REPAIR OF RADIATION-INDUCED DNA DAMAGE IN NONDIVIDING POPULATIONS OF HUMAN DIPLOID FIBROBLASTS

G. J. KANTOR, R. S. PETTY, C. WARNER, D. J. H. PHILLIPS, AND D. R. HULL, *Department of Biological Sciences, Wright State University, Dayton, Ohio 45435 U.S.A.*

ABSTRACT The occurrence of DNA repair in UV- (254 nm) and X-irradiated normal human diploid fibroblasts maintained in a quiescent, nondividing state using low serum (0.5%) medium was ascertained. Techniques that detect different steps of the excision repair process were used so that the extent of completion of repair at single sites could be determined. These included measuring the disappearance of pyrimidine dimers by chromatography, detecting repair synthesis by density-gradient and autoradiographic methods and detecting the rejoining of repaired regions and repair of x-ray-induced single-strand DNA breaks using alkaline sucrose gradients. Results show that dimer excision occurs and the subsequent steps of repair synthesis and ligation are completed. About 50% of the dimers formed by exposure to 20 J/m^2 is excised in the initial 24-h post-UV period. DNA repair (unscheduled DNA synthesis) can be detected through a 5-d post-UV period. The fraction of damaged sites eventually repaired is not known. X-ray-induced single-strand DNA breaks are repaired rapidly.

INTRODUCTION

Most studies of the effects of radiation on human cells in vitro are done using actively dividing, exponential cell populations (for reviews, see references 1–3; for some exceptions, see references 4–10). Recently, Dell'Orco and colleagues described a cell culture system that permits the maintenance for long periods (>1 yr) of human diploid fibroblasts (HDF)[1] in a quiescent nondividing state (11, 12). Cells in these nonmitotic populations maintain metabolic function without degenerative changes, remain viable, and can be returned to an actively proliferating state if incubated in the appropriate medium. The nondividing state has no effect on the limited in vitro life span of HDF as determined by the eventual number of population doublings achieved before culture senescence. Dell'Orco and colleagues have proposed this culture system as a model for the study of normal human cell populations usually found as nonmitotic in vivo (11–13).

In some initial studies with radiation, an effect of ultraviolet light (UV, 254 nm) on the ability of these nondividing cells to remain attached to the surface of the culture vessel was observed (14). The fraction of exposed cells that detach was found to be dose-dependent. The detached cells are not viable as determined by uptake of viable stains and unsuccessful attempts at cultivation. In addition, the fraction of exposed cells that detach is strain-

[1] *Abbreviations used in this paper:* AT, ataxia telangiectasia; BrdUrd, 5-bromodeoxyuridine; dThd, thymidine; HBS, Hepes-buffered saline; HDF, human diploid fibroblasts, MEM-H, Eagle's minimum essential medium buffered with Hepes; UDS, unscheduled DNA synthesis.

dependent. DNA excision repair-proficient strains are better able to withstand UV exposure in this assay than are DNA repair-deficient strains. These results suggested that DNA repair may play an important role in the maintenance of irradiated nondividing cells. The purpose of this paper is to present data that demonstrate that excision repair of radiation-induced DNA damage occurs in quiescent, nondividing cell populations of DNA repair-proficient HDF strains and to a lesser extent in DNA repair-deficient strains (for reviews of the mechanisms of DNA repair and its occurrence in human cells, see references 1, 2, 15, 16). Techniques that detect different steps of the excision repair process were used so that the extent of completion of repair at single sites could be ascertained. These include measuring the disappearance of pyrimidine dimers by chromatography, detecting repair synthesis by density-gradient and autoradiographic methods, and detecting the rejoining of repaired regions and repair of x-ray-induced single-strand DNA breaks using alkaline sucrose gradients. Some of the results presented were the subject of an earlier preliminary report (17).

MATERIALS AND METHODS

Cell Strains and Culture Conditions

Cell strains used were HDF in the phase II stage of the proliferative life span (18). Strain WS-1 was isolated by Hay (19) from embryonic skin tissue. Strain WI-38 (ATCC No. CCL75), a normal embryonic lung strain (18), XP12BE (ATCC No. CRL 1223), isolated from skin tissue of a xeroderma pigmentosum patient (20), and Se Pan, (ATCC No. CRL 1343), isolated from skin tissue of an ataxia telangiectasia (AT) patient, were obtained from the American Type Culture Collection, Rockville, Md. Further descriptions of these cell strains and detailed cell culture conditions have been presented previously (14, 21). Briefly, cells were cultured at 37°C in a humidified incubator using Eagle's minimum essential medium supplemented with nonessential amino acids (Grand Island Biological Co., Grand Island, N.Y.; F-16 powdered media), streptomycin (50 mg/liter) and penicillin (61 mg/liter), and either 10 or 0.5% fetal calf serum (Grand Island Biological Co.). This medium, buffered with 30 mM Hepes at pH 7.5, is referred to as MEM-H.

When required, cells were released from the plastic culture vessel surface by treatment with a trypsin solution (0.25% in 0.01 M Hepes-buffered saline [HBS] containing 0.01 M trisodium citrate adjusted to pH 7.5 by the addition of NaOH).

Procedures for establishing nondividing populations using serum-deficient medium (MEM-H supplemented with 0.5% fetal calf serum) have been described in detail (11, 14). Actively proliferating cultures inoculated at a density of $\sim 4.6 \times 10^3$ cells/cm^2 were placed in serum-deficient medium in plastic tissue culture dishes (Falcon Plastics, Oxnard, Calif.) and incubated at 37°C for 10–15 d, with medium changes every 5 d.

Arrested populations with radioactively labeled DNA were obtained by culturing actively proliferating populations (inoculation density, 1.8×10^4 viable cells/cm^2) in MEM-H supplemented with 10% fetal calf serum and the appropriate radioactive precursor before placing cells in serum-deficient medium. For tritium labeling, cells were cultured with [^{3}H]dThd ([*methyl*-^{3}H] thymidine, 36.2 or 2 Ci/mM, Amersham Corp., Arlington Heights, Ill.) at a final concentration of 1 μCi/ml for 48 h; for ^{14}C labeling, [^{14}C]dThd ([2-^{14}C] thymidine, 58.2 mCi/mM, Amersham Corp.) at a final concentration of 0.1 μCi/ml for 48 h was used.

Routine tests of cultures for mycoplasma contamination using procedures outlined by Hayflick were negative (22).

UV- and X-irradiation Techniques

Techniques used for UV irradiation have been described previously (14). Culture vessels were drained of medium, rinsed once with prewarmed (37°C) HBS, and then exposed to UV. The UV source was a

Westinghouse 15-W germicidal lamp (Westinghouse Electric Corp., Pittsburgh, Pa.). Incident intensities were determined using a Yellow Springs Instruments radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). In those experiments requiring a post-UV incubation period, prewarmed (37°C) serum-deficient medium was added immediately and incubation at 37°C continued.

The source of x rays was a Hewlett-Packard Faxitron model 43804N x-ray unit (Hewlett-Packard Co., Palo Alto, Calif.) operated at 110 kV and 3 mA. Cells were usually irradiated while attached to the culture vessel surface in MEM-H through the plastic culture dish lid at a dose rate of 480 rads/min at either 23° or 2°C (incubated on ice). Dose rate was determined using a Victoreen R-meter and thimble chamber model 365 (Victoreen Inc., Sheller-Globe Corp., Cleveland, Ohio). Immediately after irradiation, the medium was removed, fresh serum-deficient medium was added and cultures were then incubated for desired times at 37°C. In some experiments, cells were irradiated while in suspension in HBS. Cells were released from the culture vessel surface with trypsin, collected by centrifugation, resuspended in HBS at ~1 × 10^5 cells/ml, and maintained at 23°C during irradiation.

Thymine Dimer Measurements

Thymine-containing pyrimidine dimers in UV-irradiated nondividing populations were detected and quantitated using the chromatography procedures of Carrier and Setlow (23) as modified for thin-layer cellulose sheets by Goldmann and Friedberg (24). Arrested populations were prelabeled with 2 Ci/mM [^{3}H]dThd before incubation for 10–15 d in serum-deficient medium. Routinely, arrested populations in 9-cm tissue culture dishes (Falcon Plastics) at a density of ~4.6 × 10^3 cells/cm^2 were used. Irradiated cultures were either harvested immediately for dimer analysis or incubated in serum-deficient medium for desired times. For each analysis, cells from four identically treated culture dishes were removed from the culture surface with trypsin, combined, collected by centrifugation, resuspended in 5 ml HBS, and precipitated by the addition of 5 ml ice-cold 10% trichloroacetic acid (TCA) for 30 min. The TCA-precipitate was washed once with 5 ml ice-cold 5% TCA and hydrolyzed with 97% formic acid as described by Carrier and Setlow (23). In some experiments, cells were lysed in a 1% sodium lauryl sulfate solution with incubation at 37°C for 5 min and the DNA extracted using Marmur's procedure (25). DNA was precipitated from the aqueous layer with ice-cold 10% TCA and hydrolyzed. Samples were chromatographed and chromatograms were analyzed as described by Goldmann and Friedberg (24).

Autoradiography

Culture vessels with glass coverslips attached to the bottom were inoculated with cells and treated as previously described to obtain nondividing cells. Usually serum-deficient medium containing [^{3}H]dThd (36.2 Ci/mM, 1 μCi/ml) was added immediately after UV irradiation to cultures that were subsequently incubated at 37°C for the desired times. In some experiments, the radioactivity was added at later times. After a defined incubation period, the medium was removed, cells were washed three times with HBS, fixed for 1 h in Carnoy's solution (three parts ethanol: one part glacial acetic acid), hydrated through an ethanol series (100, 95, 70%, and water, 5 min each in the respective order), and then air dried. Coverslips were mounted on glass slides, dipped in Kodak NTB-2 emulsion (Eastman Kodak Co., Rochester, N.Y.), dried at room temperature, and stored in light tight boxes at 4°C for 7 d. Photographic emulsion was developed as described by others (26). Slides were stained with toluidine blue before microscope observation at 400 magnification. 400–600 cells were scored for each determination. Backgrounds were usually low enough so that any nucleus containing greater than three grains above it was considered labeled.

Alkaline Sucrose Gradients

The techniques for DNA sedimentation in alkaline sucrose density gradients are modifications of the McGrath and Williams technique (27) described by Walters and Hildebrand (28). Cells from pre-[^{3}H]dThd-labeled nondividing populations were maintained in the arrested state for specified times after irradiation, released with trypsin, and then suspended in HBS. For the study of postirradiation DNA synthesis, arrested populations were pulse-labeled for 3 h with [^{3}H]dThd (10 μCi/ml, 36 Ci/mM)

immediately after irradiation. After harvesting with trypsin, samples of cell suspension (20 μl, = 1–2 × 10^3 cells) and ^{14}C-labeled T2 phage (20 μl, labeled as described by Rupp and Howard-Flanders [29]) were layered into 200 μl of a lysing solution (0.1% sarkosyl, 1 mg/ml heparin) on top of 4.7 ml 5–20% (wt/vol) linear alkaline sucrose gradients (0.4 M NaOH, 0.1 M versene, 0.1% sarkosyl). After 15 min at 20°C, 200 μl of a second lysing solution (1 M NaOH, 0.2 M versene) was gently added. The complete gradient was maintained for 45 min at 20°C before centrifugation. Gradients were centrifuged at 25,000 rpm at 15°C for 2.5 h in an SB-283 rotor equipped with 5-ml tube adaptors in an International B-35 centrifuge (International Equipment Co., Needham Heights, Mass.). Gradients were fractionated and the acid-insoluble radioactivity in each fraction determined as previously described (30). Number average molecular weights of the DNA distributions were calculated as described by Rupp and Howard-Flanders (29).

Density-Gradient Equilibrium Sedimentation

Nondividing cell populations prelabeled with [^{14}C]dThd as previously described were incubated in serum-deficient medium containing 1.6 × 10^{-5} M 5-bromodeoxyuridine (BrdUrd) for 1 h before UV irradiation. Immediately after UV irradiation, the BrdUrd-containing serum-deficient medium was returned to each culture and [^{3}H]dThd (10 μCi/ml, 36 Ci/mM) was added. After 3 h at 37°C, cells were harvested with trypsin, collected by centrifugation, resuspended in a salt solution containing 0.1 M NaCl, 0.05 M Tris and 0.1 M versene, and lysed by the addition of sodium lauryl sulfate (final concentration, 1%) and incubation at 37°C for 5 min. DNA was extracted using the procedure of Marmur (25). The chloroform:octanol mixture was reextracted once with the salt solution and the aqueous layers were combined. The appropriate volume of the DNA solution was added to 4.9 g of CsCl (model 219649, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) in a 5-ml volumetric flask. The solutions were subjected to centrifugation in an AH 650 rotor using a Sorvall OTD-2 ultracentrifuge (DuPont Instruments, Sorvall DuPont Co., Newtown, Conn.) at 30,000 rpm, 20°C for 45 h. Gradients were fractionated and the acid-insoluble radioactivity in each fraction determined as previously described (30).

RESULTS

Dimer Excision

Results of several experiments for measuring UV-induced pyrimidine dimers in irradiated arrested populations of XP and WS-1 are shown in Fig. 1. The results are presented as the percent of tritium-labeled thymine appearing as pyrimidine dimers vs. time after exposure to a 20-J/m^2 dose. Lines are drawn to connect the average values for each strain. Results obtained for WI-38 are not shown but were similar to those obtained for WS-1. The fraction of radioactivity appearing as pyrimidine dimers immediately after exposure to UV, ~0.005%/J per m^2, was similar for all three strains. This fraction was constant up to at least 40 J/m^2 (data not shown). A loss of ~50–70% of the dimers from the TCA-insoluble material during a 48-h period was observed for irradiated WS-1 populations. No loss of dimers was observed during this period in irradiated arrested XP populations.

Sedimentation in Alkaline Sucrose

No variations from that observed for control populations in the DNA alkaline sucrose sedimentation profiles were observed for any UV-irradiated population. Results of a typical experiment for arrested WS-1 populations using 40 J/m^2 and various post-UV incubation times are shown in Fig. 2. All sedimentation profiles are similar. Although a 40-J/m^2 dose caused ~70–80% of the WS-1 cells to detach eventually, little or no detachment occurred in

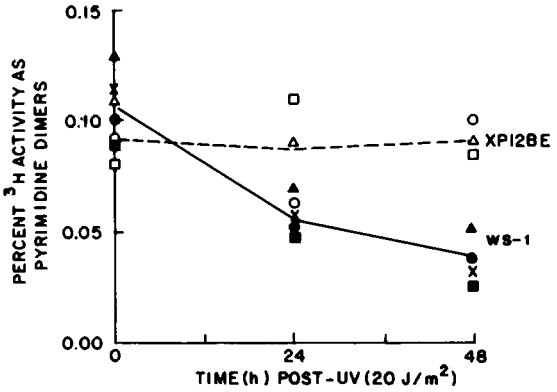

FIGURE 1 Excision of pyrimidine dimers in UV-irradiated nondividing HDF populations. Cells in serum-deficient medium for 10 d were irradiated (20 J/m^2) and maintained in serum-deficient medium for specified times before analysis of dimer content in either TCA-insoluble material or isolated DNA (■, □, x). Open symbols, XP12BE; closed symbols and (x), WS-1. Different symbols indicate separate experiments. Lines are drawn through the average value for XP12BE and WS-1. The percent of ^{3}H activity in the pyrimidine dimer region of the chromatograms for nonirradiated cells varied from 0.005 to 0.02% (total activity per chromatogram varied from 10^5 to 2×10^5 cpm).

these experiments before 48 h after UV. We can detect no loss of acid-precipitable DNA in irradiated populations through a 48-h postirradiation period (Fig. 2 and unpublished results).

Similar results were obtained for XP populations irradiated with 10 J/m^2 or less. Higher doses were not used with this strain because 10 J/m^2 causes an eventual detachment of >90% of the cells. Results of molecular weight calculations from sedimentation profiles for WS-1

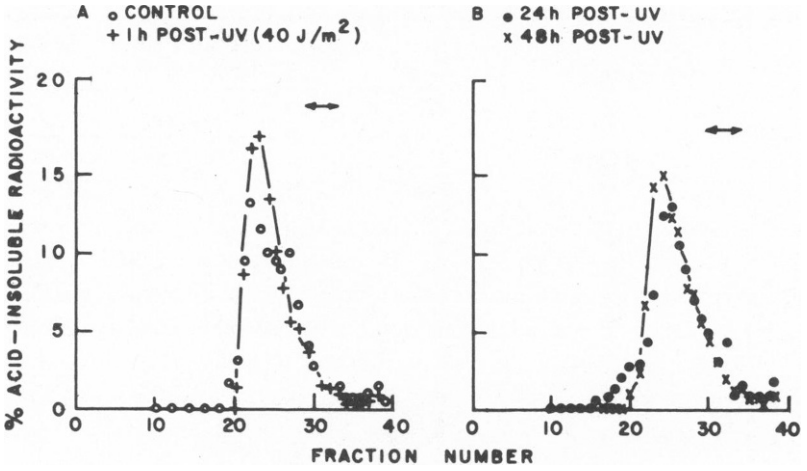

FIGURE 2 Sedimentation in alkaline sucrose of DNA from UV-irradiated nondividing WS-1 populations. Populations in serum-deficient medium for 12 d were irradiated (40 J/m^2) and incubated for the desired times before preparation for centrifugation. (○), control unirradiated arrested cells, 1600 cpm; (+), 1 h after UV, 1,490 cpm; (●), 24 h after UV, 1,630 cpm; (x), 48 h after UV, 1,500 cpm. The counts per minute are the total ^{3}H activity recovered from each gradient. All populations were labeled with [^{3}H]dThd before incubation in serum-deficient medium. The bar represents the region containing marker T2 DNA. Sedimentation is from right to left.

and XP are presented in Table I. Although some variations occur, none are significant and are within the range of values we have observed for controls in these and other experiments.

Repair Synthesis

Unscheduled DNA synthesis (UDS) was detected autoradiographically in arrested WS-1 and WI-38 populations and to a lesser extent in XP populations. Typical results are shown in Fig. 3. Although only 0–1% of the nuclei in controls had grains above them in the first 3-h period of the experiment, 95–100% of the UV-irradiated (10 J/m^2) WS-1 and WI-38 cells were labeled in the same period. After a 24-h post-UV period, 5–10% of the controls vs. 100% of the irradiated populations were labeled. In contrast only 14–18% of the XP cells were labeled in a 24-h post-UV period. Irradiation of XP with a higher dose (40 J/m^2) increased the fraction of labeled nuclei only slightly. No control cells were observed as labeled when incubated in the presence of hydroxyurea (Fig. 3 B) whereas no change in the fraction of nuclei labeled in UV-irradiated populations was observed.

The post-UV time-course during which UDS can be detected was determined using pulse-labeling procedures. Results of several experiments using 12- and 24-h pulses are presented in Fig. 4. Experiments with 12-h labeling periods show that a rapid decline, from 100 to ~40%, in the fraction of cells with labeled nuclei occurs after the initial 12-h post-UV period. A similar rapid decline is not observed until after at least 48-h after UV when 24-h labeling periods are used. Some UDS can be detected in irradiated arrested populations for up to 5 d after UV when 24-h pulse-labeling times are used. A reasonably large fraction of the

TABLE I
NUMBER AVERAGE MOLECULAR WEIGHTS DETERMINED FROM SEDIMENTATION PROFILES

Strain	UV dose	Molecular weight ($\times 10^8$) at the following postirradiation times			
		0 h	1 h	24 h	48 h
	(J/m^2)				
WS-1	0	2.8	4.0	3.5	3.0
	10	2.8	3.1	2.8	
	10	2.6	2.8	2.5	2.1
	40	2.6	2.9	2.2	3.1
XP12BE	10	2.1		2.4	2.3
	10	2.3		2.5	2.2
	X-ray dose *(krad)*				
WS-1	0	3.5			
	20	0.8 (0 C)*			
		0.9 (22 C)‡			
		3.7 (22 C)*		3.5	3.5
AT (Se Pan)	0	3.3			
	20	0.9 (0 C)*			
		0.9 (22 C)‡			
		2.7 (22 C)*		4.0	2.8

*Samples were irradiated attached to the culture vessel surface at the temperature indicated.
‡Samples were irradiated in suspension at the temperatures indicated.

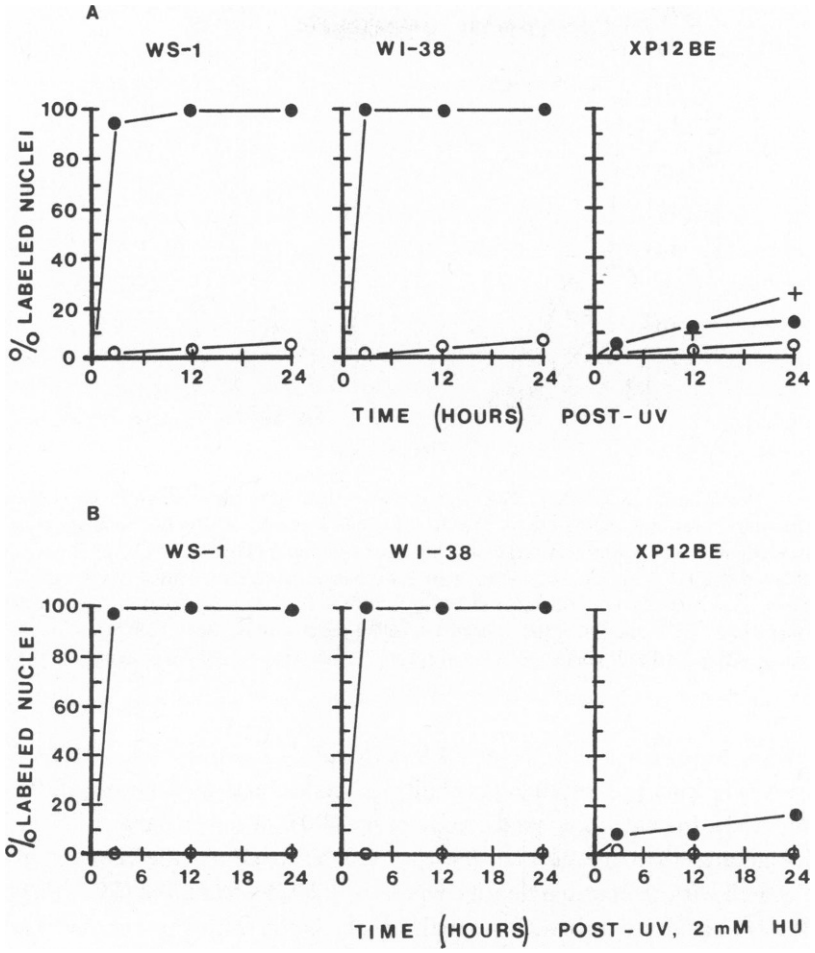

FIGURE 3 Unscheduled DNA synthesis in UV-irradiated nondividing HDF populations. Populations in serum-deficient medium for 10 d were UV-irradiated and subsequently incubated with [^{3}H]dThd for the times indicated before preparation for autoradiography. Nuclei with four or more grains over them were scored as labeled. Background levels varied from 0 to 2. At least 400 cells were observed per datum point. (A) Post-UV incubation in normal serum-deficient medium; (B) Incubation in medium supplemented with 2 mM hydroxyurea (HU). (○) control unirradiated populations; (●) UV-irradiated (10 J/m^2); (+), 40 J/m^{-2}.

control cells (~20% during the 24–48-h period) was also labeled. Although significantly less than that observed for UV-irradiated populations, it is considerably larger than the 0.3% that we reported as labeled in a 3-h pulse ([14] and Fig. 3). All of the control cells are heavily labeled, with an uncountable number of grains above their nuclei. In contrast, the average number of grains detected in UV-irradiated populations was 21, with a minimum of 5 and a maximum of 42.

To ascertain the nature of the UDS, DNA synthesized in a 3-h post-UV period was labeled with BrdUrd and [^{3}H]dThd and subjected to isopycnic density gradient centrifugation in CsCl (31). Results are shown in Fig. 5. WS-1 populations prelabeled with [^{14}C]dThd were

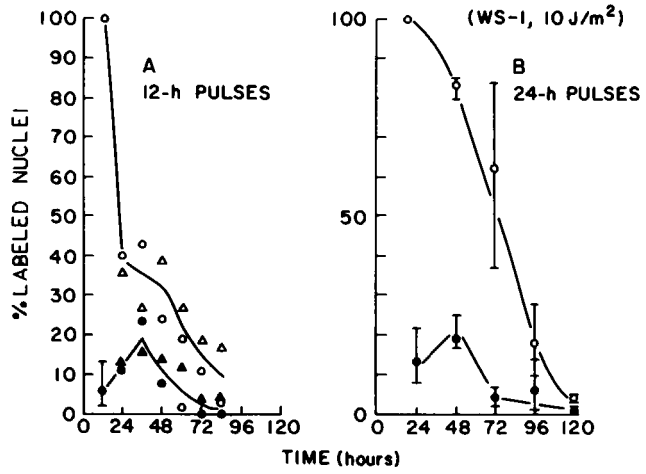

FIGURE 4 Detection of unscheduled DNA synthesis in arrested HDF cultures as a function of time after UV irradiation. The experiments are as described in Fig. 3 except that WS-1 populations in serum-deficient medium for 11 d were UV-irradiated and subsequently incubated for (A) 12-h periods or (B) 24-h periods with [^{3}H]dThd before preparation for autoradiography. Closed symbols, control unirradiated populations; open symbols, UV-irradiated (10 J/m^2); different symbols represent different experiments. Data points are placed at the end of the respective labeling periods. In A, the 0–12-h points are an average of five experiments. In B, all points are the average of four experiments; the bars represent the extreme values.

incubated with BrdUrd for 1 h before UV exposure to density label DNA synthesized semiconservatively and prevent the possibility of end-to-end association of ^{3}H to normal density DNA (32). In controls, a band representing ~90% of the ^{3}H-labeled DNA is observed at a density greater than the normal density ^{14}C-labeled DNA; the remaining 10% of ^{3}H activity is located with the normal density DNA. In the UV-irradiated (20 J/m^2) population, ~94% of the ^{3}H-activity bands with normal density DNA with the remainder at the same greater density as that observed in controls. Although the ratio of ^{14}C activity recovered from the gradients was ~1 (UV:control), indicating that nearly equal amounts of DNA per gradient were used, the ratio of ^{3}H activity was 4.7, reflecting a measure of UDS. Results similar to those shown here were observed in two other experiments.

The sedimentation properties in alkaline sucrose of DNA synthesized after UV were also determined. In preliminary experiments, it was found that when using our normally prepared nondividing populations, the sedimentation profile of DNA synthesized in a 3-h post-UV period was similar to that observed for DNA synthesized in an identical period in control unirradiated populations and for DNA from prelabeled cells (Fig. 2). However, if the medium in nondividing populations was not renewed for 10–12 d, the sedimentation profile for newly synthesized DNA in control cells was considerably different, having a lower average molecular weight than that observed for similarly treated prelabeled control cells. Using this variation in culture procedures, the DNA synthesized after exposure to UV can be distinguished from that occurring in unirradiated cells. Results of a typical experiment are presented in Fig. 6. Sedimentation profiles for DNA synthesized in a 3-h post-UV (10 J/m^2) period in WS-1 populations previously incubated for 10 d in the same serum-deficient

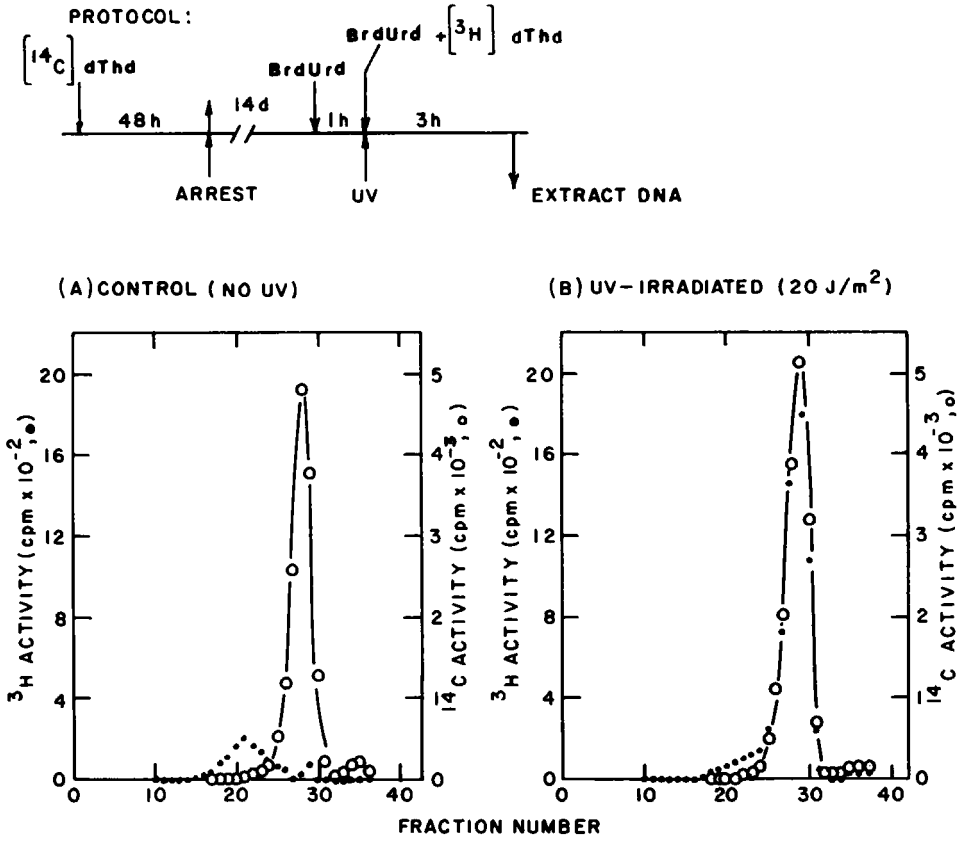

FIGURE 5 Density gradient equilibrium sedimentation of DNA from UV-irradiated populations of nondividing cells. Pre-[^{14}C]dThd-labeled populations of WS-1 maintained in serum-deficient medium for 14 d were incubated with BrdUrd for 1 h before exposure to UV, followed by a 3-h post-UV period with BrdUrd and [^{3}H]dThd (10 μCi/ml). Extracted DNA was subjected to equilibrium sedimentation in CsCl. Sedimentation is from right to left. (O), pre-[^{14}C] labeled DNA; (●), post-UV (20 J/m^2)^{3}H-labeled DNA.

medium are presented in Fig. 6 A. The controls were treated identically but were not irradiated. The profile for DNA from pre-^{3}H-labeled control cells treated in culture similarly to those in Fig. 6 A, but excluding the 3-h pulse labeling, are presented in Fig. 6 B. The control profile in Fig. 6 A is broad with a small peak coincident with T2 DNA. In contrast, the profile for UV-irradiated populations has a much larger average molecular weight, similar to that observed in Fig. 2. Most of the post-UV DNA synthesis occurs in large molecular weight DNA. A small peak coincident with T2 DNA, not observed in the prelabeled DNA profile (Fig. 6 B), is also evident. In this experiment (Fig. 6 A), ~0.25% of the control cells and 100% of the irradiated cells were labeled in the 3-h pulse as determined autographically. Results of an experiment similar to that of Fig. 5 (repair synthesis detected by CsCl density gradient centrifugation) using the culture protocol described for Fig. 6 (not renewing the medium) gave density-labeling results that were similar to those of Fig. 5.

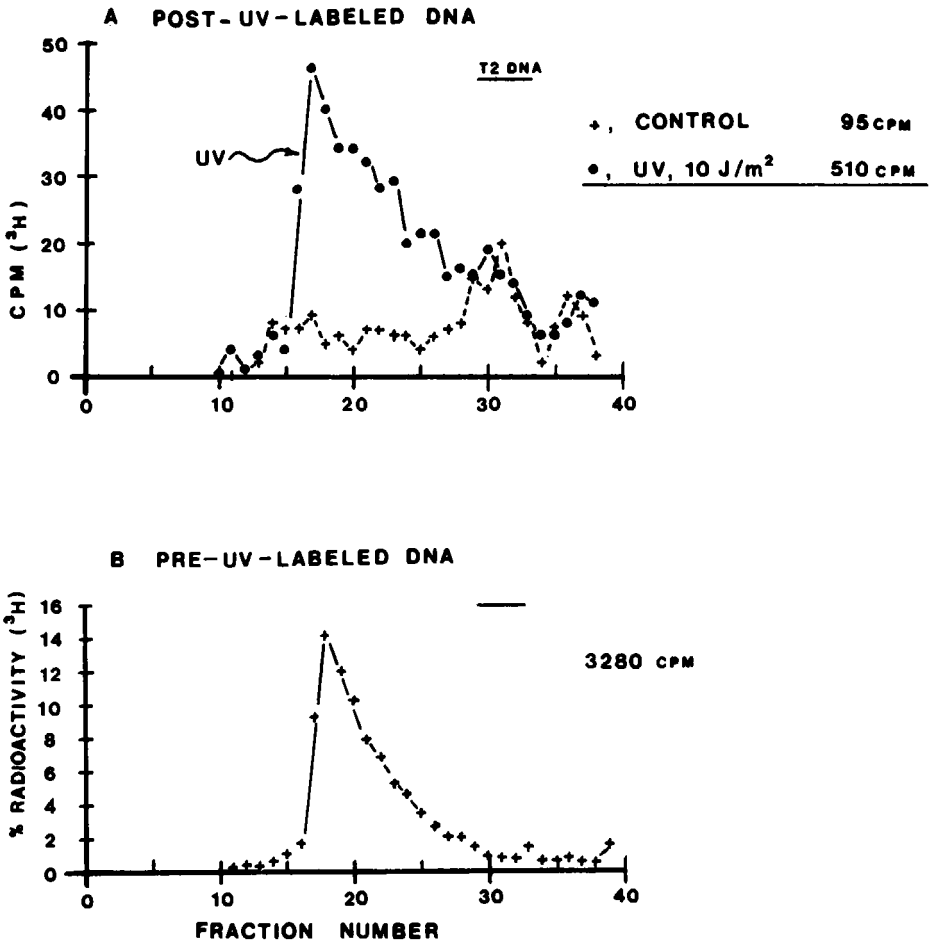

FIGURE 6 Sedimentation in alkaline sucrose of DNA synthesized in nondividing HDF populations after UV irradiation. (A) WS-1 populations in serum-deficient medium for 10 d, without the routine every 5 d medium change, were irradiated (●, 10 J/m^2) or used as unirradiated controls (+), returned to the same medium containing [^{3}H]dThd (10 μCi/ml) and incubated for 3 h before preparation for sedimentation in alkaline sucrose. (B) Control pre-[^{3}H]dThd-labeled WS-1 populations in serum-deficient medium for 10 d, without the routine every 5 d medium change.

Repair of X-Ray-induced Single-Strand Breaks

We could detect no single-strand DNA breaks in nondividing HDF exposed while attached to the culture vessel surface at 23°C to x-ray doses of up to 20 krads. Single-strand breaks could be detected if attached cells were irradiated at 2°C (on ice) or if cells were placed in suspension (HBS) and irradiated at 23°C. These results are presented in Fig. 7. At the dose rate used, a 41.7-min exposure time was required to irradiate cells with 20 krads. The data of Fig. 7 A illustrate that single-strand breaks not seen in unirradiated controls can be detected if cells are irradiated with this dose while cold or in suspension. The data of Fig. 7 B illustrate that single-strand breaks are not detected if attached cells are irradiated at 23°C. No breaks are evident after a postirradiation incubation period. Similar results were obtained using

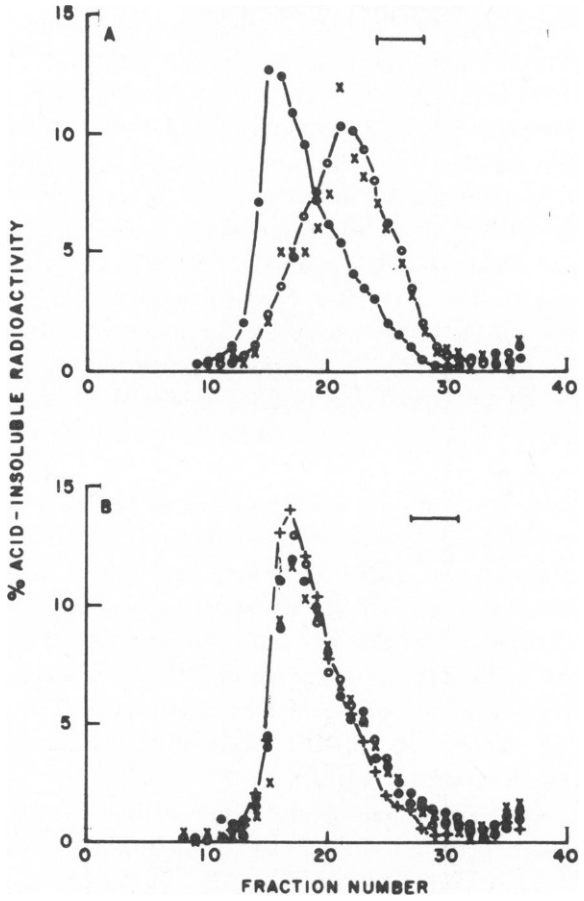

FIGURE 7 Sedimentation in alkaline sucrose of DNA from X-irradiated nondividing HDF. WS-1 cells in low serum medium for 12 d were X-irradiated (20 krads) and treated as indicated before preparation for centrifugation in alkaline sucrose gradients. (A) Detection of single strand DNA breaks (no post-irradiation incubation). (●), control, no irradiation; (○), irradiated at 2°C while attached to the culture vessel surface; (x), irradiated at 23°C while in suspension (HBS). (B) Repair of single-strand DNA breaks. All samples were irradiated at 23°C while attached to the culture vessel surface. (●), control, no irradiation; (x), 0 h after irradiation; (+), 1 h after irradiation; (○), 24 h after irradiation. Sedimentation is from right to left. The bar represents the region containing marker T2 DNA.

nondividing XP12BE and AT cells. Results using WS-1 and AT (Se Pan), summarized as average molecular weight determinations, are presented in Table I.

DISCUSSION

The occurrence of DNA repair in low serum-induced nondividing human fibroblast populations has been implied from measurements of UDS detected autoradiographically (17) or as incorporation of a precursor into DNA (7). Results of survival studies, measuring the ability of nondividing HDF to remain attached to a culture vessel surface and employing dose

fractionation procedures, have also suggested that DNA repair occurs in these nondividing HDF (17). DNA repair evidently also occurs in nondividing HDF maintained either in arginine-deficient medium (33) or in confluent monolayers using medium with 10% fetal calf serum (9, 34, 35). However, several studies employing other types of nondividing human cells (4–6, 9, 10) suggest that repair may not occur or may occur at a slower rate in nondividing cells compared to dividing or division-stimulated cells. The evidence presented here shows that low serum-induced nondividing HDF, which retain the capacity for further division upon appropriate stimulation, repair both UV- and x-ray-induced DNA damage. The excision repair process at individual sites is complete. Pyrimidine dimers are excised (Fig. 1), repair synthesis occurs (Figs. 3–6), and because no single-strand DNA breaks are detected (Fig. 2), the rejoining step occurs (also, Fig. 7). The excision of pyrimidine dimers occurs at about the same rate (50–70% in 48 h) as that reported for proliferating cells maintained in 10% serum-containing medium (36–38). A more rapid excision rate (50–70% in 1 h) observed by others (39) was not detected.

The detection of UDS in a fraction of the UV-irradiated nondividing XP12BE cells suggests that some excision repair occurs in this strain. This is consistent with the results of others using proliferating populations (40). Although no loss of dimers was observed in irradiated XP12BE populations (Fig. 1), the occurrence of a small amount of excision that was below the level of detection for the dimer assay cannot be excluded. Our results with irradiated XP12BE and UDS (Fig. 3) should not be interpreted to mean that only a small fraction of the XP12BE cells are capable of repair. Further results, not presented here, indicate that the level of repair is less in XP12BE than in WS-1 and that it occurs in the majority of the XP12BE cells, consistent with an earlier report (40).

Because UV irradiation causes nondividing cells to eventually degenerate and detach, we thought that a UV-induced degradation of DNA may occur. We have detected no solubilization of DNA in control or irradiated WS-1 or XP12BE populations (unpublished data). The data of Fig. 2 demonstrate this and also show that there is no degradation of the DNA into insoluble pieces, at least not into pieces smaller than can be detected in controls. No DNA degradation in WS-1 was detected after a 40-J/m^2 UV dose that causes a majority (70%) of the cells to detach. Detachment starts at ~48 h after UV. In similar experiments using XP12BE and appropriately lower UV doses, no DNA degradation or breaking was detected.

The UDS detected by autoradiography is indicative of repair synthesis (32). It is not blocked by hydroxyurea and, as shown in Fig. 5, bands with normal density DNA in CsCl density gradients. Whereas the synthesis that occurs in control cells is characterized by an incorporation of enough BrdUrd to alter its density, the amount of BrdUrd entering DNA in UV-irradiated cells is too small to affect its density and is probably repair synthesis (31, 32). The results obtained by sedimentation in alkali (Fig. 6) are consistent with this interpretation. DNA synthesized in the post-UV period sediments with the bulk of the DNA in alkaline sucrose gradients, suggesting that the UDS occurs in normal sized DNA fragments. Most of the DNA synthesized in unirradiated controls can be detected as smaller molecular weight pieces. The small peak of newly synthesized DNA in control cells (Fig. 6 A) is coincident with T2 DNA and therefore has a molecular weight of ~59 × 10^6 (41), consistent with a size known for some DNA replicative intermediates (42). The data of Fig. 6 B show that in these serum-starved cells, there is no general breakdown of existing DNA in unirradiated cells; the

DNA sediments as expected. The DNA synthesized in irradiated populations is indeed with the bulk of the DNA.

DNA repair as detected by autoradiography (i.e., repair synthesis, Fig. 4) can be observed through 4–5 d after irradiation. This experiment suggests that use of these nondividing cell populations to determine rates and extent of repair throughout long postirradiation periods is feasible. These data also show that a stimulation of DNA synthesis occurs in controls (i.e., ~20% of the nuclei are labeled in the 24- to 48-h period after the medium change). A further characterization of DNA synthesis in these control nondividing populations, using autoradiography and alkaline sucrose gradients, will be reported later.

Repair of single-strand breaks caused by x rays evidently occurs very rapidly. This is consistent with that found for normal human cells in a proliferating state (or in 10% serum medium) (for a review, see reference 43). Single-strand DNA breaks can be detected in our irradiation conditions only if cells are irradiated on ice and immediately subjected to lysis and analysis in alkaline sucrose gradients. This suggests that single strand breaks do occur in populations irradiated at 23°C and that because none is detected (Fig. 7 B), they are repaired during the radiation exposure time. In addition, because single-strand breaks can be detected in cells irradiated in suspension at 23°C but not in those irradiated attached to the culture vessel surface, unattached cells must be incapable of repairing these breaks. Results similar to those in Fig. 7 were obtained using XP12BE and AT cells indicating that neither of these two strains are deficient in sealing single-strand breaks in serum-deficient medium.

In conclusion, our results show that DNA repair occurs in UV- and X-irradiated HDF populations maintained in a nondividing state using low serum medium. The repair process is complete at individual sites. DNA repair as evidenced by UDS occurs at least through a 5-d post-UV period. Whether all sites are repaired is not known. The data suggest that these quiescent nondividing populations may be useful for a more detailed study of both rates and extent of repair in human cells.

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