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Defective Excision Repair of Pyrimidine Dimers in the Ultraviolet-Sensitive *Escherichia coli ras*⁻ Mutant

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The *ras*⁻ mutant of *Escherichia coli* K-12 is sensitive to ultraviolet (UV) light but only slightly sensitive to X-irradiation (1.5-fold increase). Other phenotypic properties include normal recombination ability and normal host cell reactivation ability but an abnormally high frequency of UV-induced mutation. The response of the *ras*⁻ mutant to UV has been studied biochemically. After low doses of UV, the *ras*⁻ mutant degraded excessive amounts of deoxyribonucleic acid, and long delays in resumption of deoxyribonucleic acid synthesis occurred. Pyrimidine dimers were excised at the normal rate. Although the mutant had the capability of initiating repair replication, the process was not completed after the high UV dose required to allow detection of repair replication. The *ras*⁻ mutant, after low UV doses, left three to four times as many single-strand breaks not rejoined as did the wild-type strain.

The *ras*⁻ mutant of *Escherichia coli* K-12 is sensitive to ultraviolet (UV) light but only slightly sensitive to X-irradiation (1.5-fold increase). Other phenotypic properties include normal recombination ability and normal host cell reactivation (Hcr⁺) ability, but an abnormally high frequency of UV-induced mutation. The increased lethality after UV results from the formation of pyrimidine dimers since direct photoreactivation reversed the inactivation of colony-forming ability (24).

There are several systems which repair pyrimidine dimers in *E. coli*. The excision repair system consists of (i) a single strand incision near the dimer, (ii) excision of a single-stranded deoxyribonucleic acid (DNA) segment containing the dimer, (iii) repair replication, and (iv) rejoining the 3′-5′ phosphodiester linkage by action of DNA ligase (1, 19). Ultraviolet-sensitive *E. coli uvr*⁻ mutants presumably are defective in the incision or excision steps (7, 8). Pauling and Hamm (14, 15) isolated a mutant which might be defective in the synthesis of DNA ligase.

Defects in other pyrimidine dimer repair systems lead to increased UV-sensitivity. The UV-sensitive *rec*⁻ strains might be defective in sister strand exchanges which possibly result from the generation of gaps (opposite dimers) in DNA strands synthesized after UV (17). Strains mutant at the *exr* locus have a defect in a dimer repair system, the biochemical nature of which is uncertain (25).

The *ras*⁻ mutation, a priori, might create a defect in one of the known repair systems; alternatively, the *ras*⁻ mutant might lack a previously undiscovered repair mechanism. The response of the *ras*⁻ strain to UV has been studied biochemically. After UV there is an excessive amount of DNA degradation. DNA synthesis was delayed by UV to a much greater extent than that which occurs in the wild-type strain. Delay of DNA synthesis occurred even after UV doses too low to cause detectable DNA degradation. The excision of pyrimidine dimers occurred at the normal rate. Although the *ras*⁻ mutant initiated repair replication, the process was not completed after the high UV dose needed to detect repair replication. Although many gaps resulting from excision were rejoined, the rate of closure of the gaps was reduced in the mutant, even after low UV doses. Thus, the *ras*⁻ mutation affects the excision repair system. Possibly, a defect is created such that excision is predominant over successful repair synthesis in a small proportion of the excision repair events.

MATERIALS AND METHODS

Bacteria. The strains, all F⁻ and *thi*⁻, were AX83 and AX100, *ras*⁻ and *ras*⁺, respectively; and AX84 and AX103, *ras*⁻ *thy*⁻, and *ras*⁺ *thy*⁻, respectively.

Media. Minimal medium base (13) and the low-phosphate minimal medium base (11) with peptone omitted were supplemented with separately autoclaved glucose (10 mg/ml), thiamine-hydrochloride (5 μg/ml), and Casamino Acids (5 mg/ml). Deoxyadenosine (250 μg/ml), adenosine (250 μg/ml), uridine (250 μg/ml), thymine (50 μg/ml), thymidine (25 μg/ml), and 5-bromodeoxyuridine (BUdR) (60 μg/ml) were added separately as desired.

UV irradiation. Cells were resuspended in minimal medium base and irradiated with light from a 15-w General Electric germicidal lamp. Samples were continuously stirred magnetically. Incident intensity was 13 ergs per mm² per sec. All manipulations were carried out under dim light from General Electric Gold lamps.

DNA degradation. Strains AX83 and AX100 were grown for four generations in minimal medium plus deoxyadenosine and 0.8 μCi of ^{3}H-thymidine per ml to about 4×10^8 cells/ml. A 5-ml culture was filtered, washed with 25 ml of minimal medium base, and then resuspended in 5 ml of minimal medium base. Portions of the suspension were irradiated and then diluted into four volumes of warm, concentrated minimal medium containing deoxyadenosine and unlabeled thymidine. (After dilution, the thymidine concentration was 100 μg/ml.) At intervals, 0.2-ml samples were withdrawn, precipitated with trichloroacetic acid, filtered, and counted.

DNA synthesis. Strains AX83 and AX100 were grown as for measurements of DNA degradation, except that after UV-irradiation they were diluted into minimal medium plus deoxyadenosine and ^{3}H-thymidine to give the same specific activity as that which existed before irradiation. Samples were withdrawn during incubation after irradiation, precipitated with trichloroacetic acid, filtered, and counted.

Pyrimidine dimer excision. The procedure of Boyce and Howard-Flanders (1) was modified as suggested by Patrick (*personal communication*). The bacteria were grown four generations to about 4×10^8 cells/ml in minimal medium plus deoxyadenosine and 4 μCi of ^{3}H-thymidine per ml. They were filtered onto nitrocellulose filters, washed with two volumes of minimal medium base, resuspended in one volume of minimal medium base, and irradiated. They were diluted two-fold in warm minimal medium containing double-strength glucose, thiamine-hydrochloride, and Casamino Acids. Incubation at 37 C followed, and 1.5-ml samples were removed at 0, 40, 80, and 120 min and mixed with one volume of ice-cold 10% trichloroacetic acid. After 15 min in the cold, precipitates were centrifuged at 8,000 × *g* for 10 min, washed once with cold 5% trichloroacetic acid, washed once with diethyl ether-ethanol (1:1, v/v), and dried under vacuum over P_2O_5.

After drying, precipitates were dissolved in trifluoroacetic acid (0.3 ml) by heating at 45 C for 30 min. They were then hydrolyzed at 160 to 165 C for 90 min. The samples were evaporated under N_2, dissolved in 0.04 ml of 0.1 N HCl, and chromatographed on Whatman no. 1 paper (descending technique) in the *n*-butanol-acetic acid-water (80:12:30) system (21). Areas containing pyrimidine dimers were located with a chromatogram scanner, cut out, and counted in a liquid scintillation counter.

Background values were determined from labeled but unirradiated samples.

Repair replication. The procedure was adapted from Pettijohn and Hanawalt (16), Kanner and Hanawalt (10), and Pauling and Hamm (14). Thymidine-requiring strains AX84 and AX103 were grown about four generations in minimal medium plus ^{3}H-thymidine to about 5×10^8 cells/ml. The ^{3}H-thymidine concentration was 0.5 μCi/ml for cultures to be irradiated and 2.5 μCi/ml for control cultures which received no UV. The cells (10 ml) were centrifuged at 500 × *g* for 10 min at room temperature, washed once, and then resuspended in one volume of low-phosphate minimal medium base. The suspensions were irradiated with 1,600 ergs/mm² or kept as unirradiated controls. Irradiated and unirradiated suspensions were centrifuged and resuspended in 10 ml of warm low-phosphate minimal medium plus BUdR, adenosine, and uridine. After 10 min of incubation at 37 C, ^{32}P was added. For unirradiated cultures, 2 μCi of ^{32}P per ml was added, and an additional 13 min of incubation period was allowed; for irradiated cultures, 10 μCi of ^{32}P per ml was added and an additional 20-, 40-, or 60-min incubation period was allowed.

The cells were then centrifuged at 500 × *g* at 0 C for 10 min, washed once in 10 ml of NET-CN buffer (6), and resuspended in 0.95 ml of NET-CN buffer. Lysozyme to 200 μg/ml was added, and the samples were incubated for 20 min at 37 C. The samples were subjected to two cycles of freezing in CO_2-acetone and thawing at 37 C. Pronase to 200 μg/ml was added, and incubation at 37 C was continued for 30 min. The volume was then adjusted to 3.65 ml with NET-CN buffer.

An equal volume of chloroform-octanol (9:1, v/v) was added, and the preparations were mixed on a Vortex mixer for 10 min. At least 80% of the DNA was extracted by this procedure.

The aqueous layer (3.0 ml) was added to 3.900 g of CsCl and centrifuged in a Beckman SW50.1 rotor at 37,000 rev/min for 44 hr. The gradients were fractionated into about 80 two-drop fractions. Ribonucleic acid was hydrolyzed by adding 1.0 ml of 0.5 N NaOH to each fraction and heating at 37 C overnight. The DNA was then precipitated with trichloroacetic acid and filtered onto glass-fiber filters which were washed, dried, and counted. About 98% of the label was found in appropriate density bands near the center of the gradient.

Single-strand break rejoining. The McGrath and Williams (12) technique as adapted by Setlow and Carrier (20) for inferring single-strand break closure was used. The bacteria were grown in minimal medium plus deoxyadenosine and 10 μCi of ^{3}H-thymidine per ml to 5×10^8 cells/ml. The cells were filtered, washed, suspended in 10 volumes of buffer, irradiated if desired with 200 or 400 ergs/mm², mixed with concentrated minimal medium, and incubated. Portions were withdrawn at 0 and 60 min of incubation. The cells were harvested and converted to spheroplasts by the procedure of Rupp and Howard-Flanders (17). The spheroplasts were lysed on the surface of 5-ml

alkaline sucrose gradients and centrifuged for 120 min at 30,000 rev/min at 20 C in a Spinco SW50.1 rotor. About 47 two-drop fractions were collected on filter-paper discs (17) which were washed (12) and counted. Number average molecular weights of the DNA single strands were determined from the distribution of radioactivity in the gradient by the methods of Burgi and Hershey (2), Studier (23), and Rupp and Howard-Flanders (17).

Radioisotope counting. Paper strips and filters were counted in 10 ml of toluene containing 0.5% 2,5-diphenyloxazole and 0.01% 1,4-bis-[2-(4-methyl-5 phenyloxazolyl)]-benzene.

Radiochemicals. ^{3}H-thymidine (17.9 Ci/mmole) and carrier-free $H_3{}^{32}PO_4$ were obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

DNA degradation after UV. Strains *ras*$^-$ AX83 and *ras*$^+$ AX100 were grown in ^{3}H-thymidine to label DNA uniformly before irradiation. After UV irradiation with various doses, the bacteria were resuspended in unlabeled medium, and the extent of DNA degradation from trichloracetic acid-insoluble to trichloroacetic acid-soluble fragments was determined (Fig. 1).

In the absence of UV, neither the *ras*$^-$ nor the *ras*$^+$ strain degraded DNA. Thus, the *ras*$^-$ defect does not cause spontaneous DNA degradation, as occurs in certain *rec*$^-$ strains (9).

After a UV dose of 450 ergs/mm^2, too low to cause detectable degradation in the wild-type strain, 40% of the *ras*$^-$ AX83 DNA was solubilized. Possibly as much as 10% of the DNA was degraded after 130 ergs/mm^2 by strain AX83. After the high dose of 1,300 ergs/mm^2, the *ras*$^-$ strain solubilized DNA at a faster rate and to a greater extent than did the wild-type strain.

DNA synthesis after UV. The *ras*$^+$ and *ras*$^-$ strains were grown in ^{3}H-thymidine until the intracellular pools were equilibrated with label (22). Suspensions were irradiated and diluted into minimal medium plus ^{3}H-thymidine at the same specific activity as that used for growth before irradiation. Measurements of trichloroacetic acid-precipitable ^{3}H-thymidine were made at intervals after UV.

DNA synthesis in the wild-type strain was delayed 30 min by a dose of 450 ergs/mm^2 and 120 min by 1,300 ergs/mm^2, after which rate of synthesis was normal (Fig. 2). After doses of 130 ergs/mm^2 or less, there was no detectable delay or effect on rate of synthesis.

In the *ras*$^-$ mutant, DNA synthesis was greatly delayed after 450 ergs/mm^2. There was net solubilization of radioactivity after 1,300 ergs/mm^2 during the 3-hr experiment, reflecting the extensive DNA degradation at this dose (Fig. 1). It is clear that some DNA synthesis occurred after these high doses, since there was a net increase of acid-precipitable counts after 450 ergs/mm^2,

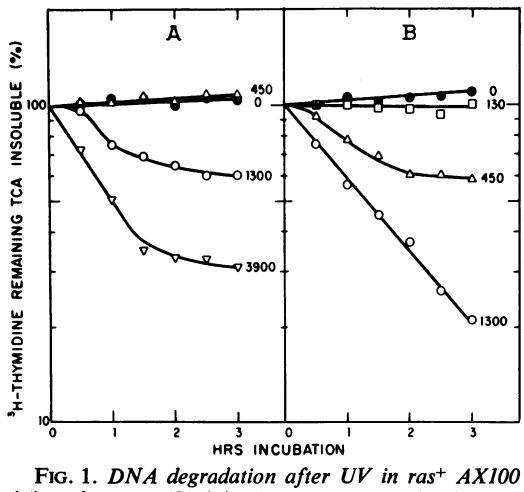

FIG. 1. *DNA degradation after UV in ras$^+$ AX100 (A) and ras$^-$ AX83 (B). A, Doses in ergs/mm^2; symbols and per cent survivors: 450 (△), 20; 0 UV (●), 100; 1,300 (○), 0.3; 3,900 (▽), 0.004. B, doses, symbols, and per cent survivors: 0 UV (●), 100; 130 (□), 0.3; 450 (△), 0.02; 1,300 (○), 0.0008. In A and B, 100% corresponds to about 2,000 counts/min in a 0.2-ml sample.*

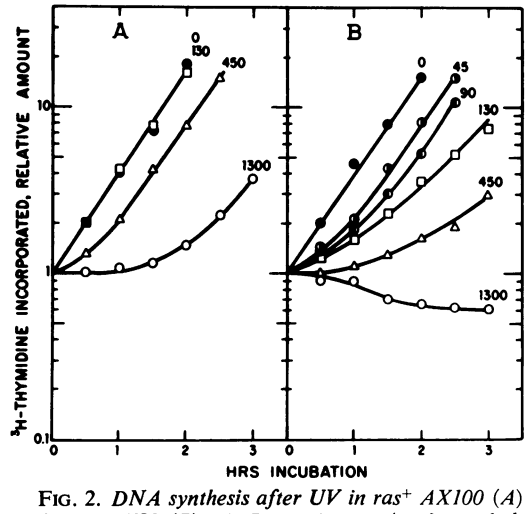

FIG. 2. *DNA synthesis after UV in ras$^+$ AX100 (A) and ras$^-$ AX83 (B). A, Doses in ergs/mm^2; symbols and relative number of survivors were: 0 UV (●), 1.0; 130 (□), 0.65; 450 (△), 0.24; 1,300 (○), 3 × 10^{-3}. B, Doses, symbols, and relative number of survivors: 0 UV (●), 1.0; 45 (◑), 0.04; 90 (◐), 7 × 10^{-3}; 130 (□), 1.4 × 10^{-3}; 450 (△), 2.1 × 10^{-4}; 1,300 (○), 6 × 10^{-6}. In A and B, relative amount 1 corresponds to about 2,000 counts/min in a 0.2-ml sample.*

whereas the DNA degradation experiment (Fig. 1) revealed solubilization of DNA after that dose.

After 130 ergs/mm², the *ras*⁻ strain synthesized DNA slowly for about 1 hr and then resumed exponential synthesis at a rate substantially less than in the unirradiated control. After doses of 45 and 90 ergs/mm², DNA synthesis was delayed about 30 and 45 min, respectively, and then resumed at the normal rate.

Since the *ras*⁻ mutant responds to UV with excessive DNA degradation and DNA synthesis delay, it is pertinent to ask whether these defects result from (or cause) a failure of some step of excision repair.

Rate of pyrimidine dimer excision. The rate of thymine-thymine and cytosine-thymine dimer excision from DNA was compared in *ras*⁻ and wild-type strains. The bacteria were labeled by growth in ³H-thymidine for about four generations, irradiated, and incubated in unlabeled medium. The amount of unexcised dimers was determined at intervals after irradiation. At least 85% of thymine-thymine and cytosine-thymine (recovered as uracil-thymine; reference 18) dimers were excised during 2 hr of incubation in both wild-type and *ras*⁻ strains (Fig. 3). Thus, the first two steps of excision repair (i.e., an incision and then excision) occur in the *ras*⁻ mutant. Both the amount of dimers formed per unit dose and the ratio of cytosine-thymine/thymine-thymine dimers were approximately the same in both strains.

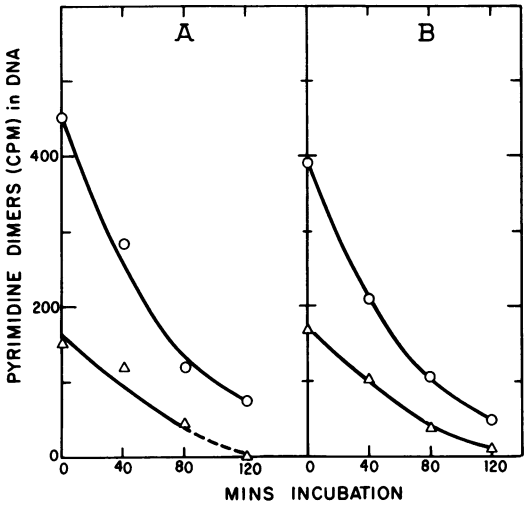

FIG. 3. *Rate of pyrimidine dimer excision from DNA in ras⁺ AX100 (A) and ras⁻ AX83 (B) strains. Thymine-thymine dimers (○); cytosine-thymine dimers (△). The dashed line of A was used since there were 0 counts above background in cytosine-thymine peak at 120 min. Dose was 1,300 ergs/mm².*

Repair replication after UV. The ability of the *ras*⁻ strain to fill the gap left by pyrimidine dimer excision, called repair replication (16), was examined. Thymine-less *ras*⁺ and *ras*⁻ strains were grown about four generations in ³H-thymidine, UV-irradiated if desired, and shifted to low-phosphate minimal medium containing BUdR and ³²P. After incubation, DNA was extracted and subjected to equilibrium density gradient centrifugation.

The wild-type strain, in the absence of UV, replicated DNA semiconservatively and ³²P was incorporated only into hybrid density material (peak at fractions 31 to 32) (Fig. 4A). No detectable ³²P counts appeared in the light density region (peak at fractions 42 to 43). Repair replication was measured during 20 min of ³²P labeling after 1,600 ergs of UV per mm². The wild-type strain incorporated ³²P only into the light density region which indicates that only repair replication occurred (Fig. 4B). The *ras*⁻ strain, after the same UV dose and labeling period, was capable of initiating repair replication since ³²P was incorporated into light density DNA (Fig. 5).

Repair replication (assayed by BUdR incorporation) in *E. coli* TAU-bar, after an initial insertion of BUdR into light density DNA, is characterized by the accumulation of DNA of density intermediate between light and hybrid (16). The total amount of BUdR inserted into intermediate density DNA before semiconservative replication resumed was greatly in excess of the photoproducts excised (16). The wild-type *E. coli* K-12 strain followed the same pattern. Even during a 20-min labeling period, a small amount of inter-

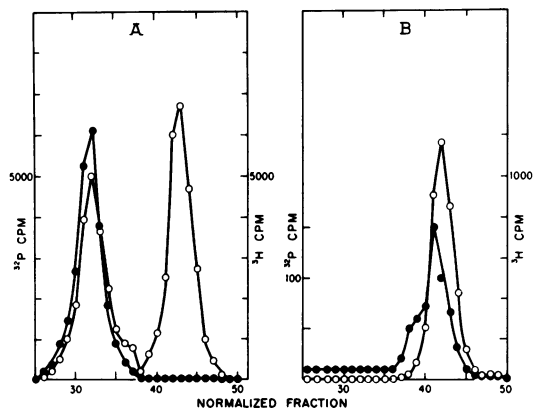

FIG. 4. *Repair replication in ras⁺ AX103. A, No irradiation; B, 1,600 ergs/mm² followed by 20 min of ³²P labeling. ³²P (●); ³H (○).*

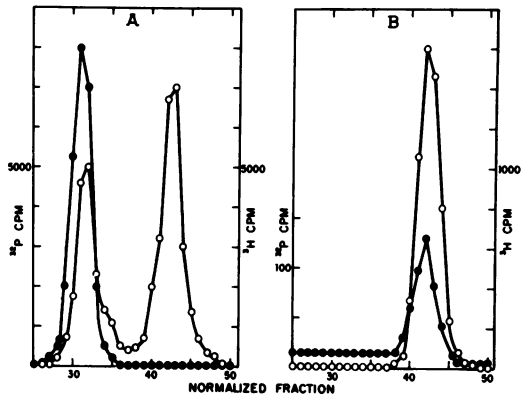

FIG. 5. *Repair replication in ras⁻ AX84. A, No irradiation; B, 1,600 ergs/mm² followed by 20 min of ^{32}P labeling. ^{32}P (●); ^{3}H (○).*

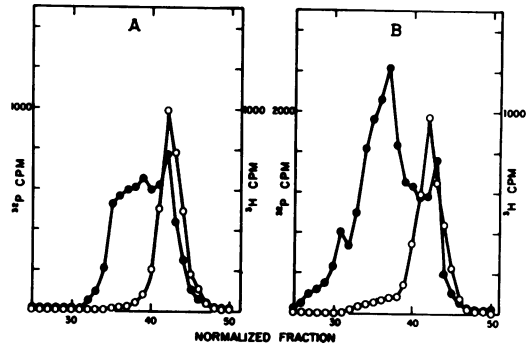

FIG. 6. *Repair replication in ras⁺ AX103. A, 1,600 ergs/mm² followed by 40 min of ^{32}P labeling; B, 1,600 ergs/mm² followed by 60 min of ^{32}P labeling. ^{32}P (●); ^{3}H (○).*

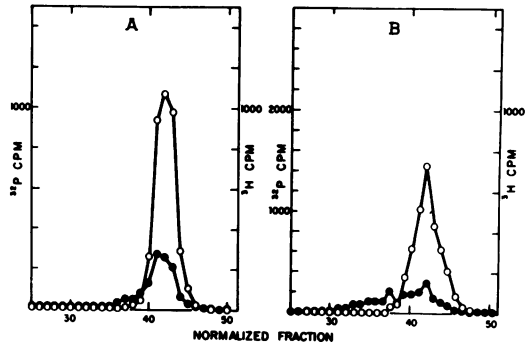

FIG. 7. *Repair replication in ras⁻ X84. A, Dose was 1,600 ergs/mm² followed by 40 min of ^{32}P labeling; B, 1,600 ergs/mm² followed by 60 min of ^{32}P labeling. ^{32}P (●); ^{3}H (○).*

mediate density DNA was synthesized, as evidenced by the shoulder on the heavy side of the ^{32}P peak (Fig. 4B). There was no shoulder on the ^{32}P peak from the *ras⁻* mutant after the same treatment (Fig. 5B).

The failure of the *ras⁻* mutant to complete the usual repair replication sequence was obvious when the ^{32}P labeling period after UV was extended to 40 or 60 min.

During a 40-min labeling period after UV, the wild-type strain increased the ^{32}P inserted into light density DNA and synthesized a great amount of intermediate density DNA (Fig. 6A). No semiconservative replication was begun. In contrast, the *ras⁻* mutant inserted only about 33% as much ^{32}P into light density DNA and synthesized only about 2% as much DNA of intermediate density (Fig. 7A; drawn to the same scale as Fig. 6A).

During a 60-min labeling period after UV, the wild-type strain continued to synthesize intermediate density DNA and semiconservative replication was begun, as evidenced by the small ^{32}P peak at the hybrid density position (fraction 31; Fig. 6B). In contrast, during the same period, the *ras⁻* mutant did not continue to insert ^{32}P into the light density position and the intermediate density DNA was only about 3% as much as that synthesized by the *ras⁺* strain (Fig. 7B; drawn to the same scale as Fig. 6B). There was no semiconservative replication during this time.

The *ras⁻* mutant solubilized more DNA after UV than did the wild-type strain, and presumably some of the excised nucleotides can be reincorporated during repair synthesis. However, after a relatively high UV dose, the extent of DNA solubilization in the *ras⁻* strain during the first 70 min after UV was only about twice that in the *ras⁺* strain (Fig. 1). Thus, excessive degradation and possible reincorporation cannot account for the reduced incorporation of ^{32}P after UV by the *ras⁻* strain.

Single-strand break rejoining. DNA of strains AX83 and AX100 was labeled by growth in ^{3}H-thymidine. The cells were collected and suspended in buffer, and a portion was withdrawn. The remainder was irradiated with 200 or 400 ergs/mm², doses too low to affect the molecular weight of DNA, and a second portion was withdrawn. The remainder was incubated in minimal medium in the dark for 60 min, by which time at least 50% of the pyrimidine dimers had been excised (Fig. 3). The cells were collected by centrifugation, and the sedimentation coefficient of DNA single strands was determined (Fig. 8). Number average molecular weights of the denatured DNA can be used to calculate the approximate number of breaks per single strand of chromosome (Table 1). The number average molecular weight of DNA

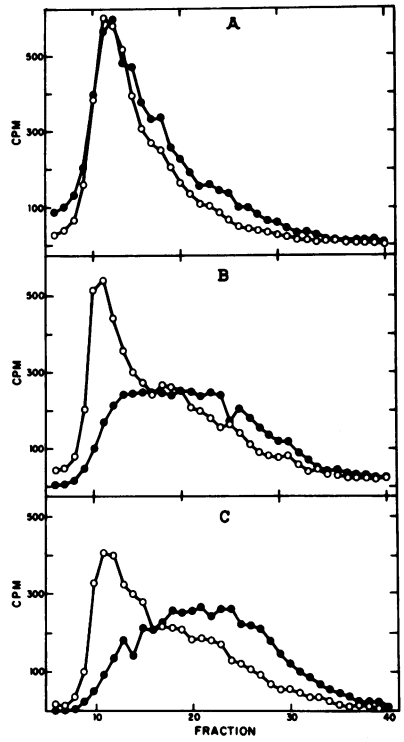

FIG. 8. *Alkaline sucrose-gradient centrifugation of strain ras⁻ AX83 (●) and ras⁺ AX100 (○) DNA extracted during excision repair. A, Dose was 0 ergs/mm²; B, 200 ergs/mm² followed by 60-min incubation; C, 400 ergs/mm² followed by 60-min incubation. Centrifugation was from right to left.*

from unirradiated cells (*ras*⁺ and *ras*⁻) was 1.6×10^8. This corresponds to about eight breaks per DNA single strand. The same results were obtained after 200 or 400 ergs/mm² with no subsequent incubation.

The wild-type strain had the ability to reseal the single-strand gaps which resulted from excision repair. After 200 ergs/mm² and 60 min of incubation, the number average molecular weight was 1.3×10^8. Thus, about 10 breaks per single strand were detected, of which 2 resulted from incomplete excision repair (Table 1). A dose of 200 ergs/mm² would have produced 600 dimers per single strand (17) and 300 of them would have been excised in 60 min (Fig. 3). A strain incapable of rejoining the gaps left by incision would be expected to have, 60 min after receiving 200 ergs/mm², about 300 breaks per DNA single strand.

The *ras*⁻ mutant also had the ability to rejoin single-strand breaks after UV, but did so with lower efficiency than normal. After 200 ergs/mm² and 60 min of incubation, the *ras*⁻ strain DNA number average molecular weight was 9.3×10^7, which corresponds to about seven breaks per strand resulting from excision repair.

TABLE 1. *Single-strand breaks in DNA*

Treatment	No. avg molecular weight[a]		Approx no. of excision-induced breaks per single strand[b]	
	ras⁺	*ras*⁻	*ras*⁺	*ras*⁻
0 ergs/mm²	1.6	1.6		
200 ergs/mm² plus incubation	1.3	0.93	2	7
400 ergs/mm² plus incubation	1.3	0.84	2	8

[a] Determined from the radioactivity in fractions 6 through 40, inclusive, which included at least 91% of the total radioactivity. Values must be multiplied by 10^8.

[b] Based on Cairns' (3) maximum value of 2.8×10^9 daltons for native DNA.

A dose of 400 ergs/mm² followed by 60 min of incubation would have provided 600 breaks per strand in a mutant incapable of rejoining excision repair gaps. Under these conditions, the *ras*⁺ strain had about two gaps per single strand (resulting from excision repair), whereas the *ras*⁻ mutant had about eight.

It is clear that, although the *ras*⁻ mutant had the capability of rejoining gaps which resulted from excision repair, it did so with less efficiency than did the wild-type strain. Three to four times more gaps remained not rejoined in the mutant than in the wild-type strain after doses of 200 and 400 ergs/mm².

DISCUSSION

In the *ras*⁻ mutant, dimer excision, which occurs at the normal rate, is accompanied by an abnormally high amount of DNA solubilized per dimer excised. After 130 or 450 ergs/mm², doses too low to cause detectable solubilization of wild-type strain DNA, about 10 and 40%, respectively, of the *ras*⁻ strain DNA was solubilized. After relatively high UV doses, the difference between degradation in *ras*⁺ and *ras*⁻ strains was not so marked. After 1,300 ergs/mm², the wild-type strain degraded 25% of the DNA during 60 min, whereas the mutant solubilized 45% of the DNA during the same time. However, after 1,300 ergs/mm², the wild-type strain ceased degradation after about 60 min, whereas the mutant continued the degradation for at least 3 hr.

It might be anticipated that, since DNA degradation after UV is increased by the *ras*⁻ mutation, the apparent rate of dimer excision might be greater in the mutant than in the wild type. How-

ever, the diminution of the difference in DNA degradation between mutant and wild type with increasing dose and the fact that dimer production is a rare event probably explain why the rate of dimer excision was about the same in both strains. A dose of 1,300 ergs/mm^2 would be expected to cause the formation of only about 7,800 dimers among the 10^7 nucleotides of the *E. coli* chromosome (17).

The DNA degradation of the *ras*$^-$ strain, although more than the normal amount, did not approach the extent which occurs in reckless-type *rec*$^-$ strains (9). The *rec*$^-$ strain AB2463 degraded about 20% of its DNA in the absence of UV, and a dose of only 10 ergs/mm^2 resulted in an additional degradation of 20% (9).

Pyrimidine dimers, although subject to excision, delayed DNA synthesis in the *ras*$^-$ mutant. After the low dose of 45 ergs/mm^2 (or 270 dimers per genome), a delay of 30 min in DNA synthesis resulted. DNA synthesis in the wild-type strain was not detectably delayed even after 130 ergs/mm^2.

In summary, the *ras*$^-$ mutant, after low UV doses, is capable of repairing most of the dimers normally. The increased DNA degradation and DNA synthesis delay after low doses probably result from defective repair of a small proportion of the dimers. This is consistent with the finding that, although most single-strand gaps are repaired after low doses, the *ras*$^-$ mutant leaves three to four times as many single-strand gaps not rejoined as does the wild type. Since most of the single-strand breaks were rejoined after low doses, repair replication probably functioned normally in the repair of most dimers after those low doses. Even after a high dose of 1,600 ergs/mm^2, repair replication was initiated by the *ras*$^-$ strain. Although apparently properly initiated, repair replication was not completed after 1,600 ergs/mm^2. [A demonstration of repair replication requires high UV doses to prevent semiconservative replication from obscuring repair synthesis (16).] After high doses, as 1,300 ergs/mm^2, excessive DNA degradation proceeded at least 2 hr past the time at which the wild-type strain stopped DNA degradation.

These findings are consistent with a model that assumes that the *ras*$^-$ mutation creates a defect such that, in a small proportion of the repair events, uncontrolled excision occurs. Even after low doses, there could be DNA synthesis delays because semiconservative replication might be expected to be inhibited at long stretches of single-stranded DNA. After high doses, excessive degradation in opposite strands could cause double-strand scissions, and repair replication might be impossible in such areas. Also, excessive degradation could interfere with repair replication at adjacent sites of excision on the same strand by re-excising repaired regions.

An alternative model assumes that increased activity of a nuclease, not necessarily specific for dimer-containing DNA, could enlarge in an uncontrolled fashion a small proportion of the temporary single-strand gaps made by excision. This model is unlikely because the *ras*$^-$ mutation increases X-ray sensitivity by a factor of only 1.5. Other models can be formulated, of course.

The *ras*$^-$ strain is more than normally susceptible to UV-induced mutagenesis (24). Mutations to valine resistance and to low thymine requirement were enhanced in the *ras*$^-$ strain by UV doses too low to cause detectable mutants of the wild-type strain. Witkin has shown that, although excision repair is an error-free (mutation-proof) process, the *exr*$^+$ gene product acting on DNA containing unexcised dimers causes frequent mutations (25, 26). Strain AX83 represents a situation in which dimers are excised at the normal rate and yet UV causes mutations. Possibly the increased DNA degradation after UV irradiation of strain AX83 is responsible for the increased UV mutagenesis.

The capability to reseal some single-strand breaks implies that DNA ligase can function after irradiation of the AX83. Direct assay of DNA ligase in crude extracts of AX83 and of the wild type indicated that both strains had the same specific activity (Gellert, *personal communication*).

The *ras*$^-$ strain is similar in some phenotypic properties to the *polA1* mutant of DeLucia and Cairns (4, 5), and it might be anticipated that DNA polymerase activity would be reduced in the *ras*$^-$ mutant. However, crude extracts of *ras*$^+$ and *ras*$^-$ strains contained the same specific activity of DNA polymerase (Walker, *unpublished results*).

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LITERATURE CITED

1. Boyce, R. P., and P. Howard-Flanders. 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. Proc. Nat. Acad. Sci. U.S.A. 51:293–300.
2. Burgi, E., and A. D. Hershey. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. 3:309–321.

3. Cairns, J. 1963. The bacterial chromosome and its manner of replication as seen by autoradiography. J. Mol. Biol. 6:208–213.
4. DeLucia, P., and J. Cairns. 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) 224:1164–1166.
5. Gross, J., and M. Gross. 1969. Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) 224:1166–1168.
6. Hanawalt, P. C., and D. S. Ray. 1964. Isolation of the growing point in the bacterial chromosome. Proc. Nat. Acad. Sci. U.S.A. 52:125–132.
7. Howard-Flanders, P., and R. P. Boyce. 1966. DNA repair and genetic recombination: Studies on mutants of *Escherichia coli* defective in these processes. Radiat. Res. Suppl. 6:156–184.
8. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53:1119–1136.
9. Howard-Flanders, P., and L. Theriot. 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics 53:1137–1150.
10. Kanner, L., and P. Hanawalt. 1968. Efficiency of utilization of thymine and 5-bromouracil for normal and repair DNA synthesis in bacteria. Biochim. Biophys. Acta 157:532–545.
11. Levinthal, C., E. R. Signer, and K. Fetherolf. 1962. Reactivation and hybridization of reduced alkaline phosphatase. Proc. Nat. Acad. Sci. U.S.A. 48:1230–1237.
12. McGrath, R. A., and R. W. Williams. 1966. Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; rejoining of broken pieces. Nature (London) 212:534–535.
13. Monod, J., G. Cohen-Bazire, and M. Cohn. 1951. Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochim. Biophys. Acta 7:585–599.
14. Pauling, C., and L. Hamm. 1968. Properties of a temperature-sensitive radiation-sensitive mutant of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 60:1495–1502.
15. Pauling, C., and L. Hamm. 1969. Properties of a temperature-sensitive radiation-sensitive mutant of *Escherichia coli*, II. DNA replication. Proc. Nat. Acad. Sci. U.S.A. 64:1195–1202.
16. Pettijohn, D., and P. Hanawalt. 1964. Evidence for repair-replication of ultraviolet damaged DNA in bacteria. J. Mol. Biol. 9:395–410.
17. Rupp, W. D., and P. Howard-Flanders. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. 31:291–304.
18. Setlow, R. B. 1966. Cyclobutane-type pyrimidine dimers in polynucleotides. Science 153:379–386.
19. Setlow, R. B., and W. L. Carrier. 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Nat. Acad. Sci. U.S.A. 51:226–231.
20. Setlow, R. B., and W. L. Carrier. 1968. The excision of pyrimidine dimers *in vivo* and *in vitro*, p. 134–141. *In* W. J. Peacock and R. D. Brock (ed.), Replication and recombination of genetic material. Australian Academy of Science, Canberra.
21. Smith, K. C. 1963. Photochemical reactions of thymine, uracil, uridine, cytosine and bromouracil in frozen solution and in dried film. Photochem. Photobiol. 2:503–517.
22. Smith, K. C., and M. E. O'Leary. 1968. The pitfalls of measuring DNA synthesis kinetics as exemplified in ultraviolet radiation studies. Biochim. Biophys. Acta 169:430–438.
23. Studier, F. W., 1965. Sedimentation studies of the size and shape of DNA. J. Mol. Biol. 11:373–390.
24. Walker, J. R. 1969. *Escherichia coli ras* locus: its involvement in radiation repair. J. Bacteriol. 99: 713–719.
25. Witkin, E. M. 1968. Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to ultraviolet light. Brookhaven Symp. Biol. 20:17–55.
26. Witkin, E. M. 1969. The role of DNA repair and recombination in mutagenesis. Proc. XII Intern. Congr. Genet. 3:225–245.