initially obtained from D. Morse.

Media. M9 medium was a minimal medium containing 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, 0.12 g of $MgSO_4$, 0.011 g of $CaCl_2$, and 4 g of glucose per liter. For most experiments, an enriched medium, EM9, was used. EM9 medium was M9 supplemented with 2.5 g of Casamino Acids (Difco) per liter. When necessary, M9 and EM9 media were solidified by the addition of 15 g of agar (Difco) per liter. EM9 medium was supplemented with 10 μg of tryptophan, 1 μg of thiamine, 1 μg of biotin, or 2 μg of thymidine per ml, as required for each strain.

LB was an enriched medium containing 10 g of tryptone (Difco), 5 g of yeast extract (Difco), 10 g of NaCl, and 1 g of glucose per liter (2).

Chemicals. Thymidine-*methyl*-^{3}H was obtained from Schwarz BioResearch, Inc. Specific activity of samples used was approximately 18 Ci/mmole.

Transduction and conjugation. P1kc was grown by the plate lysis method described by Bertani (2). For transduction, a fresh culture grown overnight in LB medium was mixed with P1 in the presence of 2.5 mM $CaCl_2$. The samples were incubated for 30 min at 37 C, mixed with an equal volume of 0.1 M sodium citrate, and plated on selective plates.

For bacterial mating experiments, the donor and recipient strains were grown in LB medium. Exponential-phase cells were gently mixed at a ratio of 1 donor for every 10 to 20 recipient cells. The cells were incubated without shaking at 37 C. Mating pairs were disrupted by mixing with a Vortex mixer, and recombinants were isolated by plating on selective plates.

Preparation of strains. Thymine-requiring derivatives of strains AX83 and N14-4 were isolated by the trimethoprim selection procedure described by Stacey and Simpson (20). Strains DY75, *ras thyA*,

TABLE 1. *List of strains*[a]

No.	Mating type	Relevant genotype	Other markers	Source
AX83	F⁻	*ras*	*thi str*	J. R. Walker
DY75	F⁻	*ras thy*	*thi str*	This paper
DY87	F⁻	*ras recC22*	*thi str*	This paper
N14-4	F⁻	*uvrD3*	*trp gal str*	H. Ogawa
DY81	F⁻	*uvrD3 thy*	*trp gal str*	This paper
DY85	F⁻	*uvrD3 recC22*	*trp gal str*	This paper
DY52	F⁻	*exrA thy*	*endA bio*	—[b]
DY89	F⁻	*exrA recC22*	*endA bio*	This paper
AB1157	F⁻		*thr leu arg his thi pro ara lac gal mtl xyl str tsx*	A. J. Clark
AB2470	F⁻	*recB21*	*thr leu arg his thi pro ara lac gal mtl xyl str tsx*	A. J. Clark
AB2463	F⁻	*recA13*	*thr leu arg his thi pro ara lac gal mtl xyl str tsx*	A. J. Clark
JC5489	F⁻	*recC22*	*thr leu arg his thi pro ara lac gal mtl xyl str tsx*	A. J. Clark
JC5495	F⁻	*recA13 recB21*	*thr leu arg his thi pro ara lac gal mtl xyl str tsx*	A. J. Clark
MM387	F⁻	*polA12 recB21*	*rha lac str*	M. Monk
MM386	F⁻	*polA12*	*rha lac str*	M. Monk
JC5426	Hfr KL16	*recC22*	*thr ilv spc*	J. D. Gross

[a] Symbols for genetic markers are as listed by Taylor (21).

[b] The *exrA* mutation of a *mal*$^+$ derivative of *E. coli* B_{s-1} was transduced into a K-12 *malB* strain (D. A. Youngs, Ph.D. thesis, The Univ. of Michigan, Ann Arbor, 1971).

and DY81, *uvrD3 thyA* were isolated by this technique.

The *recC22* mutation was introduced into strains DY75 and DY81 by transduction to *thy*$^+$ with P1kc grown on JC5489, a *thy*$^+$ *recC22* strain. When the *thy*$^+$ transductants were streaked for single colonies on selective plates (solidified EM9 medium supplemented with 1 μg of thiamine and 10 μg of tryptophan per ml), two types of colonies were observed: slow growers and normal-growing strains. The slow-growing strains were tentatively assigned the *rec* genotype, since *recB* or *recC* mutant strains grow slightly more slowly than *rec*$^+$ strains (5). The genotype of these strains was subsequently confirmed by demonstrating that P1 grown on the slow growers, but not the normal-growing strains, could transduce the *recC22* mutation into a *polA12 thyA* strain, giving characteristic minute, nonviable (at 42 C) *thy*$^+$ transductants in addition to the normal *polA12 thy*$^+$ *rec*$^+$ colonies (16).

The *exrA recC22* strain, DY89, was prepared by conjugation of the *exrA thyA* strain, DY52, with the *recC22 thy*$^+$ KL16 Hfr strain, JC5426, for 10 min. The *thy*$^+$ recombinants were selected on solidified M9 medium supplemented with 1 μg of biotin/ml. The resulting *thy*$^+$ colonies were of two types as described above, and their genotypes were confirmed in a similar manner.

The results of the confirmation experiments in each case supported the initial assignment of the *rec* genotype to the slow growers and *rec*$^+$ to normal-growing colonies. The strains isolated and used in these experiments were *uvrD3 recC22*, DY85, *ras recC22*, DY87, and *exrA recC22*, DY89.

DNA degradation experiments. In a typical DNA degradation experiment, a culture grown overnight in EM9 medium was diluted 1:100 into warm EM9 medium containing 10 or 20 μCi of thymidine-*methyl*-^{3}H and either 500 μg of deoxyadenosine or 250 μg of uridine per ml (3). Incubation at 37 C for about 2 hr gave 10^8 to 3×10^8 cells/ml. The labeled cells were collected on 0.45-μm membrane filters (Millipore Corp.), washed with warm medium, and resuspended in warm medium containing deoxyadenosine or uridine as indicated above and 2 μg of unlabeled thymidine per ml. Incubation was continued for 30 min to "chase" any residual, unincorporated thymidine-*methyl*-^{3}H.

Small samples in tubes were placed on ice and bubbled vigorously with oxygen for 1 min prior to X irradiation in an ice bath. The X-ray source was used at 250 kVp, 15 ma, with no added filtration. The exposure rate, measured with a Victoreen dosimeter, was 1,220 R/min.

Irradiated cells were diluted 1:12 into fresh, cold medium and incubated at 37 C. At various times during the incubation period, samples were withdrawn and added to an equal volume of cold 10% trichloroacetic acid. The mixtures were kept in an ice bath for at least 20 min. The resulting precipitates were collected on glass-fiber filters (Gelman Instrument Co.; type E), washed by filtration five times with cold 5% trichloroacetic acid, dried, and counted in a scintillation counter in toluene containing 4 g of 2,5-diphenyloxazole and 0.4 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene per liter. The results were plotted as the percentage of labeled material remaining trichloroacetic acid-precipitable as a function of time of incubation.

The *polA12* strains, MM386, and MM387, produce a temperature-sensitive DNA polymerase I which retains some in vivo activity at 30 C but none at 42 C (16). Furthermore, the *polA12 recB21* strain, MM387, is not viable at 42 C and grows only slowly at 30 C (16). For comparison of DNA degradation after X irradiation, these strains were grown and labeled at 30 C and incubated at 42 C after irradiation.

RESULTS

The results of DNA degradation experiments performed with each strain are shown in Fig. 1. The wild-type strain, AB1157, rapidly degraded about 30% of its DNA after an exposure of 20 kR (Fig. 1a). The *recB21* strain, AB2470, degraded DNA at a slower initial rate than strain AB1157, but after 2 hr had also degraded 20 to 30% of its DNA (Fig. 1a). These results suggest that exonuclease V is necessary for the maximal rate of DNA degradation to be obtained after X irradiation in strain AB1157, but they also demonstrate that the same level of degradation gradually occurs in the absence of this enzyme.

For each of the other strains, *recA13*, *polA12*, *exrA*, *ras*, and *uvrD3*, 70 to 90% degradation was normally observed after an exposure of 20 to 30 kR (Fig. 1b–f). The introduction of a *recB21* or *recC22* mutation into each of these strains resulted in a decrease in the initial rate and the extent of the observed degradation response. The presence of a *recB21* or *recC22* mutation in *recA13*, *polA12*, *exrA*, and *ras* strains limited the extent of degradation to 15 to 30% during the 90- or 120-min incubation period and decreased the initial degradation rate at least 10-fold (Fig. 1b–e).

The *uvrD3 recC22* strain (Fig. 1f) degraded its DNA more extensively and at a faster rate than the other *recB* or *recC* strains. Approximately 55% degradation occurred after 90 min of incubation, compared to 15 to 30% in the other *recB* or *recC* strains. The initial rate of degradation was threefold slower than in the closely related *uvrD3 rec*$^+$ strain, N14-4.

DISCUSSION

Emmerson (7) demonstrated that X-ray-induced degradation of DNA in a *recB* strain was much less extensive than in a *rec*$^+$ strain. Since *recB* and *recC* strains lack exonuclease V activity (1), his results suggested that exonuclease V may normally be an important compo-

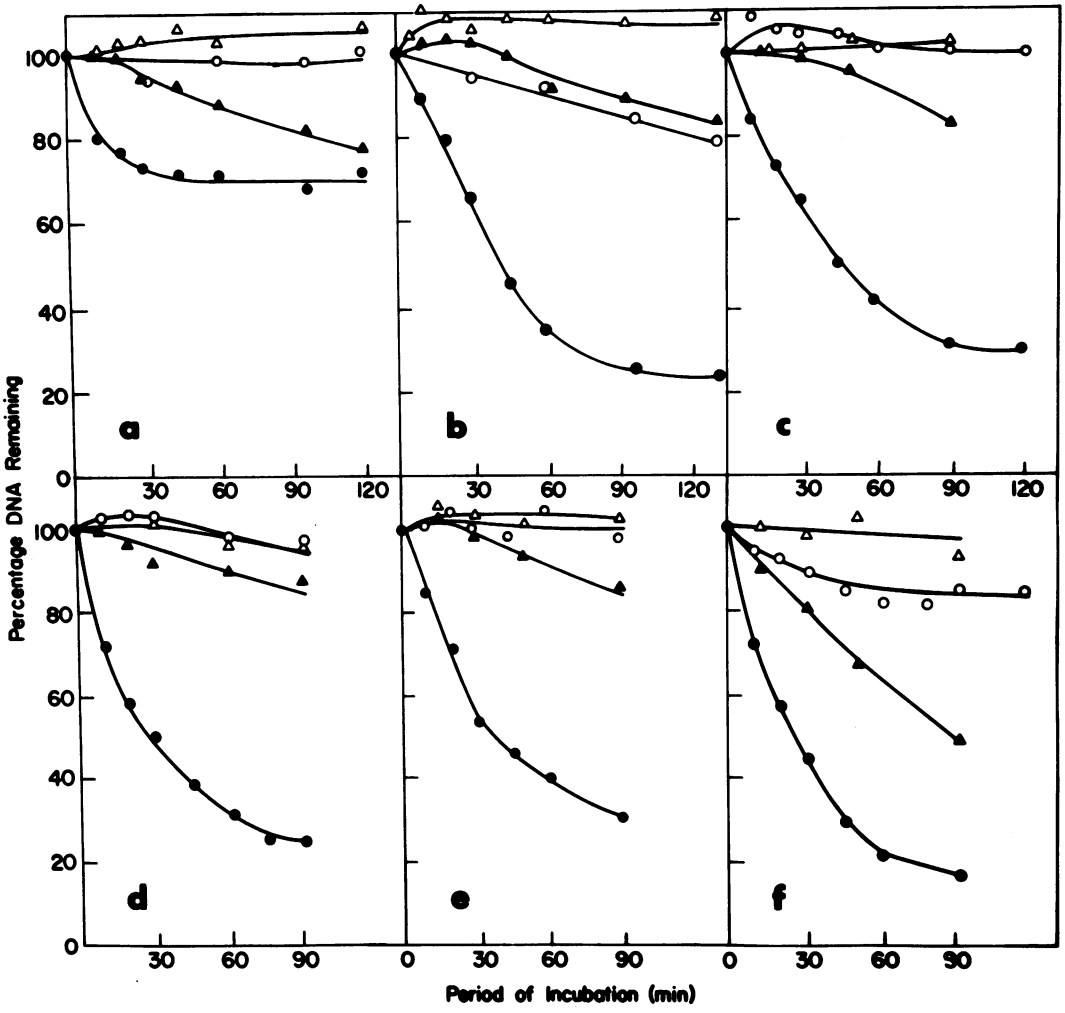

FIG. 1. *X-ray-induced degradation of DNA in recB and recC strains. Cells previously labeled with ^{3}H-thymidine were irradiated at 0 C after oxygenation. The amount of labeled material remaining trichloroacetic acid-precipitable was followed as the cells were incubated in medium at 37 C. (a) Strains recB21, AB2470* (Δ, ▲), *and rec$^+$, AB1157* (○, ●). *(b) Strains recA13, recB21 JC5495* (Δ, ▲), *and recA13 AB2463* (○, ●). *(c) Strains ras recC22, DY87* (Δ, ▲), *and ras, AX83* (○, ●). *(d) Strains polA12 recB21, MM387* (Δ, ▲), *and polA12, MM386* (○, ●). *(e) Strains exrA recC22, DY89* (Δ, ▲), *and exrA, DY52* (○, ●). *(f) Strains uvrD3 recC22, DY85* (Δ, ▲), *and uvrD3, N14-4* (○, ●). *Open symbols, 0 kR; closed, 20 kR. The only exception is* (c), *where the closed symbols indicate an exposure of 30 kR.*

nent of the degradation pathway. Similarly, the *recA13 recB21* strain was shown to degrade much less of its DNA than the *recA13* strain after UV or X irradiation, thus implicating exonuclease V in the excessive degradation of DNA exhibited by *recA13* strains (12, 24).

The results reported here are in agreement with these earlier observations and extend the information available to the other strains known to degrade DNA extensively after X irradiation. Increased DNA degradation normally occurred in *polA12*, *recA13*, *exrA*, and *ras* strains (Fig. 1c-e). Introduction of a *recB21* or *recC22* mutation into these strains drastically reduced the initial rate and extent of degradation. The results with the *polA12 recB21*, *recA13 recB21*, *exrA recC22*, and *ras recC22* strains were quite similar to those obtained with *recB21* (Fig. 1a). Thus, the extensive DNA degradation normally observed in the *polA12*, *recA13*, *exrA*, and *ras* strains after X irradiation appears to be highly de-

pendent on the presence of exonuclease V. However, in each case limited degradation of DNA, generally 15 to 30%, did occur in *recB* and *recC* strains. These results suggest that, although exonuclease V is necessary for the maximal degradation of DNA, other pathways for this degradation can exist.

Extensive degradation of DNA was normally observed in the *uvrD3* strain (Fig. 1f), as in the other radiosensitive strains. The presence of a *recC22* mutation in the *uvrD3* strain decreased the initial rate of degradation only about three-fold, compared to at least a 10-fold decrease in other double mutants containing a *recB* or *recC* mutation. The extent of degradation observed with the *uvrD3 recC22* strain was also greater than in the other *recB* or *recC* mutants; 50 to 55% degradation of DNA occurred during the 90-min incubation period compared with 15 to 30% in the other *recB* or *recC* strains. These results suggest that the *uvrD3* strain may have increased activity of a nuclease, other than exonuclease V, which partially replaces exonuclease V in the X ray-induced degradation of DNA. This appears to be detrimental to the repair processes because the radiosensitivity of *uvrD3* is greater than that of *uvrD*$^+$. The suggestion of an altered nuclease activity in the *uvrD3* strain was previously made by Ogawa and Tomizawa (17), based on the dominance of the *uvrD3* mutation to *uvrD*$^+$.

The dependence of the degradation of DNA on the presence of exonuclease V in *recA*, *exrA*, and *polA* strains indicates that this enzyme is involved in the extensive DNA degradation which occurs after X irradiation in strains deficient in Rec or Pol repair as well as in wild-type strains. Since both of these repair systems have been implicated in the repair of X ray-induced single-strand breaks in DNA (12, 22), the lesion which initiates the extensive DNA degradation, either directly or indirectly, is probably an unrepaired single-strand break in the DNA.

Since the *recB* and *recC* strains have been shown to be deficient in Rec repair of single-strand breaks in DNA (12), exonuclease V seems to be involved both in repair and in the extensive degradation of DNA which occurs in the other repair-deficient strains, *polA*, *recA*, and *exrA*. Thus, the degradation of DNA induced by X irradiation may be a result of two factors: (i) limited excision of DNA by repair systems, and (ii) more extensive degradation of the DNA initiated by unrepaired damage.

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