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Repair replication in replicating and non-replicating DNA after irradiation with UV light

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ABSTRACT

Ultraviolet light induces more pyrimidine dimers and more repair replication in DNA that replicates within 2 to 3 hr of irradiation than in DNA that does not replicate during this period. This difference may be due to special conformational changes in DNA and chromatin that might be associated with semiconservative DNA replication.

INTRODUCTION

The period of DNA synthesis (S phase) appears to be especially important in the response of cells to irradiation by ultraviolet (UV) light. Irradiation during the S phase causes severe growth delays (1) and more cell death than during other phases (2). Cells also undergo recovery from sublethal UV damage during the S phase (3).

Recently Watanabe and Horikawa (4) showed that more thymine dimers were produced in the DNA of Hela cells by UV light during the S phase than at other phases of the cell cycle. This cyclic variation in the amount of DNA damage was not reflected in the ability of cells to excise thymine dimers, which was constant throughout the cycle. We therefore decided to examine the ability of DNA damaged by UV light to undergo repair replication close to the time the DNA is involved in semiconservative replication as compared to non-replicating DNA.

MATERIAL AND METHODS

Hela S3 (mycoplasma-free) were grown as monolayers in minimal essential medium (MEM) with 15% fetal calf serum and antibiotics. For thymine dimer assays cells were labeled with ^{3}H-thymidine (1 uCi/ml, 20 Ci/mmole) for 36 hrs. Medium was removed, cells were rinsed twice with saline S, and medium without ^{3}H-thymidine was added for 4 hours. Medium containing 5×10^{-6} M unlabeled bromodeoxyuridine (BrdUrd) and 10^{-6}M fluorodeoxyuridine (FdUrd)

was then added for 45 min, removed briefly for UV irradiation with 18 J/m^2, and replaced for 180 min. Cells were collected and DNA was extracted as described previously (5). The DNA was then fractionated by neutral CsCl isopycnic gradients to separate replicated (BrdUrd-containing) DNA from unreplicated DNA (5). The DNA was further fractionated on alkaline CsCl-Cs_2SO_4 gradients (6) and the ^3H-labeled DNA strands from replicated and unreplicated DNA were harvested. The DNA was digested to bases with formic acid and analysed for thymine dimers and thymine by two-dimensional thin layer chromatography (7).

Repair replication was determined by the ^3H-bromodeoxyuridine labeling and isopycnic gradient methods previously described (6,8). Cells were labeled with ^{14}C-thymidine (0.025 uCi/ml, 36.6 mCi/mmole) for 72 hrs, rinsed and grown in unlabeled medium for 4 hrs and then in BrdUrd ($5x10^{-6}M$) and FdUrd ($10^{-6}M$) for 45 min. Cultures were drained, irradiated with 18 J/m^2, and labeled for 2 hrs with ^3H-BrdUrd (20 uCi/ml, $5x10^{-6}M$) and FdUrd ($10^{-6}M$) followed by a final incubation in BrdUrd ($5x10^{-6}M$) for 30 min. The DNA was then extracted and analyzed by neutral CsCl isopycnic gradients as previously described (5). The initial gradients consisted of a dense peak at 1.751 g/cc containing DNA that replicated during the 2 hrs period after irradiation and a light peak at 1.700 g/cc consisting of DNA that did not replicate during the ^3H-BrdUrd labeling period (9). The replicated and unreplicated DNA from several control and irradiated experiments was collected, recentrifuged at neutral pH three times to purify the DNA, denatured by alkali, and then centrifuged twice on alkaline CsCl-Cs_2SO_4 isopycnic gradients to purify normal density ^{14}C-labeled DNA (6). After the final alkaline isopycnic gradient the amount of ^3H and ^{14}C radioactivity was determined in 10 ul aliquots of the normal density DNA. Since the ^3H radioactivity comes from DNA containing small patches of ^3H-BrdUrd that has little effect on the density, the ratio of $^3H/^{14}C$ radioactivity is a measure of the relative amount of repair per unit of cellular DNA.

RESULTS

The percentage of dimers in parental DNA strands from replicated DNA (0.147 ± 0.015) is higher than from the unreplicated DNA (0.032 ± 0.006). These figures were obtained from cells harvested 3 hrs after irradiation, during which time a small amount of dimer excision will have occurred, but not enough to be the sole cause of this difference (4,10). The results suggest that more dimers are formed in those replicons that are about to replicate their DNA than in the bulk DNA, and the results are very similar to

the more extensive results of Watanabe and Horikawa (4).

The amount of repair replication that occurred during the first 2 hrs after irradiation (Fig. 1) indicates that about 7 - fold more repair replication occurs on the parental strands of replicating DNA than in bulk DNA. The ratio of $^{3}H/^{14}C$ in the peak representing replicated DNA was 2.79 while the $^{3}H/^{14}C$ ratio in the peak representing non-replicating DNA was 0.44 In addition, there appears to be a small density shift of one fraction for the ^{3}H peak relative to the ^{14}C peak only on the parental strands from replicated DNA. This suggests that not all the parental strands are repaired to the same extent, and that some have patches containing enough BrdUrd to cause a small increase in the density of the DNA. The amount of material remaining after multiple centrifugations was insufficient to allow us to determine the DNA molecular weights, so quantitative estimates cannot be made of the size of the patches involved in these density shifts (11).

Our results indicate that those replicons that replicate their DNA during the first 2 to 3 hrs after irradiation with UV light contain more dimers and undergo more repair replication, involving larger patches, than do replicons that do not replicate at that time. It seems possible that

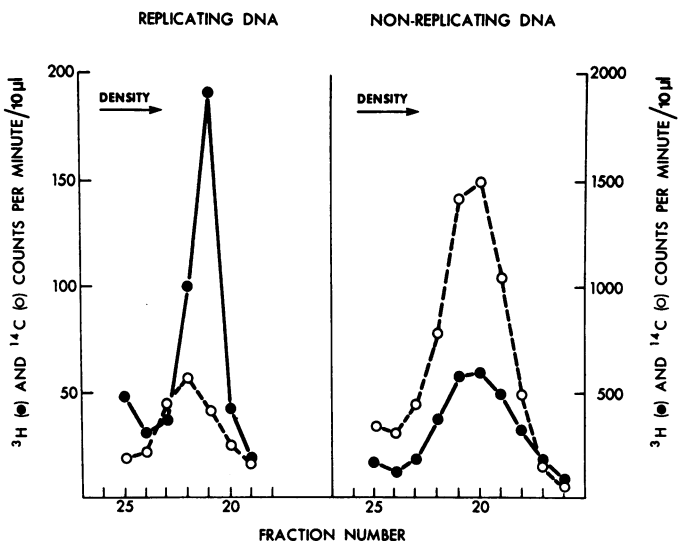

Fig. 1. Normal density regions of alkaline CsCl-Cs_2SO_4 isopycnic gradients from Hela cells grown in ^{14}C-thymidine for 72 hrs, irradiated with 18 J/m^2, and labeled for 2 hrs with ^{3}H-BrdUrd. DNA was first centrifuged 3 times on neutral CsCl isopycnic gradients and then replicating and non-replicating DNA were harvested separately and centrifuged on alkali. The radioactivity in 10 µl aliquots (out of 240 µl)of each fraction was determined. O-^{14}C counts/min/10 µl O-^{3}H counts/min/10 µl.

special conformations that may be associated with DNA replication including, for example, alterations in helicity and partial denaturation, may cause a greater efficiency in dimer formation and a concomitant increase in repair replication. We anticipate that further analysis of these phenomena with other DNA damaging agents may allow us to determine whether the unique response to the replicating DNA occurs for other kinds of damage, and what the nature of the structural alteration in DNA associated with semiconservative DNA replication may be.

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REFERENCES

1. Thompson, L.H., Humphrey, R.M. (1970)Rad. Res. 41, 183-201.
2. Djordjevic, B., Tolmach, L.J. (1967) Rad. Res. 32, 327-346.
3. Todd, P., Coohill, T.P., Hellewell, A.B., Mahoney, J.A. (1969) Rad. Res. 38, 321-339.
4. Watanabe, I., Horikawa, M. (1974) Biochem. Biophys. Res. Comm. 58, 185-191.
5. Cleaver, J.E. (1968) Biophys. J. 8, 775-791.
6. Gautschi, J.R., Young, B.R., Painter, R.B. (1972) Biochim. Biophys. Acta 281, 324-328.
7. Goldmann K., Friedberg, E.C. (1973) Analyt. Biochem. 53, 124-131.
8. Cleaver, J.E. (1974) Advances in Radiation Biology 4, ed. J.T. Lett, M.R. Zelle and H. Adler, Academic Press, New York, p. 1-75.
9. Cleaver, J.E. (1970) Photochem. Photobiol. 12, 17-28.
10. Regan, J.D., Trosko, J.E., Carrier, W.L. (1968) Biophys. J. 8, 319-325.
11. Edenberg, H., and Hanawalt, P.C. (1972) Biochim. Biophys. Acta 272, 361-372.