JOURNAL OF BACTERIOLOGY, Mar. 1970, p. 808-812

Vol. 101, No. 3
Printed in U.S.A.

Repair of Ultraviolet-Irradiated Transforming Deoxyribonucleic Acid in *Haemophilus influenzae*

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Received for publication 3 December 1969

Ultraviolet-sensitive and wild-type *Haemophilus influenzae* cells were exposed to irradiated and unirradiated transforming deoxyribonucleic acid (DNA) containing a marker which can be linked to another marker in the cells. Lysates were made after various times of incubation and assayed for transforming activity on an excisionless recipient. Repair can be noted as an increase in activity from the irradiated donor DNA after its linkage to the recipient DNA. No repair can be observed in a mutant which is unable to integrate transforming DNA. There is a little repair in another mutant which is unable to excise pyrimidine dimers. *H. influenzae* cells also repair nondimer damage, as judged by the increase in activity observed in lysates made with irradiated and maximally photoreactivated DNA.

Repair of deoxyribonucleic acid (DNA) irradiated with ultraviolet light (UV) has been inferred in *H. influenzae* from the survival of cells, phage, and transforming DNA after irradiation, because such survival is markedly lower in strains which are unable to excise UV-induced pyrimidine dimers from their DNA (7, 8). Measurement of survival can only give the end result of maximal repair. The kinetics of repair of transforming DNA can be followed by exposure of the competent cells to irradiated transforming DNA, lysis of the cells at various times, and assay of the transforming activity of the lysates in cells which are themselves deficient in repair of UV-irradiated DNA. If repair takes place in the first recipient, the number of UV-induced lesions in the irradiated transforming DNA must decrease as a function of time before lysis of the cells. After the lysates are added to the second recipient strain, which is unable to repair the DNA further, an increase in the transforming activity of the irradiated transforming DNA may be observed. In previous work (3), the second recipient was wild-type *H. influenzae* in the presence of acriflavine, a compound known to inhibit excision of pyrimidine dimers in *Escherichia coli* (9). Using mutant *H. influenzae* deficient in ability to repair DNA as the second recipient (7, 8), we have shown repair of UV-irradiated DNA in wild-type and UV-sensitive *H. influenzae*. We have also demonstrated repair of maximally photoreactivated DNA and determined the dependence of repair of transforming DNA on prior integration of the DNA into the recipient cell genome.

MATERIALS AND METHODS

Microorganisms. Strains of *H. influenzae*, the cathomycin and streptomycin markers, and the conditions of growth were described by Setlow et al. (7).

Transformation. DNA was extracted and purified by the method of Marmur (4) and dissolved in 0.067 M phosphate buffer, *p*H 7. Competent cells were prepared and treated with purified DNA or lysates by the method of Steinhart and Herriott (12). To 3 ml of growth medium (7) was added 0.3 ml of DNA-treated or lysate-treated cells. After 2 hr of incubation with aeration at 37 C, the mixture was diluted and plated as previously described (7).

Photoreactivation and UV irradiation of DNA. Yeast photoreactivating enzyme was prepared according to Muhammed (5). Photoreactivation was carried on under black light for 30 min, which was sufficient time to split all the pyrimidine dimers in the UV-irradiated DNA. UV irradiation of transforming DNA at 254 nm was as previously described (7).

Preparation of lysates. The procedure used for making lysates was a modification of methods used by Voll and Goodgal (13). To 40 ml of competent cells in a 250-ml flask was added 4 ml of DNA at 10 μg/ml. After incubation for 5 min with aeration at 37 C, deoxyribonuclease was added to a final concentration of 1 μg/ml. After 2 min more of incubation, the cells were rapidly centrifuged at 5 C, washed three times with ice-cold M-II salts (11), resuspended in 40 ml of M-IV medium (12) in a 250-ml flask, and incubated with aeration at 37 C. After various intervals,

5 ml of cells was removed, added to 10 ml of ice-cold 1 M NaCl, rapidly centrifuged and resuspended in 0.5 ml of 1 M NaCl containing 0.01 M ethylenediaminetetraacetic acid, and incubated at 37 C for 15 min with 0.012 ml of 5% sodium dodecyl sulfate. Lysates were stored frozen and diluted 1/15 in 0.067 M phosphate buffer (*p*H 7) before the assay for transforming activity in the excisionless strains, DB116 or DB112.

RESULTS AND DISCUSSION

Repair in wild-type cells. Figure 1 shows repair of irradiated transforming DNA in wild-type *H. influenzae* (strain Rd). Transforming DNA bearing the cathomycin marker was added to the first recipient, wild-type cells resistant to streptomycin. At intervals the cells were lysed, and the lysates were assayed for transforming activity in the second recipient, DB116, which is deficient in DNA repair. The fate of the total incoming donor marker in the first recipient was followed by measurement of the number of DB116 cells transformed to cathomycin resistance (C) by the lysates. The transforming activity of the integrated donor marker was followed from the number of DB116 cells transformed to both streptomycin and cathomycin resistance (SC) by the lysates, since these two markers are linked in *H. influenzae*. Both irradiated and unirradiated transforming DNA were added to wild-type cells. As seen in Fig. 1, the transforming activity of the total donor marker from irradiated DNA increased, whereas that from unirradiated DNA decreased. The activity of the integrated donor marker from irradiated transforming DNA (SC, UV) increased considerably more than that from unirradiated DNA (SC, no UV). We believe that this increased activity resulted from repair of the irradiated DNA in the wild-type recipient cells.

The cathomycin transforming activity from the unirradiated donor DNA (top line of Fig. 1) decreased rapidly after the entrance of the DNA into the cell and leveled off after approximately 30 min. The loss of activity of the incoming marker is similar to that found previously (13). The total loss of activity from the incoming unirradiated DNA cannot be calculated from data such as those of Fig. 1, since at time zero the cells and DNA have already undergone 5 min of incubation for DNA uptake plus 2 min of incubation for degradation of unbound DNA. The activity remaining after 30 min probably represents only integrated DNA, since the final ratio of SC to C activities was approximately 1:3, which is the same ratio observed when SC and C activities are measured from transforming DNA containing linked S and C markers. Note that the number of C transformations (C, no UV) did not

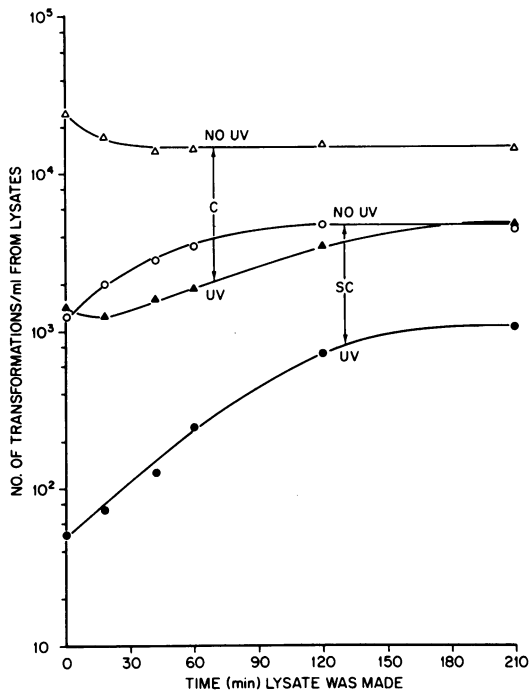

FIG. 1. *Single and double transformations from lysates of streptomycin (S)-resistant wild-type H. influenzae (strain Rd) exposed to irradiated or unirradiated transforming DNA from cathomycin (C)-resistant cells as a function of time of incubation before the cells were lysed. The lysates were assayed in strain DB116. At time zero, the cells had already been incubated with transforming DNA for 5 min and then with deoxyribonuclease for 2 min. The UV dose was 3,000 ergs/mm². Survival of the irradiated transforming DNA on wild-type cells was 15.5%.*

increase after leveling off, indicating that DNA synthesis did not take place under the conditions of the experiment. Similarly, the number of S transformations from such lysates, which are caused by the DNA of the first recipient cells, showed little or no increase.

The repair phenomenon is seen more clearly when the data of Fig. 1 are normalized, as in Fig. 2a and 2b. Here the transforming activity is expressed as relative activity, N/N_0, where N is the activity at the time the lysate was made, and N_0 is the activity of the time "0" lysate. The relative activity of the irradiated marker linked to the recipient marker (SC) increased much more than that of the total irradiated donor marker (C). However, the ratios (SC, UV)/(SC, no UV) and (C, UV)/(C, no UV) are both 5.5 after 210 min, indicating that linked and total donor DNA were both repaired to the same extent. This occurrence

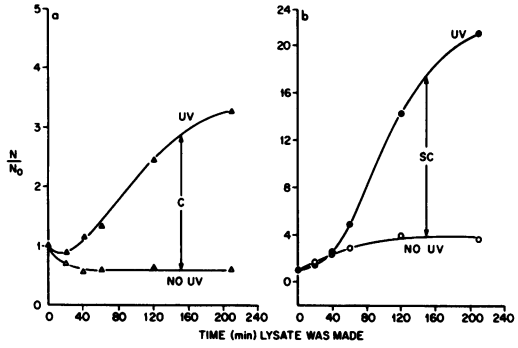

FIG. 2. *Normalized data from Fig. 1. N and N_0 are the number of transformations from the lysates at a given time and at time zero, respectively.*

is to be expected, since all unlinked donor DNA was broken down soon after its entrance into the cells. The formation of stable recombinant molecules which cause double transformations (SC, no UV, of Fig. 2b) occurred slowly under the conditions used for these experiments, whereas recombination occurs very rapidly under normal growth conditions (13).

Repair in UV-sensitive cells. Two strains of *H. influenzae*, DB112 and DB116, lack the ability to excise pyrimidine dimers from their DNA (8). Strain DB112 is slightly more sensitive to cell inactivation than is DB116 (7); similarly, UV-irradiated transforming DNA appears slightly more sensitive when assayed on DB112 than it is on DB116 recipients (7). If repair occurs in DB116, its detection should be possible by the lysate technique, with DB116 as the first recipient and DB112 as the recipient cell for assay of the lysates. Normalized data from such an experiment are shown in Fig. 3, in which the relative number of double transformations is plotted against time before the lysate was made, as in Fig. 2b. A smaller UV dose was used in this experiment than in the experiments of Fig. 1 and 2 because the survival of irradiated DNA is lower in DB112 than in DB116. There was considerable increase with time in the number of double transformations from the lysate made with cells exposed to irradiated DNA, so that clearly there was repair amounting to a factor of about three [the ratio (SC, UV)/(SC, no UV)]. On the other hand, Fig. 4 shows that the similar experiment performed with DB112 as the first recipient and DB116 as the recipient cell for the lysates gives no such evidence of repair.

The small amount of repair in strain DB116 is probably the result of some process other than excision of pyrimidine dimers, although the possibility cannot be excluded that there is a small amount of excision in this strain. A mechanism of repair involving recombination could be present in DB116 and lacking in DB112. We have observed that the frequency of transforma-

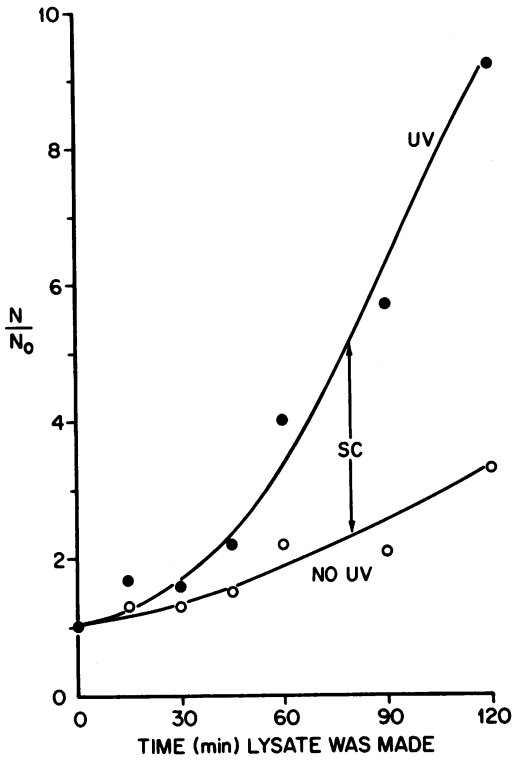

FIG. 3. *Relative number of double transformations from lysates made in strain DB116 and assayed in strain DB112, normalized as described for Fig. 2. The UV dose was 1500 ergs/mm². Survival of the irradiated transforming DNA on DB116 was 0.5%.*

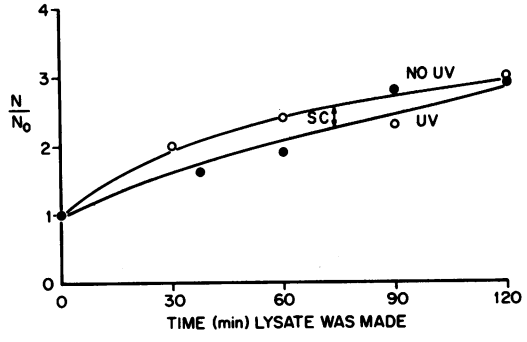

FIG. 4. *Relative number of double transformations from lysates made in strain DB112 and assayed in strain DB116, normalized as described for Fig. 2. The UV dose was 600 ergs/mm². Survival of the irradiated transforming DNA on DB112 was 6.3%.*

tion in DB112 is approximately 10% that in DB116 or in wild-type cells. Furthermore, there is some indication that DB112 is a double mutant (8). It has been postulated that the relatively slight UV sensitivity of the recombination-deficient strain DB117 is the result of its lack of a recombination repair mechanism (2).

Dependence of repair on integration. In strain DB117, there is no physical integration of transforming DNA into its genome, although it takes up DNA normally (7). Figure 5 shows the relative activity of donor marker after its entrance into cells of this strain. Whether the transforming DNA was unirradiated or irradiated, the activity decreased with time. The increased rate of inactivation in the case of the unirradiated DNA may be explained by the fact that UV-irradiated *H. influenzae* DNA is more resistant to breakdown by *H. influenzae* nucleases than unirradiated DNA (J. Setlow, *unpublished data*). In the absence of integration, inactivation of donor marker continued in DB117, whereas in wild-type cells where there is integration, such inactivation stopped after a time (*see* Fig. 5). Although DB117 has what appears to be a normal mechanism for repair of pyrimidine dimer damage (8), it cannot repair transforming DNA. On the other hand, repair of transforming DNA can be observed in strain DB115, which is considerably more sensitive to inactivation than DB117 (7) and also has an excision mechanism (8). We conclude that integration is necessary for repair of transforming DNA. Dependence of repair of transforming DNA on integration was previously hypothesized after observing that (i) only DNA which can itself integrate can compete with transforming DNA for repair enzymes (Beattie and Setlow, *unpublished data*) and (ii) the loss of integration of transforming DNA caused by UV irradiation of the DNA is independent of the presence or absence of an excision mechanism in the recipient cell (6).

Repair of nondimer UV lesions. Since the majority of the UV-induced biological damage to *H. influenzae* transforming DNA results from the formation of pyrimidine dimers (10), the repair which we have reported so far has mostly involved elimination of pyrimidine dimers from transforming DNA. Using the lysate technique, we have also been able to demonstrate repair of nondimer (nonphotoreactivable) damage in DNA maximally photoreactivated with yeast photoreactivating enzyme. Figure 6 shows the result of an experiment in which wild-type cells were exposed to DNA irradiated with 9,000 ergs/mm² and maximally photoreactivated, and

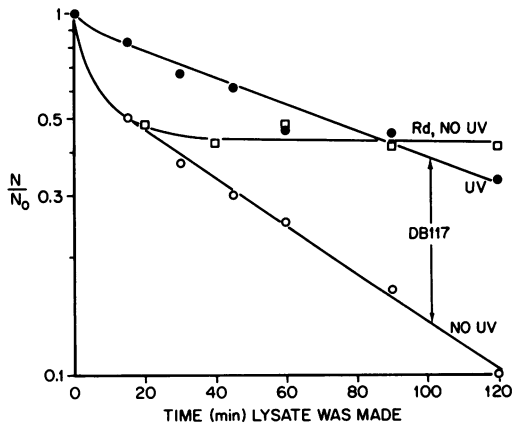

FIG. 5. *Relative number of single transformations from lysates made in strain DB117 and assayed in strain DB116, normalized as described for Fig. 2.*

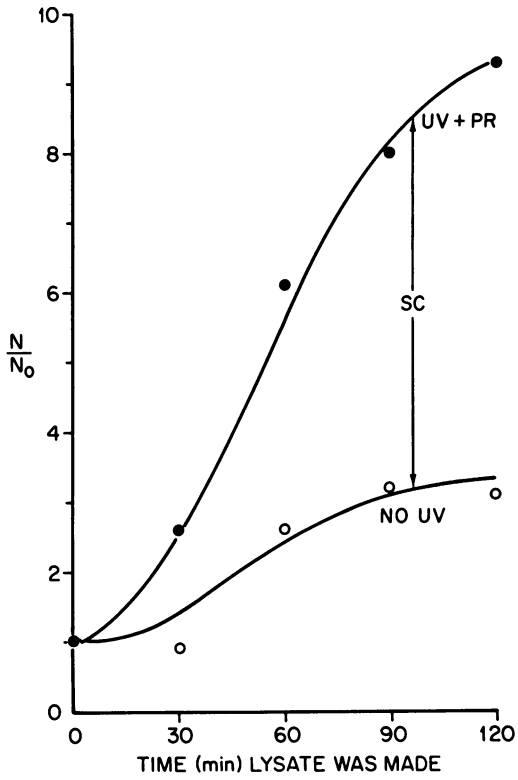

FIG. 6. *Relative number of double transformations from lysates made in wild-type cells and assayed in strain DB116, normalized as described for Fig. 2. The UV dose was 9,000 ergs/mm², and the DNA was photoreactivated with yeast photoreactivating enzyme for 30 min under black light at 37 C. Survival of the irradiated photoreactivated transforming DNA on wild-type cells was 3.3%.*

the lysates were assayed on DB116. The increase with time in amount of double transformations from the lysates was about three times as great with irradiated as with unirradiated transforming DNA. These data show that wild-type cells repair nondimer damage to transforming DNA.

Figure 7 shows the same experiment performed with DB116 and assayed on DB112. Again there appears to be evidence of repair of nondimer damage, although the rate of repair seems lower than for the wild-type cells, as judged by the decreased slope of the curve for the relative number of double transformations from the lysates containing irradiated DNA (Fig. 6 and 7). This difference between DB116 and wild-type *H. influenzae* is in accord with a previous observation that wild-type cells are not killed by nonphotoreactivable integrated lesions from transforming DNA, whereas DB116 cells are (1). There was no evidence of any repair of nondimer damage when lysates were made in DB112 and assayed in DB116. This is to be expected, since UV-irradiated and maximally photoreactivated transforming DNA appears more sensitive on DB112 than on wild-type cells (7).

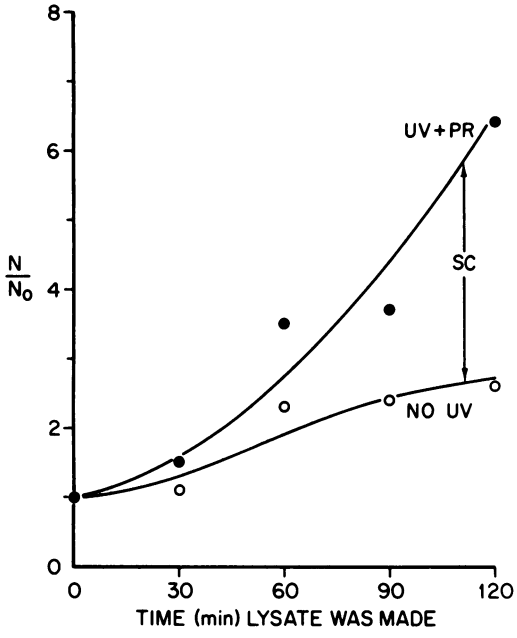

FIG. 7. *Relative number of double transformations from lysates made in strain DB116 and assayed in strain DB112, normalized as described for Fig. 2. The UV dose was 9,000 ergs/mm², and the DNA was photoreactivated with yeast photoreactivating enzyme for 30 min under black light at 37 C. Survival of the irradiated photoreactivated transforming DNA on DB116 was 2.2%.*

ACKNOWLEDGMENT

This investigation was supported by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.

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