RECOMBINATION OF UV-INDUCED PYRIMIDINE DIMERS IN HUMAN FIBROBLASTS*

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Summary—The appearance of UV-endonuclease-sensitive sites in DNA synthesized after UV irradiation has been monitored in human fibroblasts. The data indicate that 5% of the sites originally induced can be found in this DNA. Such sites are found in both normal excision-proficient fibroblasts and in those from xeroderma pigmentosum patients lacking the ability to excise UV-induced pyrimidine dimers.

Following UV irradiation, excision-deficient bacteria exhibit a postreplication repair mechanism involving single-strand recombination between sister molecules (1). Such events have been shown to result in the appearance of UV-induced pyrimidine dimers in the daughter DNA synthesized after irradiation (2). Similar exchanges have not been demonstrated in mammalian cells (3), and the majority of UV-induced gaps in DNA synthesized after UV irradiation are filled in to a large extent by de novo synthesis (4, 5). However, more recently, recombinant-like DNA molecules have been detected at low frequencies in mammalian cells (6). Furthermore, this frequency was stimulated by UV irradiation, and the occurrence of small exchanges in these cells could not be excluded.

Repair-endonuclease sensitive sites have been shown to occur at a low frequency in DNA synthesized after the UV irradiation of xeroderma pigmentosum (XP) cells, but not in normal fibroblasts (7). The crude endonuclease extract used, however, did not permit such lesions to be classified unequivocally as pyrimidine dimers, and the assay system was able to detect only 30–40% of the expected number of pyrimidine dimers when analyzed immediately after specific UV doses (8). In normal cells after 10 J/m^2 and 20 hr of incubation, this method indicated that 90% of the lesions were removed (8) whereas actual

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dimer analysis reveals only 50–60% are removed (9). In order to clarify whether or not pyrimidine dimers could be found in the DNA of human fibroblasts synthesized after UV, the following experiments utilizing extracted DNA and a purified UV endonuclease were undertaken.

Fibroblasts plated at 80,000/dish were prelabeled with [^{14}C]thymidine (sp. act. 551 mCi/mmol, Amersham/Searle) overnight, this being replaced by unlabeled medium 2 hr before UV irradiation. After irradiation, cells were labeled with 12.5 μCi/ml of [^{3}H]thymidine (sp. act. 17 Ci/mmol, Amersham/Searle) for 9 hr and chased with unlabeled medium for 14 hr. The DNA was then extracted (10) and a purified UV endonuclease (11, kindly provided by W. L. Carrier) was used to determine the numbers of endonuclease-sensitive sites in both ^{14}C- and ^{3}H-labeled DNA. This UV endonuclease has a high specificity for pyrimidine dimers and, by crude assay, has less than 10% activity on the DNA strand complementary to that containing the pyrimidine dimers (11). A maximum of one break in the unirradiated, as opposed to 70 breaks in the UV-irradiated, strands occurred

Table 1

Endonuclease-sensitive sites

UV dose (J/m^2)	Immediately after UV		23 hr after UV	
	Normal	XP	Normal	XP
5	13.5	15.2	5.1	13.6
10	27.8	29.9	12.0	30.0
20	55.0	56.5	24.5	54.1
30	81.0	83.2	42.8	81.7

UV-endonuclease-sensitive sites/10^8 daltons of total DNA in normal and XP fibroblasts either immediately or 23 hr after UV irradiation. Numbers of endonuclease sites were calculated as

$$\frac{2}{M_w \text{ of DNA + UV endonuclease}} - \frac{2}{M_w \text{ of DNA - UV endonuclease}}$$

from computed M_w's (15, 16), as $M_n = \frac{M_w}{2}$ when M_w is random.

[M_w is random if one assumes pyrimidine dimers, and hence UV-endonuclease-induced breaks, are randomly distributed in the DNA]

M_n = number average molecular weight;
M_w = weight average molecular weight.

when heavily UV-irradiated bacterial DNA of approximately 10^6 daltons was analyzed (12) More precise studies with the UV endonuclease isolated from T4 phage have revealed that less than 2% of the breaks occur in the strand opposite the dimer as opposed to that containing the dimer (9). A comparison in our system of the M. luteus enzyme with that from T4 (kindly provided by E. C. Friedberg) gave the same results. In some experiments, the [^{14}C]thymidine prelabel was omitted. This did not modify the appearance of endonuclease-sensitive sites in the ^{3}H-labeled DNA synthesized after UV. In all instances unirradiated controls ± UV endonuclease were run in parallel, and no more than 0.1 breaks/10^8 daltons were introduced into unirradiated DNA by the endonuclease.

The induction of pyrimidine dimers and their excision from total DNA at 23 hr after UV irradiation for normal and XP cells are given in Table 1. These results correlate with those obtained by direct dimer estimations (13) or by similar endonuclease assays on extracted DNA (14). Normal cells have excised approximately 50% of the pyrimidine dimers at 23 hr after the UV doses given. As expected, the XP cells have removed virtually none of these lesions following the same incubations. No cell death, as measured by detachment of cells from the petri dish, had occurred in either cell line by 23 hr after irradiation.

Sucrose-gradient profiles of DNA synthesized after various UV doses, and incubated

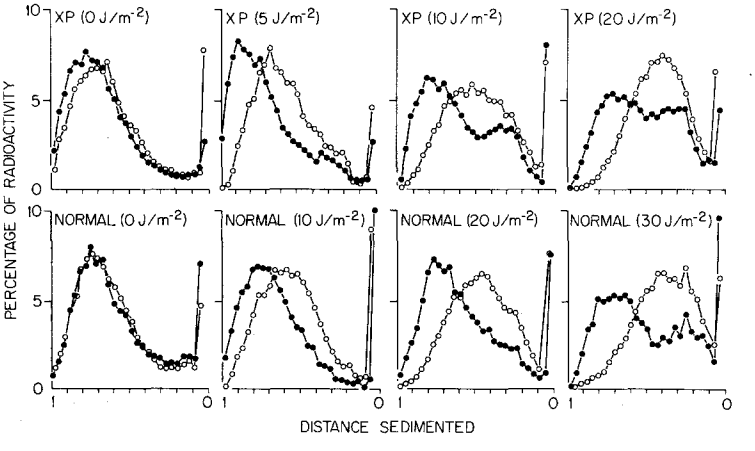

Figure 1—Alkaline sucrose sedimentation: Cells were pulse chased with [^{3}H]thymidine after the UV doses shown. DNA was extracted and incubated with (O) and without (●) UV endonuclease as described in the text. A minimum of 8 X 10^3 cpm occurred in cells receiving the higher UV doses. Gradients were run in 5–20% sucrose, 2 M NaCl at 30,000 rpm for 170 min. The unirradiated, extracted DNAs have molecular weights of approximately 1.5 X 10^8 daltons.

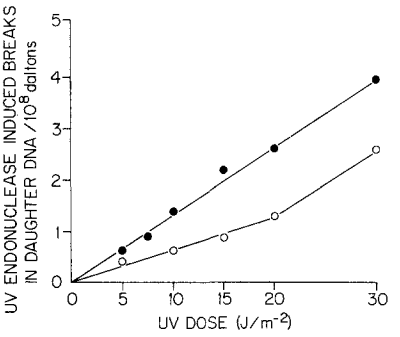

Figure 2—The increase of UV-endonuclease-sensitive sites in DNA synthesized after UV as a function of initial UV dose. XP (●); normal (○).

with or without UV endonuclease following DNA extraction, are given in Figure 1. Figure 2 shows that the number of UV-endonuclease-sensitive sites found in DNA synthesized after UV irradiation of excision-deficient XP cells increases linearly with UV doses ranging from 5–30 J/m^2, 5% of the sites induced having been exchanged. Only 2.5% of similar sites are found in the daughter DNA of normal cells after the same UV doses. However, as shown in Table 1, the normal cells have excised approximately 50% of their pyrimidine dimers at the time of sampling. Hence, numbers of sites in the daughter DNA in both XP and normal cells at the time of sampling are 5% of the total sites left in the total DNA at this time after UV. If one assumes that recombined dimers are equally accessible to excision in the normal cells, the exchange of dimers has occurred at the same frequency in both normal and XP fibroblasts. This frequency (5%) is an order of magnitude less than observed in excision-deficient E. coli K12 (2).

The number of breaks induced by the M. luteus UV endonuclease corresponds to the number of pyrimidine dimers in the DNA ± 10% (11), and the comparison with the T4 UV endonuclease (9) revealed that less than 2% of these breaks occur in the DNA strand opposite to that containing the dimer. Therefore the results presented indicate that a small percentage of UV-induced pyrimidine dimers can be found in the DNA of human cells synthesized after UV irradiation. The mechanism responsible for this exchange operates in both normal and XP cells and is thus independent of the incision step required for the excision repair of such dimers.

Note: After this manuscript was completed, some of these findings were reported by R. Meneghini and P. Hanawalt (Biochim. Biophys. Acta, 425, 428, 1976).

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