DNA CHAIN ELONGATION AND JOINING IN NORMAL HUMAN AND XERODERMA PIGMENTOSUM CELLS AFTER ULTRAVIOLET IRRADIATION

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ABSTRACT DNA synthesized in human cells after ultraviolet (UV) irradiation is made in segments of lower molecular weight than in unirradiated cells. Within several hours after irradiation these smaller units are both elongated and joined together. This repair process has been observed in normal human fibroblasts, HeLa cells, and fibroblasts derived from three types of xeroderma pigmentosum patients—uncomplicated with respect to neurological problems, complicated (de Sanctis-Cacchione syndrome), and one with the clinical symptoms of xeroderma pigmentosum but with normal repair replication. The ability of human cells to elongate and to join DNA strands despite the presence of pyrimidine dimers enables them to divide without excising the dimers present in their DNA. It may be this mechanism which enables xeroderma pigmentosum cells to tolerate small doses of UV radiation.

INTRODUCTION

UV radiation makes dimers between adjacent pyrimidines in DNA. The damage may be repaired before replication by two known mechanisms (reviewed by Witkin, 1969): photoreactivation (reviewed by J. K. Setlow, 1967), which does not occur in mammalian cells except those of marsupials (Cook and Regan, 1969; reviewed by Cook, 1970), and excision repair (reviewed by R. B. Setlow, 1968). If a dimer is not removed by either of these mechanisms before replication, it is thought that a gap is formed opposite the dimer in the daughter strand during the next round of DNA synthesis (Rupp and Howard-Flanders, 1968). These gaps are filled by recombination repair in bacteria (Rupp et al., 1971; Howard-Flanders et al., 1970). In Chinese hamster cells and in mouse lymphoma cells these gaps are also filled (Cleaver and

Thomas, 1969; Rupp et al., 1969), but the mechanism involves base insertion rather than recombination (Lehmann, 1972).

Normal human cells are capable of pyrimidine dimer excision, whereas this ability is diminished in cells derived from patients with xeroderma pigmentosum (Regan et al., 1968; Setlow et al., 1969). Recently, cells from a patient clinically diagnosed as having xeroderma pigmentosum have been found to exhibit normal unscheduled DNA synthesis (Burk et al., 1971). It is also known that cells from xeroderma pigmentosum patients survive small doses of UV radiation (D_0 = 9 ergs/mm^2) even though many pyrimidine dimers are made in the cells' DNA (Cleaver, 1970). This may be due to a less restrictive mutation or to the presence of a system that circumvents or ignores dimers, possibly by closing the gaps left opposite the pyrimidine dimers in DNA synthesized after UV irradiation. To close the gaps, each short segment would be elongated and these would then be joined to form high molecular weight DNA. This report describes experiments to determine whether cells derived from normal human individuals and xeroderma pigmentosum patients can elongate and join the small DNA units synthesized after UV irradiation.

MATERIALS AND METHODS

Cell Lines and Media

The cell lines used are listed in Table I. Cells were normally grown in minimum essential medium (Eagle, 1959) supplemented with 10% fetal calf serum and the nonessential amino acids at 37°C in a water-saturated atmosphere of 2% CO_2 in air. 1 or 2 days before each experiment the cells were trypsinized (0.25% trypsin) and plated in minimum essential medium supplemented with 10% calf serum and the nonessential amino acids on plastic Petri dishes (Permanox, Lux Scientific Corp., Thousand Oaks, Calif.) at approximately 20,000 cells per dish.

Prelabeling

In some cases the DNA was bulk labeled by incubation of the cells in medium containing 0.5 μCi/ml of methyl-^{14}C–thymidine, (SA, 35 Ci/mole) for more than 16 hr.

TABLE I
CELL LINES

Name	Origin
HeLa S3	Human cervical carcinoma
HSB	Normal human fibroblast
SS-XP1	Uncomplicated xeroderma pigmentosum
JP-XP2	deSanctis-Cacchione syndrome
WY-XP3	Clinically diagnosed as xeroderma pigmentosum but having normal repair replication

UV Irradiation, Pulse Labeling, and Incubation

Each dish of cells was washed with prewarmed phosphate-buffered saline (Dulbecco and Vogt, 1954) and exposed to UV radiation from a germicidal lamp at a dose rate of 10 ergs/mm^2·sec. The cells were then washed and incubated in prewarmed medium for 45 min. The DNA was pulse labeled by addition of 12–50 μCi/ml methyl-^{3}H-thymidine (SA, 20 Ci/mmole) for a prescribed time (usually 30 min). After pulse labeling, the cells were washed with prewarmed medium and incubated in medium supplemented with 2.5 μg/ml deoxycytidine and 2.5 μg/ml thymidine and, when indicated, 10^{-3} M hydroxyurea.

Preparation of Cells before Sedimentation

The cells were washed with and suspended in an ice-cold isotonic ethylenediaminetetraacetate (EDTA) solution (Setlow et al., 1969), centrifuged in a refrigerated centrifuge (Sorvall RC-2B, Ivan Sorvall, Inc., Norwalk, Conn.), and resuspended in cold EDTA solution. They were then irradiated with 2-krad gamma rays from a ^{60}Co source at a rate of 3.85 krad/min. The gamma irradiation causes single strand breaks in the DNA to permit sedimentation without excess entanglement of the DNA strands (Elkind and Kamper, 1970; Lehmann, 1972). The cells were then immediately lysed in a 0.2% sodium dodecyl sulfate solution on top of a 3.6 ml gradient of 5–20% sucrose, 0.1 M NaCl, and 0.1 M NaOH in a 4 ml polyallomer tube.

Sedimentation

The gradients were centrifuged at 40,000 rpm for 75 min at 20°C in the SW56 rotor of a Spinco model L3-50 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). 8-drop fractions were collected through a hole punched in the bottom of the tube onto Whatman No. 17 paper strips (Carrier and Setlow, 1971). The strips were washed in 5% trichloroacetic acid, twice in ethanol, and once in acetone (Bollum, 1966); dried, cut, and placed into vials with a toluene-2,5-bis-2-(5-*tert*-butylbanzoazolyl)-thiophene (BBOT) scintillator; and the radioactivity was counted in a liquid scintillation spectrometer. The molecular weights were calculated by computer, using the relationship observed by Abelson and Thomas (1966) and confirmed by Setlow (unpublished results), with single strands of T4, λ, and φX174 DNA's as standards (Freifelder, 1970). The relationship found was that single strand molecular weights are proportional to the 2.62 power of the distance sedimented.

RESULTS

DNA profiles on alkaline sucrose gradients of pulse-labeled DNA from UV-irradiated cells show more low molecular weight material than do profiles from unirradiated DNA (Fig. 1). The differences may be simply due to a slower rate of synthesis in the irradiated cells (reviewed by Rauth, 1970) or to the synthesis of DNA in smaller pieces as a result of gaps left opposite pyrimidine dimers (Rupp and Howard-Flanders, 1968). To eliminate the former consideration, we determined the pulse lengths that would give the same amounts of synthesis for irradiated and unirradiated cells. Fig. 2 shows the relative rates of DNA synthesis in irradiated and unirradiated cells. The DNA was prelabeled with thymidine-^{14}C irradiated with UV, and pulse

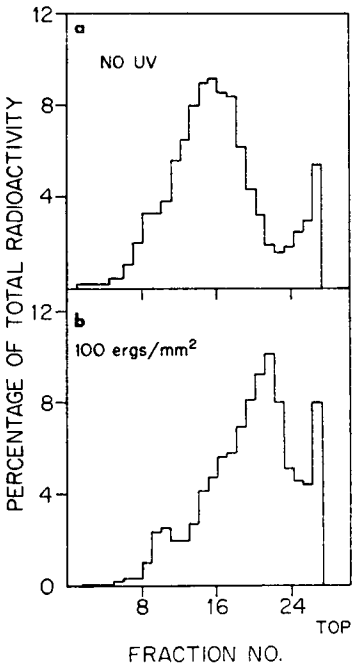

FIGURE 1

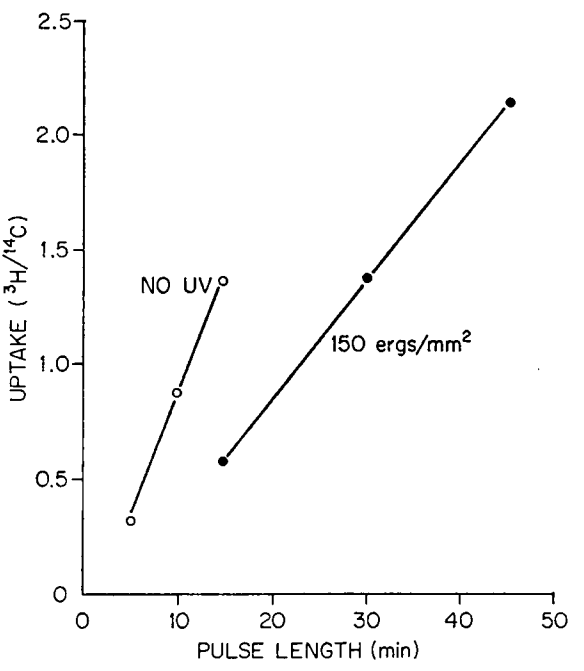

FIGURE 2

FIGURE 1 Sedimentation profiles of DNA from (*a*) unirradiated and (*b*) UV-irradiated (100 ergs/mm²) HSB cells. The cells were irradiated and washed, and the DNA was pulse labeled with 12 μCi/ml thymidine-^{3}H for 30 min before gamma irradiation (2 krad), lysis in sodium dodecyl sulfate on top of a 5–20% alkaline sucrose gradient, and sedimentation. Sedimentation is from right to left.

FIGURE 2 Relative rates of DNA synthesis in irradiated and unirradiated cells. HeLa cells were prelabeled with 0.5 μCi/ml thymidine-^{14}C for 16 hr before pulse labeling with 50 μCi/ml thymidine-^{3}H or irradiation with 150 ergs/mm² of 254 nm light and pulse labeling with thymidine-^{3}H. The cells were sonicated, placed on discs, washed in trichloroacetic acid, ethanol, and acetone, dried in air, and the radioactivity was determined.

labeled with thymidine-^{3}H. The $^3H/^{14}C$ ratio is an accurate measure of the amount of DNA synthesis, independent of sampling errors.

The rate of DNA synthesis in HeLa cells irradiated with 150 ergs/mm^2 of UV is approximately one-half that of unirradiated cells. The molecular weight profiles of DNA from irradiated and unirradiated cells having the same $^3H/^{14}C$ ratio and therefore the same amount of DNA synthesis are shown in Fig. 3. The lengths of these shorter pieces were estimated and correlated with the expected percentages of pyrimidine dimers for several UV doses (Setlow et al., 1969). Table II shows the agreement between the number average molecular weight of newly synthesized DNA and the average distance between pyrimidine dimers for the cell lines used. These data agree with those of Rauth (1970) and Rupp and Howard-Flanders (1968) and with the

suggestion that DNA synthesized after UV irradiation is made more slowly and contains gaps opposite the pyrimidine dimers. Upon incubation of irradiated, pulse-labeled cells, there is an increase in the molecular weight of the DNA. Fig. 4 shows the process of chain elongation and joining of the DNA strands and the inhibition

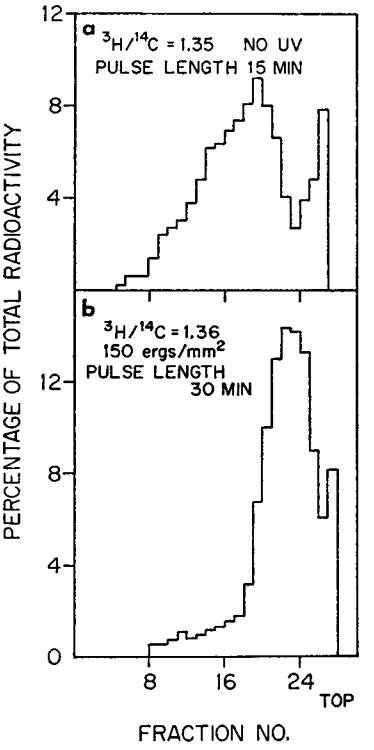

FIGURE 3 Sedimentation profiles of DNA from (*a*) unirradiated and (*b*) UV-irradiated (150 ergs/mm^2) HeLa cells showing the same amounts of DNA synthesis. The unirradiated cells were pulse labeled for 15 min and the irradiated cells for 30 min with 50 μCi/ml thymidine-^{3}H before gamma irradiation, lysis, and sedimentation.

TABLE II

MEASURED DISTANCE BETWEEN GAPS AND DISTANCE BETWEEN DIMERS

UV dose	Measured dimers*	Calculated distance between dimers‡	Measured distance between gaps§
ergs/mm^2	%	*10^6 daltons*	*10^6 daltons*
100	0.04	4.2	4.5
75	0.03	6.0	5.2–4.6

* Measurements provided by W. L. Carrier.

‡ From Setlow et al. (1969).

§ Number average molecular weight of the peak, not corrected for the number average molecular weight resulting from pulsing unirradiated cells.

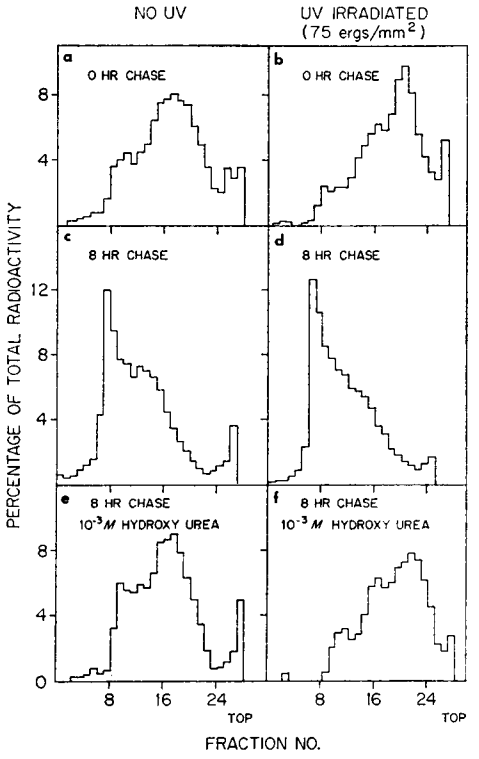

FIGURE 4 Sedimentation profiles of DNA from HSB cells. (*a*) Unirradiated, pulse labeled for 30 min with thymidine-^{3}H, harvested immediately, gamma-irradiated, lysed, and sedimented. (*b*) UV-irradiated with 75 ergs/mm^2, pulse labeled for 30 min with thymidine-^{3}H, and harvested immediately. (*c, d*) Profiles for (*a*) and (*b*), respectively, after 8 hr incubation as described in Materials and Methods. (*e, f*) DNA profiles after 8 hr incubation of unirradiated and irradiated cells, respectively, in the presence of 10^{-3} M hydroxyurea, as described in Materials and Methods.

of this process by 10^{-3} M hydroxyurea (Pfeiffer and Tolmach, 1967; Lehmann, 1972).

Fig. 5 shows that cells from the three types of xeroderma pigmentosum patients examined fill the gaps in the DNA synthesized after UV irradiation. These types were uncomplicated (Figs. 5 *a, b*), deSanctis-Cacchione syndrome (Figs. 5 *c, d*), and xeroderma pigmentosum pathology but with normal unscheduled DNA synthesis (Figs. 5 *e, f*). In addition to the above cell lines, the following cell lines have also been examined and have been found to exhibit chain elongation and joining after UV irradiation: Detroit 98 (human bone marrow), RPMI 4265 (human leukemia), GS-XP4 (uncomplicated xeroderma pigmentosum), FP-XP5 (de-Sanctis-Cacchione syndrome), and HeLa S3 (human carcinoma).

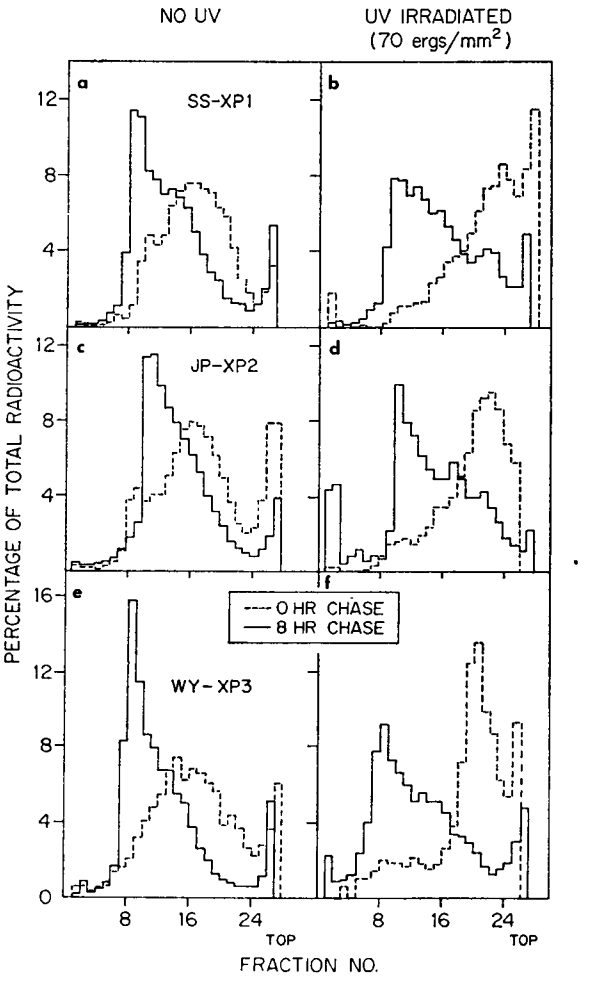

FIGURE 5 Sedimentation profiles of DNA from cells of three different xeroderma pigmentosum patients. The cells were either unirradiated (left column) or UV-irradiated (70 ergs/mm², right column), pulse labeled for 30 min with thymidine-³H, and either harvested immediately (----) or incubated for 8 hr (——) as described in Materials and Methods before harvesting. The patients' cells were (*a*, *b*) SS-XP1 (uncomplicated); (*c*, *d*) JP-XP2 (deSanctis-Cacchione Syndrome); and (*e*, *f*) WY-XP3 (xeroderma pigmentosum pathology but normal repair replication).

DISCUSSION

Our results agree with the findings of Cleaver and Thomas (1969) and Rupp et al. (1969) that DNA synthesized by mammalian cells after UV irradiation is in pieces of lower molecular weight than in unirradiated cells, and that these smaller pieces are elongated and joined during subsequent incubation, even though the dimers are

not removed (Lehmann, 1972). Our results show that this repair mechanism is independent of the ability to excise dimers.

The ability to complete DNA synthesis in cells that contain dimers may enable both normal human and xeroderma pigmentosum cells to divide without excising all the pyrimidine dimers from their DNA. This speculation is supported by the facts that 24–48 hr after UV irradiation of HeLa cells only 50% of the dimers are excised (Regan et al., 1968), and cell division is delayed only 6 hr after irradiation with 70 ergs/mm^2 (Djordjevic and Tolmach, 1967), even though unirradiated cells have a generation time of approximately 20 hr. The ability to complete DNA synthesis while there still are pyrimidine dimers in the DNA may explain why xeroderma pigmentosum cells are able to survive low doses of UV radiation (Cleaver, 1970).

Experiments to identify the defect in cells from a xeroderma pigmentosum patient that carry out normal repair replication have shown that these cells also exhibit normal gap-filling repair (Figs. 5 *e*, *f*). Our results show that gap-filling repair is present in normal, carcinoma, lymphoblast, and human bone marrow cells, as well as in xeroderma pigmentosum cells.

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