Conf-780226--1

THE REMOVAL OF UV-INDUCED PYRIMIDINE DIMERS FROM THE REPLICATED AND UNREPLICATED DNA OF HUMAN FIBROBLASTS[1]

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ABSTRACT Excision repair in UV irradiated human fibroblasts has been examined in portions of DNA replicating after irradiation versus those remaining unreplicated. Two approaches, one using a UV-endonuclease to estimate pyrimidine dimers remaining in DNA, the other using density labeling to measure excision resynthesis, indicate that the extent of repair is the same for both replicated and unreplicated DNA.

INTRODUCTION

Studies involving the effects of UV irradiation on DNA replication in mammalian cells have shown that CHO cells, which excise only a small percent of the pyrimidine dimers induced, replicate most of their genome after 5 or 10 $J \cdot m^{-2}$ of UV (1). As the DNA synthesized is larger than interdimer distance, replication must get past dimers in parental DNA (2) and, unlike bacteria (3), do so without exchanging many of the lesions to daughter strands (2).

Various experiments have suggested that DNA replication in normal human cells reacts similarly (4,2). However, interpretations of data are difficult for at least three reasons. First, normal human fibroblasts can excise half of the dimers induced by 10 $J \cdot m^{-2}$ UV during twenty four hours after irradiation (5). Second, after this dose, only half the amount of DNA is made during this period as compared to unirradiated cells. Third, DNA replication is affected to greater extent in UV irradiated excision defective Xeroderma pigmentosum cells (6). These facts suggest a close correlation between excision and DNA replication. Hence, it was possible that DNA synthesis seen in UV-irradiated normal cells only occurred in portions of the genome that had undergone excision-repair to remove dimers prior to replication, or that replicating regions were being preferentially repaired by excision. In order to investigate these possibilities the repair of both the DNA replicated and that remaining unreplicated has been monitored at various times following irradiation.

[1]Research sponsored by the Department of Energy under contract with the Union Carbide Corporation.

MATERIALS AND METHODS

Cell Culture and Radioactive Labeling: Normal human fibroblasts, HSBP were routinely grown in Dulbecco's modified medium plus 10% fetal calf serum at 37° in an atmosphere containing 5% CO_2. Cells grown to a monolayer in 75 cm^2 flasks were subcultured at a ratio of 1:3. ^3H-thymidine (s.a. 3 Ci mmol) at 0.03 µCi/ml or ^{32}P ortho phosphate at 0.5 µCi/ml were added and the cells grown to a monolayer.

Cell Plating UV Irradiation and Density Labelling: Cells were plated at 7.10^5 per 150 mm petri dish and forty hours later UV irradiated. Some ^3H labeled cells were sampled to determine the dimers induced, others were incubated in medium containing Fluorodeoxyuridine (0.25 ug/ml) and bromodeoxyuridine (3 ug/ml). ^{32}P prelabeled cells for repair replication studies were similarly incubated except that ^3H thymidine (s.a. 55 Ci/mmol) at 5 uCi/ml was also included in the medium.

Extraction of DNA, Separation of Replicated and Unreplicated Portions: Cells were lysed with 0.5 M EDTA, 0.5% sarkosyl plus 200 ug/ml Proteinase K (Merck), and left at 37° for 8 hrs prior to phenol extraction. Cesium chloride (1.25 g/ml) was dissolved in each sample and centrifuged in a 40 rotor of a Beckman 5-50 at 30,000 rpm for 40 hours. Gradients were collected as 30 fractions of 0.13 ml, 10 λ of each fraction acid precipitated to determine the heavy/light and light/light peaks, and these portions pooled and dialysed against .01M TrisHCl, .001M EDTA, .04M NaCl pH 8.0.

Estimation of Pyrimidine Dimers: This was carried out by determining the number of UV endonuclease sensitive sites (7).

Determination of Repair Replication: Unreplicated and replicated DNA from ^{32}P prelabeled cells was obtained as above. The light strand from heavy/light DNA, and both light strands from light/light DNA were further purified by 2 centrifugations in alkaline cesium chloride (8). ^3H activity found banding with light ^{32}P labeled strands was used as a measure of repair.

RESULTS

The Effect of UV on DNA Synthesis: Table 1 gives the amounts of DNA synthesised in cells during 12 or 24 hours after 5 or 10 $J \cdot m^{-2}$. Cells receiving 5 $J \cdot m^{-2}$ by 24 hours have synthesized as much as unirradiated controls, but after 10 $J \cdot m^{-2}$ only half this amount is made during the same period.

TABLE 1
THE PRECENT OF DNA SYNTHESISED IN UV IRRADIATED HUMAN FIBRO-BLASTS

	Time after irradiation (hours	
UV dose ($J\ m^{-2}$)	12 hrs	24 hrs
0	100	100
5	56 ± 10	85 ± 13
10	25 ± 6	50 ± 6

Incidences of Pyrimidine Dimers and Repair Replication in Replicated and Unreplicated DNA: Table 2 presents the numbers of dimers left in the DNA replicated or remaining unreplicated at 12 and 24 hours after irradiation. Slightly fewer dimers were frequently observed in replicated as opposed to unreplicated strands but differences never exceeded 20%.

TABLE 2
THE INCIDENCE OF PYRIMIDINE DIMERS AND EXTENT OF REPAIR REPLICATION IN DNA REPLICATED (HL), OR REMAINING UNREPLICATED (LL) AFTER UV.[a]

UV Dose ($J \cdot m^{-2}$)	Time (hrs)	Dimers Remaining per 10^8 daltons		$^3H/^{32}P$	
		HL	LL	HL	LL
5	12	8	7	1.4	1.5
10	12	12	14	2.0	1.8
5	24	4	5	1.8	2.1
10	24	9	10	3.0	3.0

[a]Treatments were as indicated in the text and time refers to the length of post UV incubation in BrdUrd containing medium. The number of dimers immediately after UV is $3.0/10^8/J \cdot m^{-2}$. $^3H/^{32}P$ denotes the degree of excision resynthesis associated with light DNA strands.

Hence, regardless of dose or time after UV, dimers are found in both the replicated and unreplicated DNA. Following 10 $J \cdot m^{-2}$ the DNA replicated during 12 hours after irradiation was incubated with or without UV endonuclease. The heavy daughter strand was larger than the interdimer distance observed in the replicated light strand.

Table 2 also gives the amount of repair synthesis in the replicated and unreplicated portions of the DNA that received UV. The data indicate that after a given dose and post

irradiation incubation the extent of repair is similar regardless of whether the DNA has undergone semiconservative replication

DISCUSSION

The effect of UV irradiation on DNA replication in human cells (Table 1) is greater than observed in CHO cells receiving equivalent doses (1), despite the fact that human cells are able to excise more lesions than CHO cells (5). Following UV, E. coli exhibits a two fold increase in the repair synthesis seen in replicated versus unreplicated DNA, this being absent in a uvrB excision less mutant (9). Such a result would be expected if a preferential excision repair of replicating DNA occurred. In human fibroblasts, although pulse chase experiments inferred semi-conservative replication could get past dimers (5), a preferential excision repair of replicating DNA in normal cells has been suggested by experiments with normal and excision defective XP fibroblasts (10). However, the data presented here clearly indicate that replication gets past dimers and that both the incidence of dimers remaining, and the repair replication of those removed, is the same in portions of DNA replicated or left unreplicated after the UV irradiation of normal human fibroblasts.

ACKNOWLEDGEMENTS

The author wishes to thank J. D. Regan for the fibroblast line used in these experiments.

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