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Some Properties of Excision-defective Recombination-deficient Mutants of *Escherichia coli* K-12

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Strains of *Escherichia coli* that carry the mutation *uvrA6* show no measurable excision of pyrimidine dimers and are easily killed by ultraviolet (UV) light, whereas strains that carry *recA13* are defective in genetic recombination and are also UV-sensitive. An Hfr strain carrying *uvrA6* was crossed with an F^- strain carrying *recA13*. Among the recombinants identified, one carrying *uvrA recA* proved to be of exceptional sensitivity to UV light. It is estimated from the UV dose (0.2 erg/mm^2 at 253.7 nm) required to reduce the number of colony-forming cells by one natural logarithm that about 1.3 pyrimidine dimers were formed in a genome of 5×10^6 base pairs for each lethal event. This double mutant is 40 times more UV-sensitive than the excision-defective strain carrying *uvrA6*. The replication of one pyrimidine dimer is generally a lethal event in strains carrying *recA13*. Spontaneous breakdown and UV-induced breakdown of the deoxyribonucleic acid (DNA) of cells of the various genotypes were estimated by growing the cells in medium containing ^{3}H-thymidine and measuring both acid-precipitable and acid-soluble radioactivity. The UV-induced degradation in strains with *recA13* did not require the *uvr*$^+$ genes and hence appears to depend upon a mechanism other than dimer excision. The greater level of survival after irradiation in Rec^+ as compared to Rec^- bacteria may be due to a recovery mechanism involving the reconstruction of the bacterial chromosome through genetic exchanges which occur between the newly replicated sister duplexes and which effectively circumvent the damaged bases remaining in the DNA.

Escherichia coli is able to repair its own deoxyribonucleic acid (DNA) after damage by ultraviolet (UV) light. It is believed that UV-induced dimers are formed between adjacent pyrimidine bases and that these pyrimidine dimers are excised in normal cells and the DNA is repaired. Wild-type *E. coli* K-12 has an effective repair system and may retain the ability to form colonies after exposure to a UV dose which forms several thousand dimers in each bacterial genome. Strains mutant at one of the *uvr* loci (e.g., *uvrA*) are unable to excise dimers and fail to survive a small UV dose (4, 12, 13, 16, 19, 20).

Mutants of *E. coli* K-12 that are defective in their ability to form recombinants when mated in suitable genetic crosses have been obtained. These strains have also proved to be UV- and X-ray-sensitive (6, 9, 14). The high sensitivity of these mutants may be due to a defect in a common enzyme system required for repair and recombination, or to a defect in a second genetic repair mechanism which in some way depends upon the machinery of recombination. If the *recA* gene (11, 23) affects DNA repair only through control of the enzymes common to repair by excision and to recombination, the *uvrA recA* double mutant might be no more sensitive than strains carrying either *recA* or *uvrA* mutations. If the *recA* gene controls a second mechanism of repair, the double mutant might be much more UV-sensitive.

Wild-type bacteria, mutants carrying *uvrA6* or *recA13*, and recombinants carrying *uvrA6 recA13* were investigated for their sensitivities to UV light or ionizing radiation and for their ability to incorporate or release radioactive thymidine (Tdr). We found that the *uvrA recA* cells were 40 times more UV-sensitive than *uvrA recA*$^+$ mutants. This result indicates that the *recA* gene may control a genetic recovery mechanism which can be detected in excision-defective as well as in normal bacteria. It is suggested that this recovery occurs after DNA replication and depends upon exchanges between sister duplexes. In cells carry-

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ing *recA* mutations, however, attempted sister exchanges appear to prevent colony formation.

MATERIALS AND METHODS

Bacterial strains. The list of strains of *E. coli* K-12 and their characteristics and origins are given in Table 1.

Media and methods. The complete and selective media and the methods used in mating the bacteria were as previously described (1, 14). Cultures of the strains to be tested for survival after UV irradiation were grown overnight without aeration in broth containing 1% NaCl, 0.5% yeast extract, and 1% tryptone (YET broth). They were diluted as appropriate and spread on YET agar plates, exposed to various doses of UV light from a 15-w, low-pressure mercury germicidal lamp giving predominantly 253.7-nm light, and then incubated in the dark overnight. The UV dose rate was measured with a General Electric germicidal light meter. To test for photoreactivation, the cells were plated, UV-irradiated, and then incubated in light of a wavelength greater than 300 nm at a distance of 4 inches from two 40-w fluorescent lamps. The fraction of cells retaining the ability to form colonies was determined from the number of colonies visible on the following day. Cultures of the strains to be tested for survival after high-energy electron irradiation were grown overnight in YET broth, and diluted 10-fold into 3XD medium (8). The cell suspensions were bubbled slowly with oxygen and exposed to (6-Mev) electrons from a linear accelerator. After exposure to doses of 10 or 20 krads, the cell suspensions were diluted and plated on YET medium. The fractions of cells surviving were again calculated from the numbers of visible colonies after incubation overnight.

To determine the amount af acid-precipitable DNA in the various strains of bacteria after UV irradiation, they were grown overnight in a glucose-salts-Casamino Acids medium EM9 (2) and were then diluted 1:10 into EM9 containing 5 μc of ^{3}H-TdR (specific activity, 7 c per mmole) and 250 μg of deoxyadenosine per ml. After growth to about 4×10^8 cells/ml, the cells were washed three times and resuspended in five times the original volume. Samples of the cell suspension were then exposed to appropriate doses of UV light and were supplemented with 40 μg of nonradioactive Tdr per ml. The control and treated cultures were then aerated at 37 C and sampled at 0.5-hr intervals. An equal volume of 10% cold trichloroacetic acid was added to each sample, which was then allowed to stand at 0 C for 1 hr. After adding sufficient serum albumin to give bulk to the precipitate, the sample was centrifuged in the cold at 10,000 rev/min for 20 min. To determine the ratio of the radioactivity in the supernatant fluid and pellet, 0.1 ml of the supernatant fluid was added to 10 ml of liquid scintillator. This scintillator contained 670 ml of toluene, 330 ml of ethyl alcohol, 2.7 g of 2,5-diphenyloxazole, and 33 mg of 1,4-bis 2-(5-phenyloxazolyl) benzine per liter. The remaining supernatant fluid and pellet were heated to 90 C for 1 hr to hydrolyze the radioactivity in the pellet, and again a 0.1-ml sample was taken and added to liquid scintillator. The radioactivity in both samples was measured in a scintillation counter, and the ratio of cold acid-precipitable to hot acid-soluble radioactivity was calculated.

To measure the uptake of radioactive Tdr after exposure to UV light, the bacteria were grown in EM9 medium to about 5×10^8 cells/ml, centrifuged, and resuspended in five times the volume of EM9. Portions of the suspension were exposed to UV light and then supplemented with 0.25 mg of deoxyadenosine per ml plus 10 μc of ^{3}H-Tdr per ml (specific activity, 7 c per mmole). The suspensions were incubated at 37 C with aeration, and 0.1-ml samples were taken at intervals up to 3 hr; these samples were pipetted onto 3-mm filter discs (25 mm in diameter) previously treated with nonradioactive Tdr and 0.1 M NaOH and were washed in cold 5% trichloroacetic acid to remove soluble radioactivity. The discs were placed in the liquid scintillator fluid and counted as before.

RESULTS

Construction of uvrA recA strains. Recombinants carrying *uvrA6* and either *recA13* or *recA14* were constructed by mating AB2437 Hfr J2 *uvrA6* with AB3033 F$^-$ *recA13* or AB3034 F$^-$ *recA14*. This was possible because, although recombination-deficient, the *recA* females form about 0.01% of the normal number of recombinants with this Hfr male when selecting for the entry of *his*$^+$ which follows *recA*$^+$. After 2 hr of mating, Arg$^+$ His$^+$ recombinants were selected and inoculated onto patch plates for replica plating. The recombinants were tested by replica plating for sensitivity to UV light, ability to propagate UV-irradiated T1 bacteriophage, and ability to form Pro$^+$ Met$^+$ recombinants on plates spread with AB2383 Hfr J2. They were tested for their ability to show plaques when plated with the male-specific phage MS2 and for their sensitivity to ionizing radiation (6-Mev electrons). The genotypes of the recombinants could be distinguished, as only those recombinants that carried a *recA* mutation were killed by the exposure to this ionizing radiation, whereas only *uvrA* recombinants showed confluent growth when printed onto UV-irradiated T1 phage (i.e., were host cell reactivation$^-$). Both *uvrA6* and *recA13* genes segregated in these crosses, and recombinants were recovered of all four possible genotypes. Single colony isolates were made of recombinants of each type and were called A_1, B_1, C_1, and D_1. The results of further tests on the newly isolated recombinants are shown in Table 2. The last column shows the numbers of recombinants formed when parental strains and recombinants of the four types were mated with AB2383 Hfr J2 for 2 hr and were plated on agar selective for Pro$^+$ Met$^+$ Str$^+$ cells. Strains A_1 and C_1 were found to yield

TABLE I. *Characteristics of E. coli K-12 strains*

Strain No.	Origin or synonym	Auxotrophic characters											Energy source utilization					Phage			Drug resistance		Sex	Injection sequence	Obtained from
		uvr	*rec*	*thr*	*leu*	*pro*	*his*	*met*	*thi*	*arg*	*thy*	*ilv*	*Lac*	*Gal*	*Ara*	*Xyl*	*Mtl*	T1	T6	λ	Str	Mc			
AB1157		+	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	S	R	S	R	R	F⁻		Adelberg
AB1886	Mutant from AB1157	A6	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	S	R	S	R	S	F⁻		Reference 2
AB2383	P4X6	+	+	+	+	+	+	B	−	+	−	+	+	+	+	+	+	S	S	(λ)	S	R	Hfr	*pro, leu, ara*	Eggertsson
AB2437	AB2383 × AB1886	A6	+	+	+	+	−	+	−	+	+	+	+	+	+	+		S	S	(λ)	R	S	Hfr	*pro, leu, ara*	Reference 13
AB2528	Mutant from AB313	+	+	+	−	+	+	+	−	+	+	−	−	+	+	+	+	S	S	(λ)	R	R	Hfr	*mtl, xyl, str*	Eggertsson
AB2463	Mutant from AB1157	+	A13	−	−	−	−	+	−	−	+	+	−	−	−	−	−	S	R	S	R	S	F⁻		References 14, 15*a*
AB2464	Mutant from AB1157	+	A14	−	−	−	−	+	−	−	+	+	−	−	−	−	−	S	R	S	R	S	F⁻		References 14, 11
AB3033	AB2528 × AB2463	+	A13	−	−	−	+	+	−	−	+	+	−	−	−			S	R		R	S	F⁻		This paper
AB3034	AB2528 × AB2464	+	A14	−		−	+	+	−	−	+	+	−	−	−			S	R		R	S	F⁻		This paper
AB2480	AB3033 × AB2437	A6	A13	+	+	−	+	+	−	+	+	+	−	−				S	R	S	R	S	—[a]		This paper
AB2481	AB3034 × AB2437	A6	A14	−	−	−	+	+	−	+	+	+	−	−	−			S	R	S	R	S	—[a]		This paper
W4520		+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+				S		*F gal*[a]		Adelberg

[a] These strains are sensitive to phage MS2 and may be defective males.

TABLE 2. *Characteristics of recombinants from Hfr uvrA6 × F⁻ recA*

Type of strain	Strain no.	Genotype[a]	UV dose to T1 phage (ergs/mm²)	Fraction forming plaques	High-energy electrons (krads)	Fraction forming colonies	UV light (ergs/mm²)	Fraction forming colonies	No. of *pro*⁺ *met*⁺ StrR recombinants[b]
Parental	AB1157	++	500	9.5×10^{-2}	20	4.4×10^{-1}	150	8.6×10^{-1}	3.8×10^{6}
	AB3033	+*recA13*	500	6.5×10^{-2}	20	3.2×10^{-4}[c]	30	3.1×10^{-3}	$< 10^{2}$
	AB2437	*uvrA6*+	500						
	AB1886	*uvrA6*+	500	2.4×10^{-5}			30	3.2×10^{-3}	
Recombinants from AB2437 *uvrA6* × AB3033 *recA13*	A1	++	500	10^{-1}	20	2.3×10^{-1}	150	9.5×10^{-1}	5.4×10^{6}
	AB2482 (B1)	+*recA13*	500	7.8×10^{-2}	20	6.7×10^{-4}	30	6.4×10^{-3}	4×10^{2}
	C1	*uvrA6*+	500	1.8×10^{-5}	20	2.8×10^{-1}	30	7.5×10^{-3}	3.5×10^{6}
	AB2480 (D1)	*uvrA6* *recA13*	500	1.6×10^{-5}	20	3.0×10^{-5}	2.0	4.8×10^{-5}	10^{2}
Recombinants from AB2437 *uvrA6* × AB3034 *recA14*	A2	++					150	7.6×10^{-1}	
	B2	+*recA14*					30	4.5×10^{-3}	
	C2	*uvrA6*+					30	3.7×10^{-2}	
	AB2481 (D2)	*uvrA6* *recA14*					2	1.3×10^{-4}	

[a] For the two groups of recombinants, the genotype is attributed.
[b] Mated with AB2383 Hfr J2 for 2 hr.
[c] For AB2463 *recA13*.

from 10 to 15% of the number of Pro⁺ Met⁺ Str⁺ recombinants obtained in a similar cross with the original strain AB1157 and were therefore considered to carry *recA*⁺; however, strains B_1 and D_1 yielded less than 0.01% of the normal number. The recombinants B_1 and D_1 permitted plaque formation when plated with the male-specific phage MS2. They nevertheless mate and accept F *gal*⁺ episomes from the Str^s F *gal*⁺ donor W4520 and form Gal⁺ Str^R colonies at 1 to 2% of the normal frequency. However, as the yield of Pro⁺ Met⁺ recombinants in the cross with AB2383 was less than 0.01% of normal, it appears likely that both B_1 and D_1 carry *recA13*. D_1 did not appear to be heterozygous and diploid at the *uvrA* or *recA* loci, as the wild-type allele is dominant at both loci (13, 23) and the *uvrA*⁻ and *recA*⁻ phenotypes are expressed. D_1 is therefore a recombinant and not a partial diploid of the type obtained by Low (15) in crosses with a female carrying *recA* under conditions in which the *recA*⁺ gene did not enter during mating.

The recombinants were also tested for the ability to propagate UV-irradiated T1 bacteriophage which is absent in strains carrying the *uvrA6* mutation. Table 2 lists the fraction of UV-irradiated T1 phage able to form plaques when plated on bacteria of the four recombinant types, as well as on AB1157, AB1886 *uvrA6*, and AB3033 *recA13*. The UV-irradiated T1 phage was reactivated almost as effectively in AB3033 *recA13* and in types A_1 and B_1 of the recombinants as it was in the original strain AB1157. The UV-irradiated phage was not reactivated in AB1886 *uvrA6* or in the recombinants of types C_1 and D_1 which must carry *uvrA6*.

The ability of cells of the various strains to form colonies on complete medium after irradiation with high-energy electrons was greatly reduced in strains with *recA13*, whereas that of strains with *uvrA6* was only slightly affected (Table 2, Fig. 1). The colony-forming ability of A_1 and C_1 was of the same resistance to ionizing radiation as that of the original AB1157 and AB1386 *uvrA6* strains. However, B_1 was as sensitive as AB2463 *recA13* and type D_1 was still more sensitive.

The survival of colony-forming ability after UV irradiation was the same in the recombinant A_1 as in the original AB1157, although both of its parents, AB2437 Hfr *uvrA6* and AB3033 *recA13*, were sensitive to UV light (Table 2). The levels of UV survival of recombinants C_1 and AB1886 *uvrA6* were similar, and those of the recombinant B_1 and AB3033 *recA13* were also similar. However, the recombinant D_1 which carries *uvrA6 recA13* was many times more sensitive to UV irradiation and is designated AB2480. AB2437 Hfr J2 *uvrA6* was also crossed with AB3034 F⁻ *recA14* (11) in the same way, and the corresponding set of recombinants, A_2, B_2, C_2, and D_2, were isolated and identified as before. D_2 was numbered AB2681. Both AB2480 and AB2481 were sensitive to the male-specific phage MS2, but this did not influence colony-forming ability after UV irradiation, as MS2^R derivatives were selected from these *recA uvrA* recombinants and found to

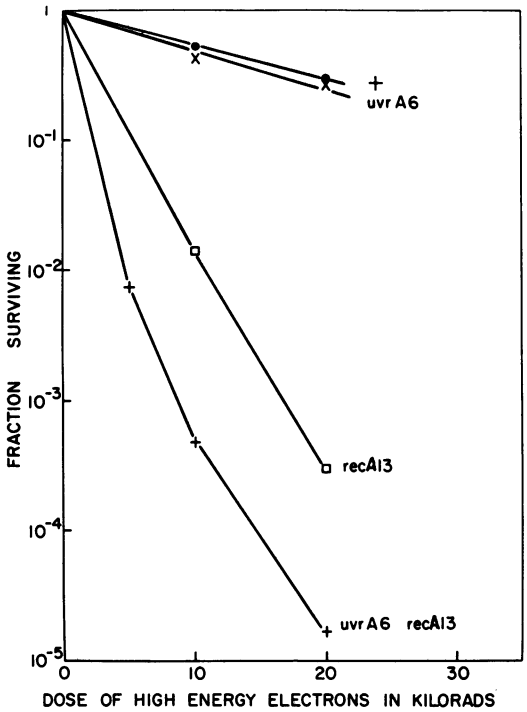

FIG. 1. *Fraction of cells forming colonies on YET agar after exposure to various doses of ionizing radiation. Overnight cultures were grown without aeration in YET broth and were resuspended in 3XD medium before being bubbled with oxygen and exposed to the electron beam from a 6-Mev electron accelerator. After irradiation, the cell suspensions were diluted, plated on YET agar, and incubated for 18 to 24 hr at 37 C. Strains used include: AB1157+, AB2463 recA13, and recombinants C1uvrA6 and AB2480 uvrA6 recA13 from the cross AB2437 Hfr uvrA6 × AB3033 recA13.*

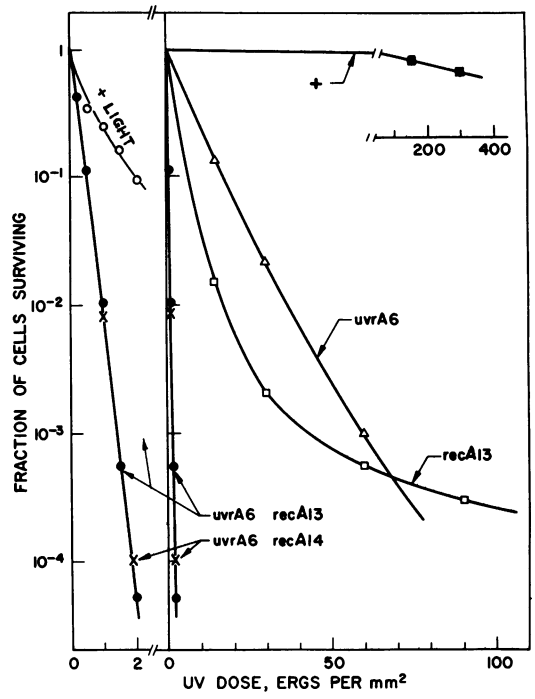

FIG. 2. *Fraction of cells forming colonies on YET agar is plotted against the UV dose. The cells were harvested after overnight growth in YET broth and were diluted and plated on YET agar. They were exposed to various doses of UV without further delay and were incubated for 18 to 24 hr at 37 C. The strains used were: AB1157, AB1886 uvrA6, AB3033 recA13, and recombinants AB2480 uvrA6 recA13 and AB2481 uvrA6 recA14. The points ○ represent AB2480 uvrA6 recA13 exposed to visible light from fluorescent lamps after UV irradiation and show that photoreactivation occurs in this strain.*

be equally UV-sensitive. Certain characteristics of these recombinants are listed in Table 2.

Figure 2 shows the fraction of cells retaining the ability to form colonies on complete medium after exposure to various doses of UV light. Results are given for the parental strains AB1157+, AB2463 *recA13*, and AB1886 *uvrA6* and for the two double mutants AB2480 *uvrA6 recA13* and AB2481 *uvrA6 recA14*, both of which are many times more UV-sensitive than the other strains. The strain AB2480 is subject to photoreactivation as the fraction of cells surviving UV irradiation is increased by incubation in the light rather than in darkness.

Figure 3 shows the effect of incubating UV-irradiated bacteria for 6 hr at 37 C in 0.02 M phosphate buffer before plating on complete medium. The number of colonies formed by AB2463 *recA13* after UV-irradiation is greatly increased by this treatment (9*a*). No effect was observed, however, with AB2480 *uvrA6 recA13*, a result in accord with the lack of any liquid holding recovery in other excision-defective mutants (17).

DNA metabolism after UV irradiation. In view of the abnormal DNA metabolism of certain recombination-deficient strains of *E. coli* (5, 14), the DNA breakdown and Tdr incorporation were investigated in AB2482 *recA13* and AB2480 *recA13 uvrA6*. To determine the spontaneous and UV-induced release of radioactive label from the DNA of these strains, the cells were labeled in their DNA by growth in medium containing ^{3}H-thymidine. The amounts of acid-soluble and acid-precipitable radioactivity were determined. As previously found with parental strain AB2463 *recA13*, only 75 to 80% of the radioactivity in the recombinant AB2482 *recA13* was acid-precipitable. The relative amount of radioactivity re-

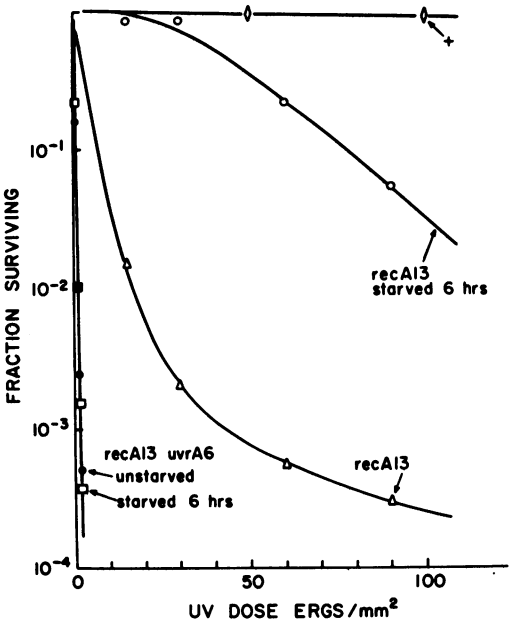

FIG. 3. *Effect of incubation in buffer on the fraction of cells forming colonies plotted against UV dose. Cells were grown and UV-irradiated as for Fig. 2, and were plated either without delay or after aeration for 6 hr at 37 C in 0.02 M phosphate buffer (pH 7.2) and 0.15 M NaCl. The strains used are AB2463 recA13, AB2480 uvrA6 recA13, and AB1157+.*

maining acid-precipitable during incubation is shown in Fig. 4. Radioactivity is released spontaneously during incubation at the rate of about 7% of the acid-precipitable radioactivity per hour (Fig. 4A). The recombinant AB2480 *recA13 uvrA6* showed about the same spontaneous release of radioactivity (Fig. 4B), but only 55 to 60% of the total radioactivity in the cell suspension was initially acid-precipitable. As exposure to UV-light increased the release of radioactivity from both strains, it appears that UV-induced DNA breakdown in the strains carrying *recA13* can be initiated by a mechanism other than dimer excision, a question to be considered in the discussion.

The extent of DNA replication after exposure to UV light in cells unable to excise pyrimidine dimers was investigated next (21, 22). Cultures of exponentially growing cells were exposed to various doses of UV light, incubated with radioactive Tdr, and sampled at intervals to determine the amount incorporated into acid-precipitable form. As shown in Fig. 5, 200 ergs/mm² inhibited uptake in all strains. Incorporation resumed after a short delay in the wild-type strain. A dose of 20 ergs/mm² reduced the uptake to about 5% of the control in the strain with *recA13* and reduced the

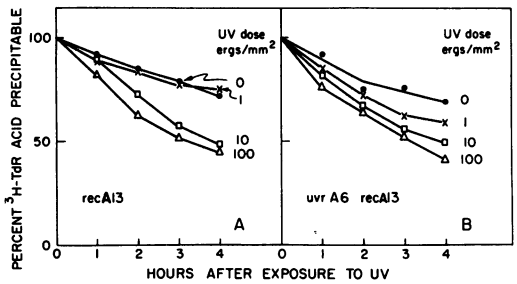

FIG. 4. *³H-radioactivity remaining in acid-precipitable form expressed as a percentage of the initial level, during incubation after exposure to UV light. Results are given for the recombinants AB2482 recA13 in Fig. 3A and AB2480 uvrA6 recA13 in Fig. 3B. Cells were labeled in their DNA by growth with ³H-Tdr to late log phase, washed, exposed to various UV doses, and incubated in M9 medium. The complete acid-precipitable and acid-soluble radioactivity was determined for the suspension containing both cells and medium. The amount of radioactivity in the washed cells that was acid-soluble after a zero UV dose was 23% in AB2482 recA13 and 44% in AB2480 recA13 uvrA6.*

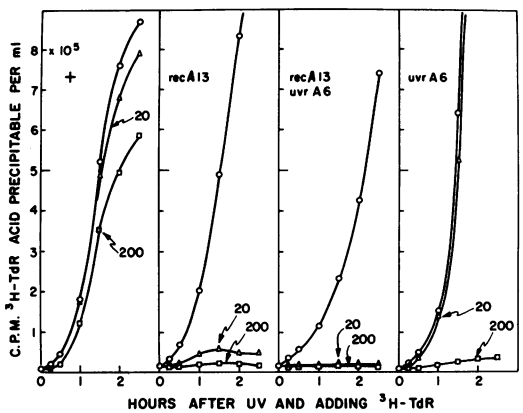

FIG. 5. *³H-Tdr radioactivity incorporated into exponentially growing cells during incubation with ³H-Tdr after exposure to UV light. The strains used were, from left to right: AB1157+, AB2482 recA13, AB2480 recA13 uvrA6, and AB1886 uvrA6. Logarithmically growing cells were harvested at about 2 × 10⁸ cells per ml, washed, exposed to UV light, and then incubated in the presence of ³H-Tdr and deoxyadenosine. The amount of the acid-precipitable radioactivity was determined at intervals during incubation. The UV doses in ergs/mm² at 253.7 nm are indicated by the numbers in the figure.*

uptake to even lower levels in the strain with *uvrA6 recA13*. However, the strain with *uvrA6* incorporated at a rate of more than 50% of that of the wild type, even though this dose produced about 130 dimers per genome and these presumably remained in situ. Evidently, dimers may delay,

but do not block, the incorporation of radioactive Tdr in this strain. A greater inhibition was found with the strains carrying *recA13*, as if the incorporation of exogenous Tdr is inhibited more by DNA breakdown than by the dimers.

DISCUSSION

The recombinants of genotype *uvrA6 recA13* and *uvrA6 recA13* are exceptionally sensitive to UV light (Fig. 2). The dose required to reduce the number of cells able to form colonies by one natural logarithm (i.e., to give a lethal hit to each cell) is 0.2 erg/mm^2. Assuming that the yield for 1 erg/mm^2 of 253.7-nm light is 1.3×10^{-6} pyrimidine dimers per base pair (2, 20, 21, 24) and that there are 4×10^6 to 5×10^6 base pairs per genome in *E. coli* (3, 7), it can be calculated that 1 to 1.3 dimers are formed per lethal event per genome in these double mutants. The extreme UV sensitivity of these cells might be due to the combined action of two mutations, each causing a partial block in DNA repair by excision. However, the fact that double mutants of the type *uvrA uvrB* are not appreciably more UV-sensitive than single mutants carrying *uvrA6* (13) renders this explanation unlikely.

In comparison with the *uvrA6 recA13* double mutant, the strains carrying *uvrA recA*$^+$ are 40 times more resistant. A UV dose of about 8 ergs/mm^2 is required to reduce the number of colonies formed by these cells by one natural logarithm (Fig. 2), so that about 50 pyrimidine dimers are formed in a bacterial chromosome of 5×10^6 base pairs per lethal event, even though the dimers are not excised in these mutants. These and other experiments may help us to understand the radiation resistance of Rec$^+$ as compared to Rec$^-$ cells and also the mechanism by which cells carrying *recA* or *recA uvrA* suffer a lethal event after the induction of small numbers of pyrimidine dimers in their DNA.

The results in Fig. 3 show that the *recA* mutant exhibits a substantial recovery in its colony-forming ability (9*a*). Cells exposed to 30 ergs/mm^2, for example, show 0.2% survival when plated on complete medium but almost 100% survival when their growth is delayed by starvation in buffer for 6 hr. This liquid-holding recovery may reflect the excision of dimers from the DNA before lethal metabolic events can take place (17). In contrast, the *recA urvA* double mutant exhibits no increase in survival after UV irradiation when incubated in buffer before plating, and no cells survived this UV dose. It appears that the replication of a single pyrimidine dimer is frequently lethal in cells carrying *recA*.

When DNA containing pyrimidine dimers is replicated in Rec$^+$ cells, the newly synthesized strands are discontinuous and appear to have gaps opposite each dimer, as shown in Fig. 6A and 6B (13*a*, 18). Genetic exchanges between newly replicated sister duplexes may be induced by the dimers and opposing gaps (13*a*, 22*a*) and may promote the reconstruction of a viable chromosome, even though dimers remain in the individual strands. This reconstruction by sister exchanges can occur in normal and in excision-defective cells and may be responsible for the greater UV resistance of Rec$^+$ as compared to Rec$^-$ organisms.

When DNA containing pyrimidine dimers is replicated in cells carrying *recA13*, however, the dimers and opposing gaps so formed may induce abortive exchanges in which one strand is cut for each new one formed at replication (Fig. 6C and 6D). This hypothetical model is consistent with the idea of a failure in a repair process (5) and is in accord with three observations: (i) about 1.3 dimers are formed per lethal event in the *uvrA recA* double mutant; (ii) these photoproducts are subject to photoreactivation by visible light; and (iii) larger numbers of dimers are tolerated in the *recA* mutant, as this strain is able to excise dimers before replication in numbers dependent upon the growth conditions and the time available. Moreover, cells carrying *recA13* exhibit a spontaneous breakdown of their DNA during growth which can be further increased by exposure to UV light (5, 14). UV-induced DNA breakdown in strains carrying *recA13* is not restricted to *uvr*$^+$ strains, but is almost independent of the capacity of the cell to excise dimers (compare Fig. 4A and 4B).

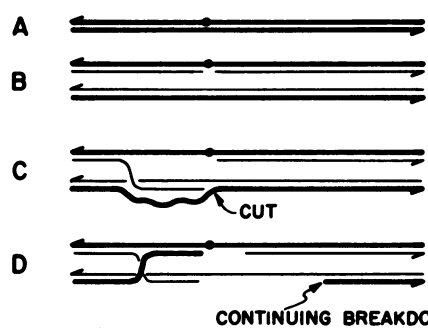

FIG. 6. *Proposed mechanism for a lethal event in the uvrA recA strain caused by a single pyrimidine dimer. (A) Bacterial DNA containing a UV-induced pyrimidine dimer. (B) After replication, a gap is left in the newly synthesized daughter strand at a point opposite the dimer. (C) One of the free ends thereby created in the daughter strand is able to pair with the complementary daughter strand. The next step in recombination may involve the parental strand being cut, as shown. (D) As the strain carries recA13, the cut is not healed and leads to continuing breakdown. Consequently, the genome is never successfully replicated.*

Thus, the breakdown of labeled DNA can be initiated in other ways than by dimer excision and might be due, for example, to the inability of these cells to join strands when recombination is attempted.

The induction of mutations by UV light is greatly reduced in recombination-deficient mutants (15*a*). This is to be expected if UV mutagenesis depends upon the replication of DNA past UV photoproducts (18, 23*a*) and if, as suggested here, this is lethal in mutants carrying *recA*.

Strains carrying *recA13* or both *recA13* and *uvrA6* have been examined for lethal sectors in colony-formation and have been found to give rise to many nonviable daughter cells (10). This is another interesting manifestation of the repair defects in these strains.

In conclusion, it appears that *E. coli* contains a mechanism for survival after UV irradiation that is additional to DNA repair by dimer excision and is effective in Rec^+, but not in Rec^-, cells. This mechanism is most easily demonstrated in excision-defective mutants. It may operate after the replication of the damaged DNA and may depend upon the newly synthesized strands being formed with gaps opposite the pyrimidine dimers. Recombination between the newly replicated sister duplexes may be induced by the dimers and opposing gaps and may lead to the reconstruction of a viable bacterial chromosome even though unexcised dimers remain in the damaged strands. Mutants carrying *recA* are frequently killed if they replicate DNA containing a single pyrimidine dimer.

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