DEOXYRIBONUCLEIC ACID SYNTHESIS IN ULTRAVIOLET-LIGHT-IRRADIATED CHINESE HAMSTER CELLS

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ABSTRACT Two mammalian cell lines, Chinese hamster ovary (CHO) which can recover colony-forming ability between fractionated doses of ultraviolet light (UV), and Chinese hamster B-14FAF28 which cannot recover, were tested for the ability to bypass UV-induced photoproducts in DNA during postirradiation DNA synthesis. The molecular weight distributions of newly synthesized DNA in UV-irradiated populations of both cell lines showed evidence for photoproduct bypass. Hence, the bypass mechanism does not correlate with recovery after UV.

INTRODUCTION

Humphrey, Sedita, and Meyn (1) reported that CHO cells recover colony-forming ability between fractionated doses of UV during the first few hours after irradiation, whereas another Chinese hamster strain, strain B-14FAF28 (B-14), which is intrinsically more UV sensitive than CHO cells, could not. These results suggested that CHO cells possess some repair capabilities that are absent in B-14 cells.

DNA repair involving the excision of pyrimidine dimers has been detected in both bacterial and mammalian cell lines. The ability of bacterial cells to excise UV-induced pyrimidine dimers from DNA is correlated with the UV sensitivity of these cells (2, 3). Cell lines of human origin have also been found to be capable of dimer excision (4) and a correlation between excision ability and UV sensitivity has been established in cells cultured from sunlight sensitive xeroderma pigmentosum patients (5, 6). Although repair replication, as shown by the Pettijohn and Hanawalt technique (7), can be detected in all types of mammalian cells (8–11), the excision of pyrimidine dimers has not been demonstrated in mammalian cells of rodent origin (12, 13). Thus, a relationship between the excision of pyrimidine dimers and UV sensitivity cannot be assumed for the rodent cell lines. The work of Rauth and Domon (14, 15) and Humphrey et al. (1) would lead one to expect that cells of rodent origin possess some mechanism for recovery from UV damage. One mechanism by which mammalian cells may recover involves the bypass of UV-induced

photoproducts during DNA synthesis following irradiation (16), a process similar to that proposed for bacterial cells by Rupp and Howard-Flanders (17).

This paper presents the results of experiments which test the supposition that the bypass of UV damage during DNA synthesis contributes to the recovery of the colony-forming ability of CHO cells. These results indicate that both the CHO and B-14 strains may be capable of bypassing UV-induced DNA damage.

MATERIAL AND METHODS

The cell lines used in this study were CHO and Chinese hamster B-14FAF28. Stock cultures were maintained as monolayers in McCoy's 5A medium (Schwarz BioResearch, Inc., Orangeburg, N.Y.) supplemented with 20% fetal calf serum.

Techniques used for irradiation with UV have been described elsewhere (18). Analysis of DNA sedimentation characteristics were obtained by the use of alkaline sucrose gradients (19). Techniques for lysing of the cells, centrifugation, fraction collection, and molecular weight determination have been reported in detail (20). Briefly, 1×10^5 cells were lysed in a solution of 2% tri-iso-propylnaphthalenesulfonic acid and 1% *p*-aminosalicylic acid adjusted to pH 12.5. Gradients were of the constant velocity exponential type described by Noll (21). Sucrose concentration at the top of the gradient was 5%. The sucrose solutions were adjusted to pH 12.5 with NaOH. Centrifugation was carried out in an SW 25.1 rotor in a Spinco Model L preparative ultracentrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) at 22,500 rpm for 5.5 hr at 20°C. Cells were usually labeled for 1 hr in medium containing 5.0 μCi/ml thymidine-^{3}H (1.9 Ci/mmole). Approximately 10,000 cpm were placed on each gradient.

RESULTS

CHO cells were tested for the ability to bypass photoproducts in the following experiment. Cell monolayers were irradiated with either 0, 50, or 100 ergs/mm^2 of UV and incubated in medium containing thymidine-^{3}H for 1 hr. The cells were washed in saline, lysed, and the DNA sedimented on alkaline sucrose gradients. The results of this experiment are shown in Fig. 1. A dose-dependent reduction in the molecular weight of newly synthesized DNA was observed in irradiated cells. The synthesis of smaller molecular weight DNA after UV irradiation may be due to the bypass of a photoproduct which leaves a gap or discontinuity in the newly synthesized strand of DNA. It should be noted that the doses of UV used in these experiments do not produce changes in the molecular weight of the parental or template DNA (22; also R. E. Meyn and R. M. Humphrey, unpublished experiments).

An alternate explanation of these results is as follows. UV irradiation slows down the rate of DNA synthesis to such an extent that during a 1 hr labeling period following irradiation, the irradiated cells synthesize lower molecular weight DNA than do unirradiated cells. In order to test this possibility, UV-irradiated CHO cells were incubated in the presence of thymidine-^{3}H for a length of time sufficient to allow the irradiated cells to synthesize an amount of DNA equivalent to that

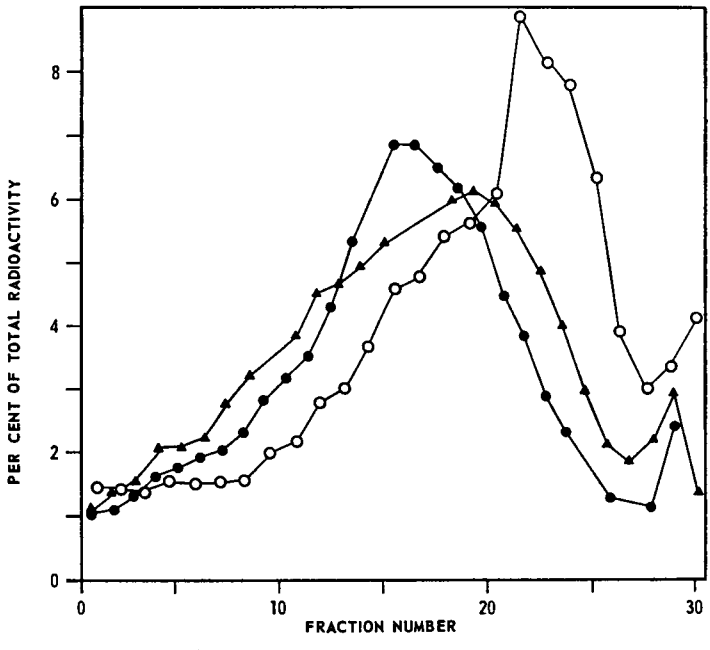

FIGURE 1 Sedimentation profile of DNA synthesized in CHO cells immediately after UV irradiation. Cells growing as monolayers on plastic dishes were rinsed once with Hanks' solution without phenol red and irradiated with UV. Medium containing 5 μCi/ml thymidine-^{3}H was added and the dishes were incubated for 1 hr at 37°C. After incubation the cells were lysed on top of alkaline sucrose gradients and centrifuged for 5.5 hr. Fractions were collected and analyzed for radioactivity in a liquid scintillation counter. Cells which received no irradiation (●). Cells which received 50 ergs/mm^2 (▲) or 100 ergs/mm^2 (○). The direction of sedimentation for this and succeeding figures is from right to left.

synthesized by unexposed cells. The cells were then lysed and the DNA sedimented on alkaline sucrose gradients (Fig. 2). The molecular weight of the DNA synthesized in irradiated cells incubated for this additional time is the same as that of DNA synthesized in irradiated cells incubated for 1 hr. This indicates that the synthesis of lower molecular weight DNA after UV irradiation is not due to the UV-induced reduction of the rate of DNA synthesis.

The discontinuities left in the newly synthesized strand of DNA by the bypass of a photoproduct would be lethal to the cell unless sealed up. CHO cells were tested for the ability to seal up the discontinuities in the newly synthesized DNA in the following experiment. Cells irradiated with 50 ergs/mm^2 were incubated for 1 hr in medium containing thymidine-^{3}H. At the end of this labeling period the labeled medium was poured off and fresh medium without label was added. The cells were then incubated for an additional 2 hr. The results are shown in Fig. 3. The data in this figure illustrate the fact that CHO cells are able to seal up the discontinuities within 3 hr after irradiation.

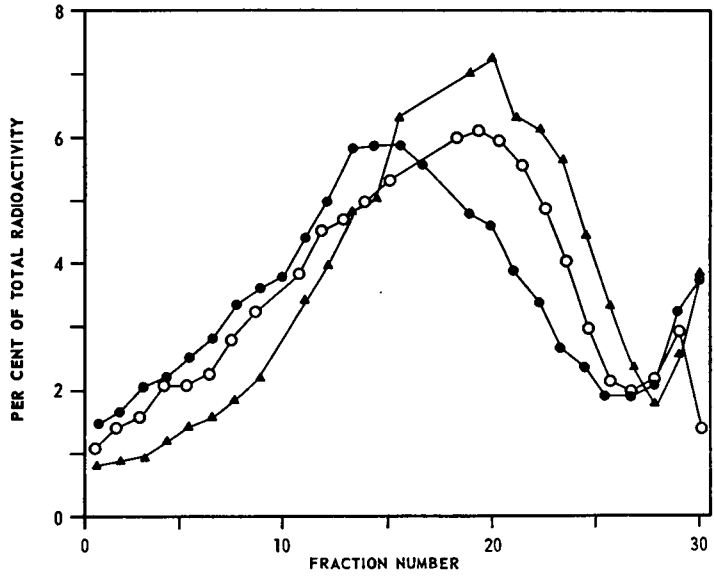

FIGURE 2 Sedimentation profile of DNA synthesized in CHO cells labeled with thymidine-^{3}H for different periods of time following irradiation. Cells which received no irradiation (●). Cells which received 50 ergs/mm^2 and labeled for 1 hr with thymidine-^{3}H (○) or labeled for 1.3 hr with thymidine-^{3}H (▲).

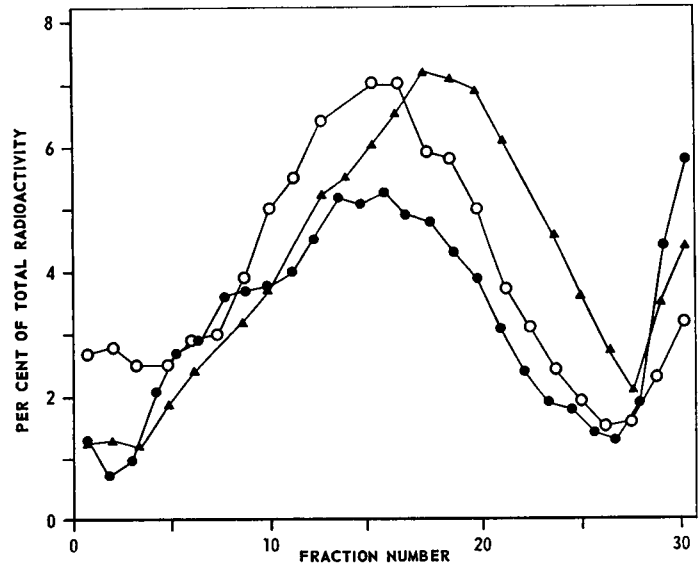

FIGURE 3 Sedimentation profile of DNA from CHO cells labeled immediately after irradiation and incubated for an additional 2 hr. Cells which received no irradiation (●). Cells which received 50 ergs/mm^2 and labeled for 1 hr and lysed (▲) or labeled for 1 hr and incubated for an additional 2 hr in medium without label and lysed (○).

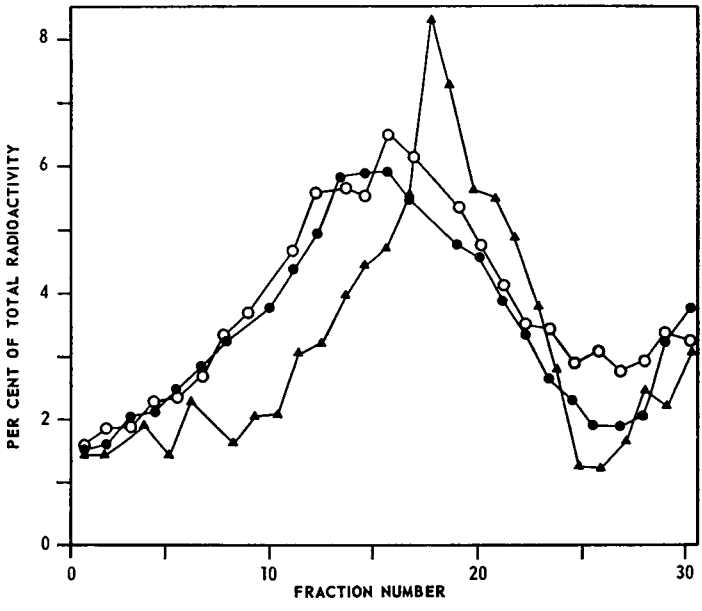

FIGURE 4 Sedimentation profile of DNA synthesized in CHO cells 3 and 6 hr after irradiation. Cells which received no irradiation (●). Cells which received 50 ergs/mm², incubated for 2 (▲) or 5 hr (○) in medium without label prior to a 1 hr labeling period.

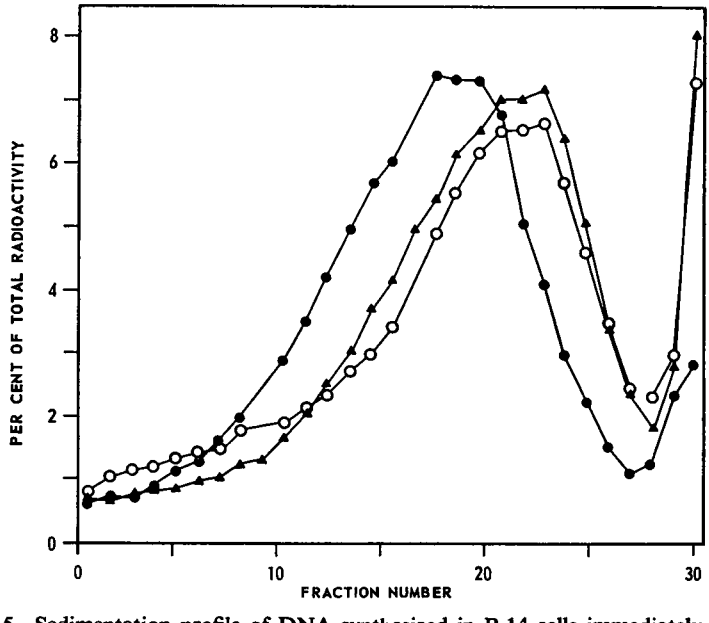

FIGURE 5 Sedimentation profile of DNA synthesized in B-14 cells immediately and 6 hr after irradiation. Cells which received no irradiation (●). Cells which received 50 ergs/mm² and labeled for 1 hr and lysed (▲) or incubated for 5 hr in medium without label prior to a 1 hr labeling period and lysed (○).

As a further test for the bypass of damage, UV-irradiated CHO cells were incubated for various lengths of time prior to a 1 hr labeling period with thymidine-^{3}H. The results of such an experiment are shown in Fig. 4. If the cells behaved according to expectation they should continue to synthesize DNA of smaller molecular weight than the control for at least 12 hr (one generation time) after irradiation. That is, the cells should bypass damage in the DNA until all the DNA in the population of cells has been replicated once. As seen in Fig. 4, UV-irradiated CHO cells synthesized DNA with the same molecular weight as unirradiated control cells during the sixth hour after irradiation.

Another strain of Chinese hamster cells, strain B-14, was tested for its ability to bypass photoproducts during DNA synthesis following UV irradiation. The results were qualitatively similar to the results with the CHO cells (R. E. Meyn and R. M. Humphrey, unpublished experiments). However, B-14 cells continued to synthesize DNA containing discontinuities throughout the first 6 hr after UV irradiation (Fig. 5), in contrast to the results with the CHO cells (Fig. 4).

DISCUSSION

Rupp and Howard-Flanders have presented evidence which suggests that excision-defective strains of *Escherichia coli* may be able to bypass UV-induced photoproducts in their DNA during postirradiation DNA synthesis (17). As a result of this bypass a gap or discontinuity would be left in the newly synthesized strand of DNA opposite the photoproduct. They suggest that these gaps are subsequently filled in with the appropriate bases by a process that may involve genetic recombination.

Since both the nature and importance of excision repair in mammalian cells of rodent origin has not yet been clearly determined it seemed appropriate to examine these cells for bypass synthesis. Thus, we examined the molecular weight distributions of newly synthesized DNA in irradiated CHO and B-14 cells for evidence of photoproduct bypass. If a bypass mechanism contributes to cell survival, we anticipitated observing the process in the CHO cell line, which exhibits recovery between fractionated exposures to UV (1). The B-14 cells, which do not exhibit recovery between fractionated exposures (1), were not expected to possess the bypass mechanism. The results (Figs. 1, 3, and 5) suggested that both CHO and B-14 cells may be able to bypass photoproducts. Hence, the bypass mechanism does not correlate with recovery. Evidence for bypass of photoproducts in two other mammalian cell lines, Chinese hamster V79 (16) and L5178Y mouse leukemia cells (22), has been reported previously.

The early synthesis of normal molecular weight DNA 6 hr after irradiation in CHO cells (Fig. 4) occurred in contrast to what one would expect. This result does not necessarily invalidate the suggestion that mammalian cells can bypass photoproducts. The other mammalian cell line used in these experiments, Chinese hamster B-14, apparently is capable of bypass of damage, but does not recover the ability

to synthesize normal molecular weight DNA within 5 hr (Fig. 5). These results suggest that another process in addition to or instead of bypass of damage is taking place in CHO cells which does not take place in B-14 cells during the same time period. One explanation of this other process would be that during the first 6 hr following UV irradiation a type of DNA repair other than the photoproduct bypass type restores the template DNA so that normal molecular weight DNA is synthesized from the template. In order for molecular repair to correlate with recovery of colony-forming ability the repair must take place during the first few hours after irradiation (1). The results of these experiments do not rule out the possibility that B-14 cells also perform this second type of repair but at a much reduced rate or at a later time. The fact that B-14 cells have a lower level of repair replication than other strains of mammalian cells (10), is consistent with the idea that this second type of repair may involve the excision of photoproducts. Experiments are currently being designed to determine if this second type of repair does indeed exist, and if it is responsible for the recovery of colony-forming ability between fractionated doses of UV in CHO cells.

This work was supported in part by National Institutes of Health grants CA 04484 and CA 05099.

Received for publication 24 June 1970 and in revised form 17 November 1970.

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