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Postreplication Repair of Ultraviolet Damage in *Haemophilus influenzae*

J. EUGENE LECLERC AND JANE K. SETLOW

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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The deoxyribonucleic acid (DNA) synthesized following ultraviolet (UV) irradiation of wild-type (Rd) and recombination-defective strains of *Haemophilus influenzae* has been analyzed by alkaline sucrose gradient sedimentation. Strain Rd and a UV-resistant, recombination-defective strain Rd(DB117)$^{rec^-}$ are able to carry out postreplication repair, i.e., close the single-strand gaps in the newly synthesized DNA; in the UV-sensitive, recombination-defective strain DB117, the gaps remain open. The lack of postreplication repair in this strain may be the result of degradation of the newly synthesized DNA.

Deoxyribonucleic acid (DNA) synthesized following ultraviolet (UV) irradiation of *Escherichia coli* cells contains discontinuities, as shown by alkaline sucrose sedimentation (10). These discontinuities were shown to be single-strand gaps (6). Upon further incubation of the cells, the single-strand molecular weight of the DNA approaches that of unirradiated cells. This postreplication repair process takes place independently of excision mechanisms that remove dimers from the DNA. Smith and Meun (13) showed that in *recA E. coli* cells gaps are not filled in the DNA synthesized from templates containing pyrimidine dimers. This observation supports the hypothesis of Rupp and Howard-Flanders that postreplication repair utilizes some of the same enzymes involved in genetic recombination and that information lost in gaps may be recovered by genetic exchanges between sister DNA duplexes (10).

We have studied postreplication repair in recombination-deficient and wild-type *Haemophilus influenzae*. Resistance to the lethal effects of UV is correlated with the ability of cells to fill in the gaps in newly synthesized DNA; but a complete recombination mechanism is not required for this process, since a UV-resistant strain, which is transformed with an efficiency of about 10^{-7} that of the wild type and permits no measurable phage recombination, fills gaps in an apparently normal fashion.

MATERIALS AND METHODS

Microorganisms. *H. influenzae* strain Rd (wild type), the recombination-defective strains DB117 and Rd(DB117)$^{rec^-}$, and Rd(DB112)^{uvs}, the UV-sensitive transformant of strain Rd with DNA from the excision-defective strain DB112, have been described (1, 11, 12).

Sedimentation of DNA synthesized after irradiation. The size of the DNA molecules synthesized after irradiation was determined by alkaline sucrose sedimentation of DNA from lysed cells. Cells were grown in Brain Heart Infusion (BHI) growth medium (11) to an optical density at 675 nm (OD_{675}) = 0.6. The cells were centrifuged and resuspended in M9 salts (11) to a concentration of about 5×10^8/ml. Five milliliters of this suspension was irradiated at 254 nm in a 9-cm petri dish under a germicidal lamp (incident intensity, 5 ergs per mm^2 per sec). One milliliter each of unirradiated (control) and irradiated cells were centrifuged and resuspended in 2 ml of growth medium. DNA synthesized after irradiation, or in control samples, was labeled with [^{3}H] thymidine from the following mixture kept at 36 C: 1.5 ml of cells, 0.75 mg of adenosine, 75 μCi of [^{3}H] thymidine (15.6 Ci/mmole). After incubation in radioactive medium for 15 min, cells were centrifuged, washed, and resuspended in 1.6 ml of growth medium at 36 C. At intervals 0.5-ml samples were removed, centrifuged, and resuspended in 0.15 ml of M9 medium. Samples of 0.1 ml (approximately 10^8 cells) were layered on alkaline sucrose gradients (7) and centrifuged at about 22 C in an SW39 or SW50.1 rotor at 30,000 rev/min for 90 min. Fractions were collected on paper strips, processed as previously described (3), and counted in toluene-2,5-bis-2[(5-*tert*-butylbenzoxazolyl)]thiophene scintillation fluid. Correction for background and spillover and calculation of number-average and weight-average molecular weights were made by a computer program with a calibration as previously described (9).

Degradation of DNA. Degradation of DNA synthesized before and after UV irradiation was meas-

ured as loss of acid-insoluble radioactivity upon incubation of cells. Cells were grown in BHI growth medium containing 0.50 mg of adenosine and 2.0 μCi of [^{14}C]thymidine (53 mCi/mmole) per ml. After 0.5-ml cultures were grown from OD_{675} = 0.05 to OD_{675} = 0.4, the cells were centrifuged, washed, and resuspended in 0.5 ml of nonradioactive medium. At OD_{675} = 0.6, cells were centrifuged and resuspended in 1.5 ml of salts of M9 to a concentration of about 5 $\times$ 10^8/ml. Samples (0.5 ml) of this suspension in a 9-cm petri dish were irradiated at 254 nm for various times. Cells were then centrifuged, resuspended in 0.5 ml of growth medium containing 0.50 mg of adenosine and 50 μCi of [^{3}H]thymidine (17.3 Ci/mmole) per ml. After incubation for 30 min at 36 C, the cells were centrifuged, washed, and resuspended in 1.0 ml of growth medium containing 10 μg of nonradioactive thymidine per ml. They were then incubated at 36 C, and 0.1-ml duplicate samples were removed at intervals. Samples were pipetted onto paper discs, which were processed like the paper strips.

RESULTS

Sedimentation of DNA synthesized after irradiation. The newly synthesized DNA from irradiated cells showed a dose-dependent decrease in single-strand molecular weight in wild-type and all mutant strains tested (results not shown). Figure 1a shows alkaline sucrose gradients for DNA of unirradiated Rd cells and cells given a UV incident dose of 25 ergs/mm^2 (254 nm), pulse-labeled with tritiated thymidine, and then incubated for various periods at 36 C. The sedimentation rate of DNA single strands from unirradiated cells corresponds to a weight-average molecular weight (M_w) of about 2 $\times$ 10^8 and a number-average molecular weight (M_n) of 7 $\times$ 10^7. The single-strand molecular weight of *H. influenzae* DNA as determined by Berns and Thomas (2) is about 4 $\times$ 10^8. After a UV dose of 25 ergs/mm^2, the newly synthesized DNA has an M_w of about 1.3 $\times$ 10^8, corresponding to an average of about six breaks in a single strand, based on an M_n of about 3.5 $\times$ 10^7 from irradiated cells. In the experiment reported in Fig. 1a, the M_w reached 1.8 $\times$ 10^8 and 2.0 $\times$ 10^8 during incubation for 20 and 40 min, respectively. Thus, as judged by the return of the single-strand molecular weight to the control value of unirradiated cells, gaps present in the newly synthesized DNA were filled in during further incubation.

DB117 is a UV-sensitive strain of *H. influenzae* that has a level of transformation about 10^{-6} that of strain Rd and exhibits no measurable phage recombination, although the cells take up DNA normally (Setlow, Boling, Beattie, and Kimball, J. Mol. Biol. *in press*). The delay in DNA synthesis after UV irradiation at an incident dose of 10 ergs/mm^2 is seven times longer in this strain than in Rd (8). Figure 1b shows sedimentation profiles for DNA from DB117 cells. After a dose of 7.5 ergs/mm^2 and

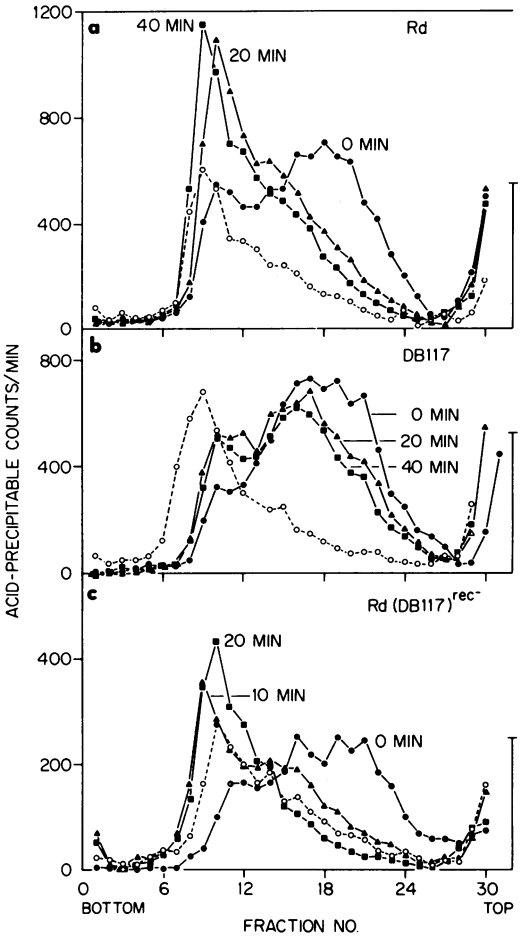

FIG. 1. *Sedimentation in alkaline sucrose of DNA synthesized by irradiated (solid lines) and control (dashed lines) cells. Cells were irradiated with UV (254 nm), labeled with [^{3}H]thymidine for 15 min, incubated at 36 C in nonradioactive medium for the periods indicated, and held at 4 C until the cells were lysed on top of gradient. Unirradiated control shown was incubated for 40 min at 36 C. The height of the vertical bar at the right represents 10% of the total radioactivity found in control cells. (a) Rd cells irradiated with 25 ergs/mm^2 (total counts per min per gradient: 10,440, 10,490, and 9,820 for 0, 20, and 40 min, respectively). (b) DB117 cells irradiated with 7.5 ergs/mm^2 (total counts per min per gradient: 10,200, 9,590, and 8,360 for 0, 20, and 40 min of incubation, respectively). (c) Rd(DB117)$^{rec-}$ cells irradiated with 25 ergs/mm^2 (total counts per min per gradient: 3,400, 3,330, and 3,200 for 0, 10, and 20 min, respectively).*

pulse label, the values of M_w do not approach the value for the unirradiated control during further incubation but remain at approximately 1.2×10^8. A dose of 7.5 ergs/mm^2 should on the average produce seven thymine-containing dimers per strand of DNA (12). The average number of breaks (calculated as above) in a single strand of DNA, newly synthesized on template DNA from DB117 cells given this dose, is six. This correlation between the number of dimers induced in the template DNA and the number of breaks in the newly synthesized DNA is consistent with the formation of a gap at each dimer passing through the replication fork. The correlation can be observed in strain DB117 because of the lack of gap filling during the period of pulse labeling. The fact that the single strands of DNA synthesized after irradiation remain short indicates a defect in the post-replication repair mechanism in these recombination-defective cells and is similar to the result for *E. coli recA*, another UV-sensitive, recombination-defective strain (13).

In strain Rd(DB117)$^{rec^-}$, a recombination-defective but UV-resistant strain of *H. influenzae*, the sedimentation patterns are similar to those of wild type, as seen in Fig. 1c. After irradiation and pulse labeling, followed by incubation in nonradioactive medium, the radioactive label rapidly goes into larger single-strand pieces of DNA, finally sedimenting at a rate corresponding to an M_w of 2.1×10^8. Thus, a functional postreplication repair mechanism is present in a strain that has no measurable phage recombination and has a transformation frequency about 10 times lower than that of strain DB117 (Setlow, Boling, Beattie, and Kimball, J. Mol. Biol. *in press*). UV sensitivity and UV-induced delay in DNA synthesis are also the same as in Rd (1).

Experiments like those described above show that gap filling in an excision-deficient strain of *H. influenzae*, Rd(DB112)^{uvs}, is like that of Rd cells. Excision of UV-induced pyrimidine dimers takes place in strains Rd, DB117, and Rd(DB117)$^{rec^-}$, although only about 15% of the dimers are excised during the time of the pulse label given after UV irradiation (12). Thus, a postreplication repair process may operate before excision mechanisms have removed most of the dimers from the DNA of the irradiated cell.

Degradation of DNA. In the experiments described in the preceding section, we noted a loss of acid-insoluble radioactivity during incubation of cells defective in postreplication repair. In order to assess the role of breakdown of DNA synthesized after UV irradiation, the degradation of DNA labeled before and after UV irradiation was measured. Figure 2 shows that in wild-type cells there is little degradation after an incident dose of 25 ergs/mm^2, whereas during 2 hr of incubation DB117 cells lose almost half of the acid-insoluble counts from the DNA synthesized after incident doses above 7.5 ergs/mm^2. However, this strain is not as subject to rapid and extensive breakdown of parental DNA following UV nor as susceptible to degradation of unirradiated DNA as is *E. coli recA* (4, 13). The UV-resistant strain Rd(DB117)$^{rec^-}$ shows the same resistance to DNA degradation as wild-type cells at the UV doses used (results not shown).

Separate experiments showed no increase in incorporation of [^{3}H]thymidine after removal of cells from radioactive media used for pulse labeling. Thus, the internal thymidylate pools are small, and it is unlikely that there is much incorporation of radioactivity while degradation is being measured.

In strain DB117 the UV-induced degradation of DNA synthesized after irradiation is more extensive than that of the template DNA synthesized before irradiation (Fig. 2b). This observation could be explained by degradation at a dimer gap site, the fraction of acid-precipitable counts lost from newly synthesized DNA appearing larger because of the smaller fraction of total radioactive label in the newly synthesized DNA. However, in strain Rd there is little difference between the breakdown of DNA made before or after irradiation, even at the largest UV dose used (Fig. 2a). We therefore conclude that the ratio of degradation in newly synthesized versus template DNA is greater in the UV-sensitive mutant than in strain Rd.

DISCUSSION

Resistance to the lethal effects of UV irradiation is correlated with a functional postreplication repair process in *H. influenzae*, as shown by the fact that the two different UV-resistant strains tested were able to fill gaps in DNA synthesized after irradiation. Conversely, a UV-sensitive strain that is unable to fill gaps in DNA synthesized from irradiated templates shows greater loss of colony-forming ability after irradiation. Another UV-sensitive strain, Rd(DB112)^{uvs}, shows normal gap filling, but the sensitivity of this mutant apparently results from its lack of ability to excise dimers (12). The observation that one of the UV-resistant strains exhibiting normal gap filling has a recombination defect even more severe

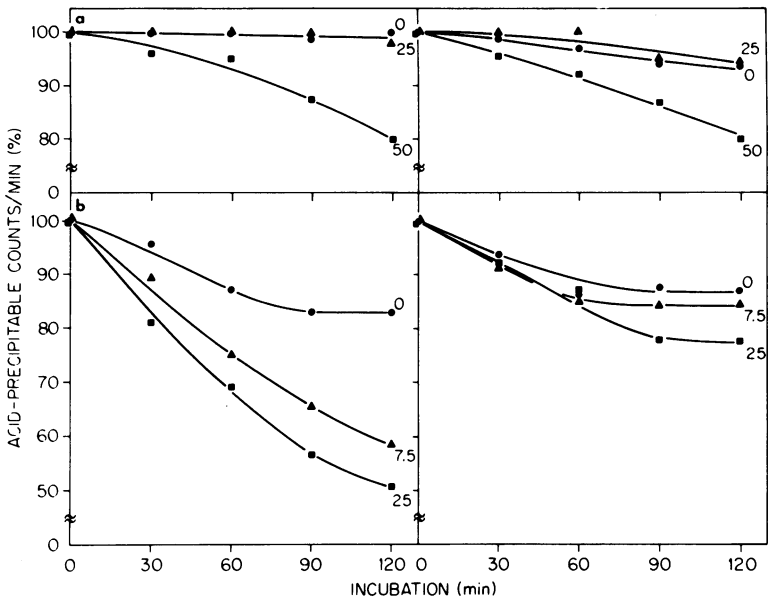

FIG. 2. *Degradation of the DNA in H. influenzae strains Rd and DB117 labeled with [^{14}C]thymidine before UV irradiation (254 nm) and with [^{3}H]thymidine after irradiation. Incident UV doses are indicated in ergs/mm^2. (a) Percentage of DNA synthesized after irradiation (left panel) and before irradiation (right panel) remaining during incubation of Rd cells. Incident UV doses: 0 ergs/mm^2 (●), 25 ergs/mm^2 (▲), and 50 ergs/mm^2 (■). (b) Percentage of DNA synthesized after irradiation (left panel) and before irradiation (right panel) remaining during incubation of DB117 cells. Incident UV doses: 0 ergs/mm^2 (●), 7.5 ergs/mm^2 (▲), and 25 ergs/mm^2 (■). One hundred per cent acid-precipitable counts/min represents about 200 ^{14}C counts/min in DNA synthesized before irradiation and about 2,000 to 4,000 ^{3}H counts/min in DNA synthesized after irradiation.*

than that of *E. coli recA* (5; Setlow, Boling, Beattie, and Kimball, J. Mol. Biol. *in press*) shows that postreplication repair in *H. influenzae* does not require a functional recombination mechanism.

The repair of single-strand gaps in DNA synthesized after UV irradiation seems to be a rapid process. Some experiments indicate that the process is nearly completed during 10 min of incubation. After irradiation of UV-sensitive strain DB117, the gaps are not filled and could be the initiation points for nucleolytic activity, thus enhancing DNA degradation which occurs preferentially in newly synthesized material. This degradation of DNA could contribute to the long delay in DNA synthesis observed after UV irradiation. Alternatively, degradation of newly synthesized DNA could prevent gap filling by the postreplication repair machinery. From our data it is not possible to determine whether the degradation causes the defect in postreplication repair or the defect in postreplication repair causes the DNA degradation. Smith and Meun (13) have attempted to solve this problem by using *E. coli* cells starved before irradiation, thus eliminating most of the degradation. They observed that under these conditions the *recA* strain was still unable to fill gaps in DNA. However, it is not clear whether the remaining small amount of degradation would be sufficient to prevent gap filling.

Genetic studies have shown that the mutations leading to the UV sensitivity and recombination deficiency of strain DB117 are not separable by transformation and thus are likely to result from a mutation in a single gene (Setlow, Boling, Beattie, and Kimball, J. Mol. Biol. *in press*). We have presented evidence that the UV sensitivity of strain DB117 results from its inability to carry out postreplication repair. However, we cannot conclude that postreplication repair directly involves the cell's recombination machinery, since (i) postreplication repair is normal in a very recombination-defective strain and (ii) a tendency toward DNA degradation at the site of single-strand breaks could cause both defective recombination and lack of postreplication repair.

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