EFFECTS OF ULTRAVIOLET IRRADIATION AND POSTIRRADIATION INCUBATION ON HETEROGENEOUS NUCLEAR RNA SIZE IN MURINE CELLS

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ABSTRACT We have analyzed the decrease in synthesis of individual size classes of heterogeneous nuclear RNA (hnRNA) in ultraviolet (UV)-irradiated Merwin plasmacytoma (MPC-11) cells at various times of postirradiation incubation. HnRNA from nonirradiated control cells is distributed over a wide range from approximately 60S to 5S, with 42S RNA carrying more label than any other size class. HnRNA from UV-irradiated cells shows a dose-dependent shift in size distribution toward lower molecular weight. The size distribution of hnRNA synthesized after prolonged times of postirradiation incubation is restored toward normal, i.e., synthesis of long RNA molecules increases relative to the synthesis of short ones. Analysis of the total number of hnRNA chains synthesized during a 20-min [^{3}H]uridine pulse shows a considerable reduction in their number with increasing UV dose. Murine cell lines are excision-repair-deficient but capable of post replication repair inhibited by caffeine. HnRNA transcripts of cells incubated in its presence were studied. The caffeine, which has no effect on hnRNA size in control cells, inhibits to a considerable extent the restoration of full-length transcripts during postirradiation incubation. The lack of excision repair in MPC-11 was confirmed by the analysis of pyrimidine dimers in trichloracetic acid-insoluble and soluble fractions within 8 h of postirradiation incubation.

The size of parental and daughter strand DNA in UV-irradiated cells was correlated with RNA transcript size. The parental DNA in these experiments does not change its size as a consequence of UV exposure and postirradiation incubation. In contrast, daughter DNA strands are short in UV-irradiated cells and they increase in size during postirradiation incubation to reach the size of parental strands after 8 h.

INTRODUCTION

Ultraviolet light (UV) irradiation of DNA causes dimerization of neighboring pyrimidines, formation of pyrimidine adducts, and occasional interstrand cross-linking and substitutions across the C_5/C_6 double bond of pyrimidines. When mam-

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malian cells replicate DNA containing such UV photoproducts, daughter chains are polymerized that are shorter than those synthesized in unirradiated control cells (Clarkson and Hewitt, 1976; Meyn et al., 1976; Walker and Sridhar, 1976; for review of earlier reports, see Painter, 1975). The formation of shorter chains is not a consequence of reduced rates of chain elongation, but is due to a failure to form long, continuous DNA chains (Povirk and Painter, 1976). It was also observed that the size of the newly synthesized DNA daughter strands is inversely related to the UV dose received by the template DNA and approximately equal to the distance between pyrimidine dimers in the parental DNA strands (Cleaver and Thomas, 1969; Meyn and Humphrey, 1971; Lehmann, 1972; Fujiwara, 1971; Buhl et al., 1972*b*). The proposal was forwarded that semiconservative replication leaves gaps in the daughter DNA strand opposite to pyrimidine dimers on the parental strand (e.g., Nilsson and Lehmann, 1975). With increasing time of postirradiation incubation, daughter strands increase in size (Buhl et al., 1972a; Lehmann, 1972) probably due to a DNA polymerization step termed "postreplication repair," which fills the gap left by semiconservative replication and joins DNA fragments.

At present, there is uncertainty as to the molecular basis for the production of discontinuous daughter strands and for the restoration of full-length DNA strands (see Painter, 1975). Models explaining the gap formation on the basis of the Okazaki et al. (1968) suggestion of discontinuous replication have been proposed recently by Meneghini (1976) and by Clarkson and Hewitt (1976). In particular, the proposal by Clarkson and Hewitt (1976) accounts for the failure of pyrimidine dimer-specific endonuclease to produce molecular weight reductions upon neutral sucrose gradient analysis of semiconservatively replicated, UV-irradiated DNA at a time after irradiation when daughter strand gaps have not yet been sealed by repair replication (Clarkson and Hewitt, 1976; Meneghini and Hanawalt, 1976). Such molecular weight reductions would be expected if daughter strand gaps were located opposite to pyrimidine dimers of the parental strand. However, molecular weight reductions of such DNA can be achieved by using single strand-specific but not pyrimidine dimer-dependent endonuclease (Meneghini, 1976). It therefore appears that daughter strand gaps are not located exactly opposite to pyrimidine dimers.

In excision repair-deficient rodent cell lines, pyrimidine dimers persist in the DNA for many hours after UV irradiation, while the processes of semiconservative replication and postreplication repair form short daughter strands and subsequently seal the gaps to form uninterrupted daughter strands. To find out how persistence of pyrimidine dimers and of DNA single strand gaps affect transcription, we have studied the template properties of DNA for in vivo synthesis of heterogeneous nuclear RNA (hnRNA) in UV-irradiated mouse Merwin plasmacytoma cells (MPC-11) at various times of postirradiation incubation; we correlate the finding on the transcript level with the physical state of the template DNA, i.e., the size of parental and daughter DNA strands. We have further investigated the inhibitory effect of caffeine on the restoration of DNA template function by assaying for the reappearance of full-length hnRNA transcripts.

UV-induced pyrimidine dimers cause transcription to terminate at the site of dimers, thus exerting a strong polar effect on the expression of promotor distal parts of transcription unit (Michalke and Bremer, 1969; Sauerbier et al., 1970; Hackett and Sauerbier, 1974). This effect leads to a UV-dose-dependent reduction in RNA chain length (Michalke and Bremer, 1969; Sauerbier, 1976). Processes that repair UV-damaged DNA are expected to restore synthesis of full-length transcripts. However, since pyrimidine dimers persist in the replicated DNA of murine cells, their transcription-terminating effect should persist at least in one of the daughter chromosomes. In agreement with this expectation, no recovery of synthesis of 45S ribosomal RNA precursor transcripts was found during 6 h of postirradiation incubation of mouse L cells (Hackett, 1974). On the other hand, recovery of the synthesis of 45S rRNA precursor during postirradiation incubation of green monkey kidney cells, CV-1, was reported recently by Nocentini (1976), and it is conceivable that this recovery was due mainly to excision repair.

We have shown recently that UV irradiation causes premature transcription termination in cultured murine cells (Hackett and Sauerbier, 1975) and we have exploited this effect in estimating the size of transcription units for hnRNA (Giorno and Sauerbier, 1976). Here, we raise the question of to what extent postirradiation incubation of murine cells will allow for the removal of the UV-induced transcription blocks. We therefore assay for the size of hnRNA transcripts in control cells and in UV-irradiated cells at various times after irradiation and we evaluate loss and restoration of synthesis of different size classes of hnRNA.

METHODS

Cell Growth and Labeling of Stable RNA

Myeloma mouse cells (MPC-11, generously supplied by Dr. M. Scharf, Albert Einstein College of Medicine, New York) were maintained at a density of 0.5–1.5 × 10^6 cells/ml in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N.Y.), supplemented with 20% dialyzed horse serum. The exponentially growing cells were prelabeled with [^{14}C] uridine (50 μCi/0.26 mg, New England Nuclear, Boston, Mass.) at a concentration of 0.05 μCi/ml before irradiation and subsequent incubation. The prelabeling period was around 22 h, which allowed for sufficient incorporation of [^{14}C]uridine into stable rRNAs, used as electrophoretic markers and internal standards for RNA recovery.

UV Irradiation and Pulse Labeling of RNA

Exponentially growing cells were harvested by centrifugation, resuspended in the medium at a concentration of 3 × 10^6 cells/ml and UV-irradiated with a $GEG_4T_4/1$ low-pressure mercury lamp (General Electric Co., Wilmington, Mass.) emitting predominantly at 254 nm at an incident dose rate of 1.3J/m^2 per s (for details, see Hackett and Sauerbier, 1975). Irradiation of the medium in this UV dose range has no effect on its ability to support cell growth (Antoschechkin, 1970). Irradiation in fluid other than growth medium was avoided, as it might lead to the leakage of cytoplasmic components or other possible unknown affects. The effective dose, determined by T7 bacteriophage inactivation in the presence of MPC-11 concentrated to 3 × 10^6 cells/ml, was 16.7 ± 3% of the incident dose (all doses are given in effective dose). The irradiated cells (2.0 ml) were transferred to 30 ml Corex centrifuge tubes (Corning Glass Works,

Corning, N.Y.) containing actinomycin D at 0.08 μg/ml. After 20 min of incubation at 37°C in a slow stream of CO_2 (5%), [^{3}H]uridine (New England Nuclear, 250 μCi/0.0035 mg) was added to a concentration of 20 μCi/ml. Label uptake was allowed for 20 min and terminated by adding 3 vol of ice-cold Earle's balanced salt solution (EBSS). Cells were harvested by centrifugation, resuspended in 1.6 ml NET (0.1 M NaCl, 0.01 M Tris-HCl, and 0.001 M EDTA, pH 7.2), 0.2 ml of lysis buffer (10% sodium dodecyl sulfate, 2×10^{-2} M EDTA, pH 7.0, adjusted with Tris base) and heated to 60°C for 65 s.

Repair Kinetics in the Absence and Presence of Caffeine

For studying repair kinetics, UV-irradiated cells were incubated with and without 10^{-3} M caffeine under a slow stream of 5% CO_2. Aliquots (2.0 ml) were withdrawn at various times of postirradiation incubation, treated with actinomycin D (0.08 μg/ml) and RNA-labeled for 20 min by addition of [^{3}H]uridine.

RNA Isolation and Size Analysis

RNA was isolated from cell lysates by the CsCl precipitation method (Sauerbier and Brautigam, 1970). The size distribution of RNA was analyzed by electrophoresis on composite 1.8% polyacrylamide–0.5% agarose gels (Loening, 1967). 3-mm fractions of the gels were solubilized by 40-min incubations at 100°C with 0.5 ml of 30% hydrogen peroxide containing 1% NH_4OH in tightly sealed vials. The radioactivity was assayed with Kinard's scintillator (Kinard, 1957) in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Test for Excision Repair

Exponentially growing cells were prelabeled with [methyl-^{3}H]thymidine (360 mCi/mM, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.) at a concentration of 1.0 μCi/ml for about 24 h. The cells were harvested by centrifugation and then washed two times with ice-cold Earle's balanced salt solution. Cells were resuspended in growth medium to a final concentration of 3×10^6 cells/ml and exposed to a UV dose of 84 J/m^2. Samples were taken immediately and after several hours of incubation in growth medium. The analysis of thymine-containing dimers was as described by Carrier and Setlow (1971). Cells were harvested and treated with 5% trichloracetic acid (TCA). The TCA-soluble fraction was repeatedly extracted with ethyl ether, which was drawn off upon phase separation. Both soluble and insoluble fractions were dried under vacuum and hydrolyzed in a sealed, evacuated ampoule with 88% formic acid at 175°C for 30 min. After hydrolysis, the contents of the ampoule were concentrated under a stream of air and thereafter applied to Whatman No. 1 paper strips (Whatman, Inc., Clifton, N.J.). Descending chromatography was carried out in *n*-butanol:water (86:14) at room temperature. Upon drying, the chromatogram was cut into 1.25-cm-wide strips starting from the origin; individual strips were placed in vials containing 1.0 ml water, and the radioactivity was counted in Kinard's (1957) liquid scintillation fluid.

Size Analysis of DNA on Alkaline Sucrose Gradients

The size of DNA synthesized before UV irradiation (parental DNA) and of DNA synthesized after irradiation (daughter DNA) was determined by centrifugation in alkaline sucrose gradients. Exponentially growing cells were prelabeled with [*methyl*-^{14}C]thymidine at 0.5 μCi/ml (54 mCi/mM, Schwarz/Mann) for about 30 h in the presence of 5% CO_2. Cells were harvested by centrifugation and then washed twice with ice-cold EBSS, resuspended in fresh growth medium to a concentration of 3×10^6 cells/ml, and irradiated with 28 J/m^2 effective UV dose. Control and UV-irradiated cells were labeled for 30 min with [*methyl*-^{3}H]thymidine at 50 μCi/ml immediately after irradiation. The cells were washed free of exogenous radioactive

thymidine by two washings with ice-cold EBSS and then incubated with fresh growth medium at 37°C under 5% CO_2. Samples were withdrawn at the requisite times, centrifuged, resuspended in EBSS, and layered directly on top of an alkaline sucrose gradient according to McGrath and Williams (1966). The cell concentration was kept low to minimize aggregation and concentration artifacts in DNA sedimentation. Sucrose gradients consisted of 5–20% sucrose containing 0.9 M NaCl, 0.01 M EDTA, and NaOH to pH 12.0 over a 1.0-ml cushion of a 50% sucrose solution. Onto it a lysis layer of 0.6 ml of 0.5 N NaOH, 0.01 M EDTA, and NaOH to give a pH of 12.0 was carefully laid. Cell lysis and DNA denaturation were allowed for 20 min at 25°C and the DNA sedimented for 3 h at 23,000 rpm, 25°C, in a Beckman SW27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Gradients were fractionated and analyzed for radioactivity in a toluene-based scintillator (21 toluene, 11 Triton X-100 [Research Products International Corp., Elk Grove Village, Ill.] 312 ml water, and 125 ml concentrated PPO-POPOP {2,5-diphenyl-oxazole–1,4-bis[2-(5-phenyloxazolyl)] benzene} liquid scintillator, Research Products International Corp., Elk Grove Village, Ill.).

Preparation of Nuclei and RNA Isolation from Nuclei

To determine the RNA content in the nuclei, prelabeled cells ([^{14}C]uridine; 0.05 μCi/ml: 30 h), either unirradiated or UV-irradiated with 28, 56, or 84 J/m^2, were labeled with [^{3}H]uridine (20 μCi/ml) from 20 to 40 min after irradiation in the presence of actinomycin D (0.08 μg/ml), added 20 min before the [^{3}H]uridine pulse. Under these labeling conditions, the ratio of ^{3}H/^{14}C counts in the whole cells (control and irradiated) was 0.1 and less. Cells were chilled, pelleted, resuspended in 1.0 ml of EBSS, and made 0.5% in NP-40 (Shell Chemical Co., Houston, Tex.), a nonionic detergent. The cells were vortexed and the nuclei sedimented. The supernatant was aspirated off, the pelleted nuclei were washed twice with NET, and resuspended in 1.6 ml NET. At this point, the ^{3}H/^{14}C ratio had changed to 20 or greater, indicating that the preparation of nuclei was almost free from ribosomes. The nuclei were disrupted by 2% sodium dodecyl sulfate and the RNA purified by CsCl precipitation. The amount of RNA was determined by measuring absorbance at 240, 260, and 280 nm; one A_{260} being equivalent to 50 μg/ml of RNA. The radioactivity was determined by liquid scintillation counting.

Dose-Effect Evaluation of RNA Gel Profiles

To provide an internal monitor for fluctuations in RNA isolation and in loading onto gels, ribosomal RNA's were prelabeled with [^{14}C]uridine for more than one cell doubling time. Correction for differences in RNA isolation and loading were based on the amount of [^{14}C]-rRNA (28S and 18S) observed on the individual gels, i.e., the ^{3}H counts in each gel profile were multiplied by the ratio (^{14}C counts in 28S rRNA from unirradiated cells)/(^{14}C counts in 28S rRNA from irradiated cells). UV irradiation (less than 100 J/m^2) of murine cells does not lead to a degradation of mature rRNA (Hackett, 1974); therefore, rRNA is a valid monitor.

The corrected gel profiles were then partitioned into various RNA size classes in increments of 10^3 nucleotides and the ^{3}H radioactivity in each size class converted into number of RNA chains based on the size of the RNA and an assumed constant specific activity of RNA (curies per mole) within the gel and for the RNA on gels from control and UV-irradiated cells. Hackett (1974) has shown that UV irradiation of murine cells has no effect on the specific activity of RNA synthesized after UV irradiation.

The next step was to add the number of RNA chains across a given gel profile, divide the total number of RNA chains in the RNA gel profile from unirradiated cells by the RNA chain number obtained from irradiated and postirradiation incubated cells with this quotient. This manipulation eliminates from consideration indirect effects of UV irradiation on hnRNA synthesis, as cell death and others, and assures that we are measuring only the UV effects on the

template DNA, i.e., we are reducing the experimental question to: "What is the size distribution of hnRNA in one of the same number of RNA chains synthesized in unirradiated, in UV-irradiated, and in postirradiation incubated cells?"

From the size-partitioned gel profiles, after above corrections for recovery and normalization for total RNA chains numbers were made, the loss of individual size classes of RNA as a function of UV dose was determined.

The loss of synthesis of individual size classes as a function of UV dose follows pseudo-first-order kinetics (Giorno and Sauerbier, 1976; this investigation), therefore, the inactivation rate constant, k, is defined by $k = -(\ln N_d - \ln N_0)/D$, where N_d is the number of RNA chains of a given size class from the UV-irradiated cells, N_0 is the number of RNA chains of the same size from unirradiated cells, and D is the UV dose.

Since the vast majority of hnRNA molecules constitutes primary transcripts, their sizes are equivalent to the sizes of the respective transcription units (Giorno and Sauerbier, 1976). Therefore, the inactivation rate constant for the synthesis of the individual size classes of hnRNA can be converted to unit length of DNA, which we have defined as 1,000 nucleotides pairs, conforming with earlier usage (Michalke and Bremer, 1969; Sauerbier et al., 1970; Hackett

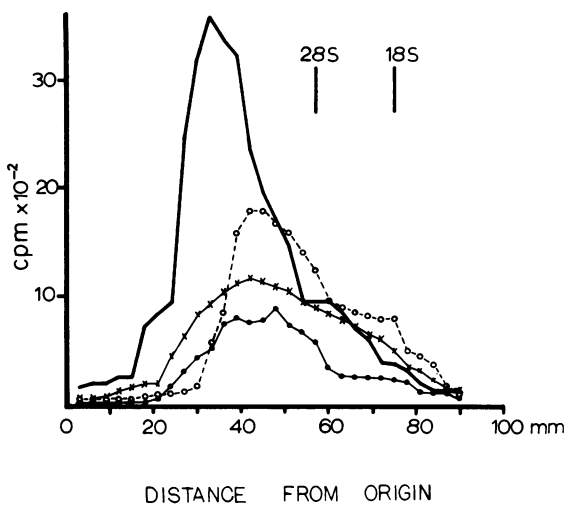

FIGURE 1 Polyacrylamide gel electrophoresis of MPC-11 hnRNA synthesized before irradiation and after 28 J/m^2 UV irradiation at various times of postirradiation incubation. Cells were prelabeled with [^{14}C]uridine (0.05 μCi/ml) for about 22 h. After concentration to 3 x 10^6 cells/ml and UV irradiation, 2.0 ml cells were incubated with actinomycin D (0.08 μg/ml) for 20 min and then pulse-labeled with [^{3}H]uridine (20 μCi/ml) for 20 min. RNA was isolated by the CsCl precipitation method and electrophoresed on 1.8% acrylamide-0.5% agarose gels at 30 V for 16 h. It was demonstrated by internal and end labeling of RNA that no aggregates are formed under these conditions of electrophoresis (Giorno et al., unpublished observation). Gels were sliced in 3-mm slices, digested with hydrogen peroxide/ammonium hydroxide, and counted in a scintillation counter. The gel profiles were normalized to an equal number of cells on the basis of internal ribosomal RNA markers. For the determination of repair kinetics, UV-irradiated cells were incubated under 5% CO_2, and aliquots of 2.0 ml were withdrawn at various times of postirradiation incubation, treated with actinomycin D, and pulse-labeled with [^{3}H]uridine. Vertical lines indicate the positions of 28S and 18S rRNAs. Migration of RNA is from left to right. Unirradiated (——); 28 J/m,^{2} (- -o- -); 28 J/m^2 after 4 (—x—) and 8 h (—●—) of post-irradiation incubation.

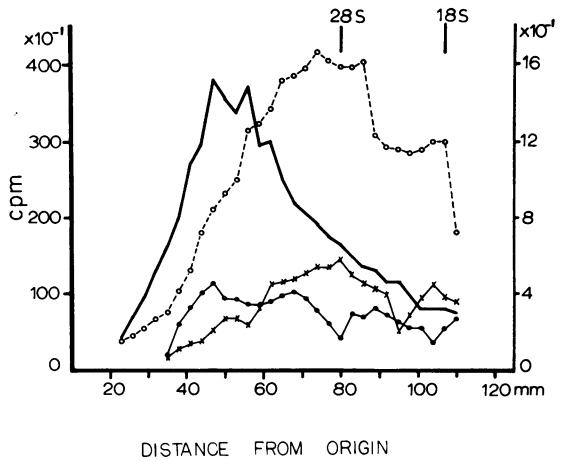

FIGURE 2 Polyacrylamide gel electrophoresis of MPC-11 hnRNA synthesized before and after 56 J/m^2 UV irradiation at various times of postirradiation incubation. The experimental conditions are the same as described in the legend to Fig. 1 except for the increased UV dose and electrophoresis at 30 V for 14 h. The actual ^{3}H in the gel profile from irradiated cells was much higher than the ordinate values indicate. The profile shows the hnRNA from identical number of cells. The left ordinate applies to the hnRNA from unirradiated cells (control), the right ordinate to RNA synthesized in 56 J/m^2 UV-irradiated cells. Unirradiated (——); 56 J/m^2 (- -o- -); 57 J/m^2 after 6 (—x—) and 12 h (—●—) of postirradiation incubation.

and Sauerbier, 1975). These facts were utilized to calculate the number of transcription terminating hits per 1,000 DNA base pairs per 100 J/m^2 effective UV dose.

RESULTS

Size of hnRNA Transcripts in UV-Irradiated Mouse Myeloma Cells at Various Times of Postirradiation Incubation

Control and UV-irradiated cells (3 × 10^6/ml) were incubated at 37°C for 20 min in the presence of actinomycin D (0.08 μg/ml) to arrest the synthesis of rRNA (Perry and Kelley, 1970) and then pulse-labeled for 20 min with [^{3}H]uridine in the continued presence of actinomycin D. Under these labeling conditions, no ribosomal RNA is made, only 5% of the radioactivity is in the cytoplasmic RNA, and 95% of the radioactivity is in hnRNA (Giorno and Sauerbier, 1976). Therefore, all of the labeled RNA will be referred to as hnRNA. RNA was extracted from whole cells and purified by precipitation through CsCl. The CsCl-purified hnRNA was then subjected to gel electrophoresis and analyzed for its size distribution.

Typical hnRNA size profiles are presented in Figs. 1–3. The radioactivity in hnRNA from nonirradiated control cells is distributed over a wide size range from approximately 60S to 5S, with 42S RNA carrying more label than any other size class (Figs. 1–3, control). HnRNA extracted from UV-irradiated cells shows a dose-dependent shift in size distribution toward lower molecular weights (Fig. 4). This agrees with our

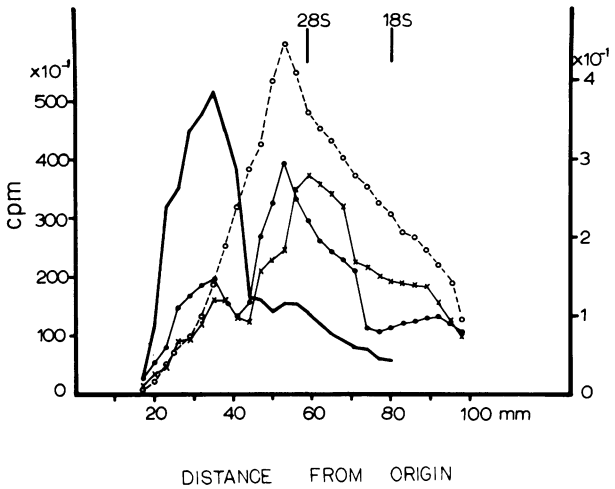

FIGURE 3 Polyacrylamide gel electrophoresis of MPC-11 hnRNA synthesized after 84 J/m^2 UV irradiation at various times of postirradiation incubation. Experimental conditions are the same as in Figs. 1 and 2 except for the extension up to 12 h of the postirradiation incubation period. Left ordinate applies to control, right ordinate to hnRNA from UV-irradiated cells. (The actual radioactivity in the gel analysis of RNA from irradiated cells was much higher than the ordinate values indicate. The profiles show the hnRNA from identical numbers of cells.) Unirradiated (——); 84 J/m^2 (- -o- -); 84 J/m^2 after 8 (—x—) and 12 h (—●—) of postirradiation incubation.

previous findings (Giorno and Sauerbier, 1976) and with the notion that UV lesions cause transcription termination.

The effect of postirradiation incubation on hnRNA transcript size was analyzed next. Cells, having received UV doses of 28, 56, and 84 J/m^2, were incubated for 0, 2, 4, 6, 8, and 12 h and treated with actinomycin D for 20 min followed by a 20-min pulse with [^{3}H]uridine. The actinomycin D was present during the pulse labeling. RNA was isolated and the size distribution of radioactively labeled RNA analyzed by gel electrophoresis. Fig. 1 shows that there is considerable restoration of synthesis of full-length transcripts within 8 h in cells having received 28 J/m^2; those having received 56 and 84 J/m^2 show less restoration of normal transcript size within 12 h of post-irradiation incubation (Figs. 2 and 3). To better illustrate this point, we replotted the hnRNA profile from cells exposed to 56 J/m^2 by size classes and as percent of total ^{3}H-radioactivity (Fig. 5). A gradual shift in synthesis from low molecular weight to higher molecular weight transcripts is seen within 8 h of postirradiation incubation. With 28 J/m^2 irradiated cells, an almost complete restoration of synthesis of high molecular weight transcripts was observed within 8 h; with 84 J/m^2 irradiated cells only incomplete recovery of synthesis of high molecular weight hnRNA was found (data not displayed as histograms).

The hnRNA size profiles in Figs. 1–3 and those for intermediate times were evaluated with respect to the following parameters: (*a*) Loss of synthesis of individual size classes of hnRNA as a function of effective UV dose: (*b*) Restoration of synthesis of

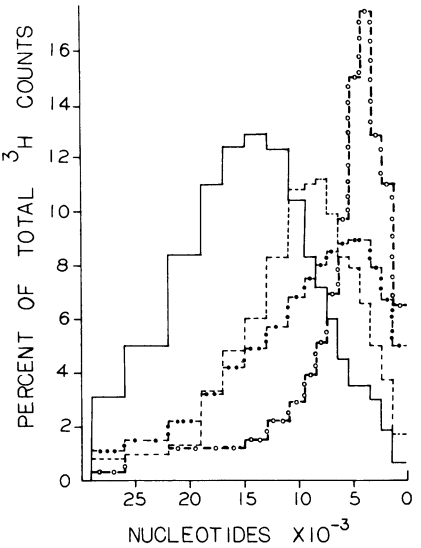

FIGURE 4

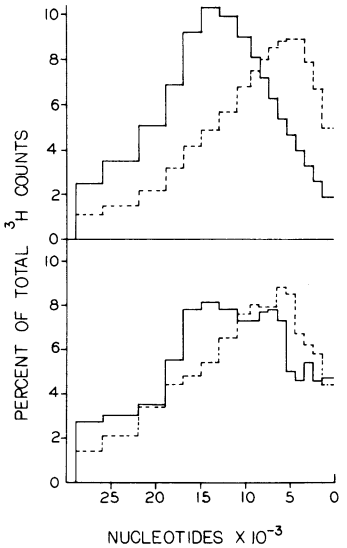

FIGURE 5

FIGURE 4 Histogram of gel profiles of hnRNA from unirradiated and UV-irradiated MPC-11 cells. Exponentially growing, [^{14}C]uridine-prelabeled cells were concentrated to 3×10^6 cells/ml, UV-irradiated, treated with actinomycin D, and pulse-labeled with [^{3}H]uridine, as described in Methods. Whole-cell RNA was isolated by centrifugation through CsCl. Aliquots were electrophoresed on acrylamide-agarose gel. The gel profiles were partitioned into various size classes of hnRNA (1.5×10^3–2.9×10^4 nucleotides) and ^{3}H was radioactivity tabulated in each size class as a percent of total [^{3}H]RNA. Control (——); 28 J/m^2 (- - -); 56 J/m^2 (-●-●-); 84 J/m^2 (-○-○-).

FIGURE 5 Histogram of gel profiles of hnRNA from unirradiated, 56 J/m^2 UV-irradiated, and postirradiation incubated MPC 11 cells. The details are as described in the legend of Fig. 4. Postirradiation incubation of UV irradiated cells was in the presence of 5% CO_2. Aliquots were withdrawn after 2 and 8 h of incubation, treated with actinomycin D, and pulse-labeled. Upper panel: unirradiated (——); 56 J/m^2 UV exposure (- - - -). Lower panel: 56 J/m^2 after 2 (- - -) and 8 h (——) of postirradiation incubation.

full-length transcripts as a function of time of postirradiation incubation; (*c*) Rate of elimination of transcription blocks during postirradiation incubation; (*d*) Apparent loss of RNA chain initiation as a function of UV dose.

The loss of synthesis of individual size classes of hnRNA as a function of UV dose follows pseudo-first order kinetics and the rate of loss is proportionate to transcript size (as reported earlier, Giorno and Sauerbier, 1976; Goldberg, Schwartz, and Darnell, personal communication). We, also, observe an apparent influx of prematurely terminated RNA chains into the low molecular weight size classes as evidenced by the negative *k* values in Table I (for data evaluation, see Methods). With increasing times of postirradiation incubation, synthesis of long hnRNA transcripts is restored, apparently at the expense of short transcripts (Fig. 6 and Table I). The rate of removal of transcription blocks was determined from these data (Fig. 7). It appears that the average lifetime of a transcription block is 12 h in MPC-11. This rate calculation as-

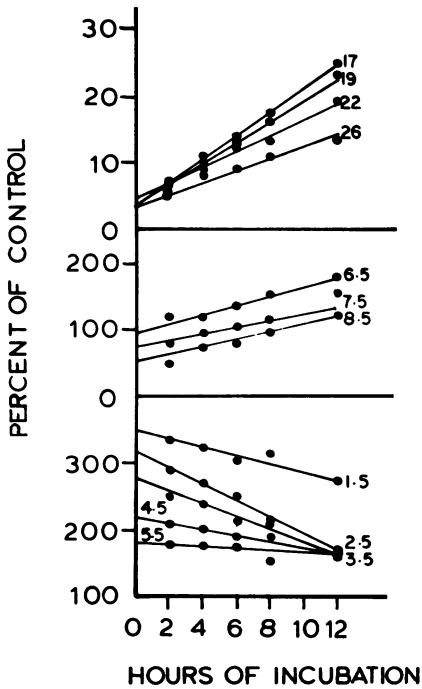

FIGURE 6

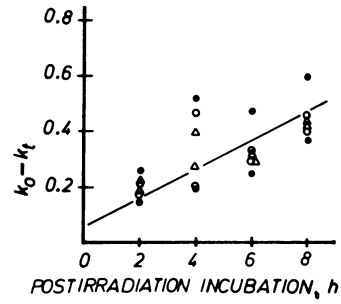

FIGURE 7

FIGURE 6 Relative rate of synthesis of various size classes of hnRNA as a function of post-irradiation incubation of 84 J/m^2 UV-irradiated MPC-11 cells. The tritium radioactivity profiles of the gels in Fig. 3 and duplicate profiles from a repeat experiment were partitioned into different size classes in increments of 10^3 nucleotides and the relative number of chains in each class was calculated as described in Methods. Since the total number of RNA chains labeled in a 20-min pulse decreases as a function of UV dose and since there is an additional decrease with time of postirradiation incubation, the number of total RNA chains for a given UV dose and time of postirradiation incubation was normalized to the number of chains in the control cell hnRNA. The relative rate of synthesis of a given size of class of hnRNA is the ratio (relative no. chains)$_i$/(relative no. chains)$_a$, where a refers to the hnRNA profile of control cells and i to the gel with the hnRNA from irradiated cells. The numbers near the lines give the size of RNA in nucleotides $\times 10^{-3}$. Similar families of curves are obtained for 28 and 56 J/m^2 (data not displayed).

FIGURE 7 Time-course of disappearance of transcription-terminating hits from DNA of UV-irradiated MPC-11 cells. HnRNA gel profiles from Fig. 1–3 (and of a duplicate for each UV dose; 28, 56, and 84 J/m^2) were partitioned into various size classes as described in Methods. After normalization on the basis of internal ribosomal RNA markers and for the number of total RNA chains synthesized during the pulse-labeling period on the basis of chain number integration over the hnRNA profile, k, the inactivation rate constant in the pseudo-first-order loss of RNA synthesis was calculated for each RNA size class, as given in Methods. The differences in the inactivation rate constant, $\Delta k = k_0 - k_t$(k_0 before postirradiation incubation and k_t at t = 0, 2, 4, 6, and 8 h of postirradiation incubation) for the hnRNA size classes between 10,000 and 22,000 nucleotides were calculated at the different UV doses. Values of Δk for the different size classes of hnRNA, after normalization to unit length, were found to be very similar; therefore, the numerical mean of Δk for a given UV dose is plotted versus time of postirradiation incubation. The line is fitted to the data points by least square approximation (correlation coefficient = 0.85). The multiple points at a given incubation period represent the duplicate experiments for 28 (open circles), 56 (closed circles), and 84 (triangles) J/m^2.

TABLE I

TRANSCRIPTION TERMINATING LESIONS,* k, PER UNIT LENGTH OF DNA‡ FOR THE DIFFERENT SIZE CLASSES OF hnRNA TRANSCRIPTION UNIT AT VARIOUS TIMES OF POSTIRRADIATION INCUBATION OF 84 J/m^2-IRRADIATED MPC-11.

hnRNA length in nucleotides $\times 10^{-3}$	Hours of postirradiation incubation					
	0	2	4	6	8	12
1.5	−0.54	−0.53	−0.51	−0.48	−0.50	−0.43
2.5	−0.51	−0.46	−0.43	−0.40	−0.33	−0.23
3.5	−0.45	−0.32	−0.38	−0.33	−0.32	−0.22
4.5	−0.41	−0.30	−0.30	−0.28	−0.28	−0.22
5.5	−0.26	−0.26	−0.25	−0.25	−0.19	−0.23
6.5	−0.02	−0.08	−0.07	−0.13	−0.18	−0.25
7.5	0.11	0.10	0.02	−0.02	−0.06	−0.19
8.5	0.24	0.19	0.13	0.10	0.009	−0.08
9.5	0.39	0.32	0.24	0.18	0.13	0.06
11	0.66	0.59	0.48	0.46	0.40	0.34
13	1.00	0.82	0.77	0.70	0.62	0.50
15	1.22	1.05	0.85	0.77	0.70	0.55
17	1.22	1.10	1.00	0.89	0.77	0.60
19	1.30	1.15	1.05	0.89	0.80	0.64
22	1.40	1.15	1.05	0.92	0.85	0.72
26	1.52	1.30	1.10	1.05	0.96	0.85
29	1.52	1.30	1.16	1.10	0.96	0.89

*Negative values result from the influx of UV-lesion-terminated transcripts from longer hnRNA transcription unit. hnRNA profiles from gels displayed in Figs. 1 through 3 were evaluated as follows: First, correction was made for RNA recovery in the isolation procedure based on the recovery of ^{14}C-prelabeled rRNA. Next, radioactivity profiles were converted to RNA chain number profiles and the total RNA chain number of any given gel set equal to the RNA chain number in the control RNA gel. This manipulation eliminates effects of UV irradiation on the rate of RNA chain initiation and leaves for consideration only those UV lesions which reside within the transcribed portion of the transcription unit. k was calculated as described in Methods.

‡Unit length of DNA is defined as 1,000 base pairs.

sumes that removal of transcription blocks is random in time, i.e., by a process approaching first-order kinetics.

Analysis of number of hnRNA chains synthesized during a 20-min [^{3}H]uridine pulse shows a considerable reduction in numbers with increasing UV dose (Fig. 8). This analysis is based on the assumption that the specific activity of the RNA remained the same within each size class.

To gain more insight into the hnRNA specific activities and labeling kinetics in UV-irradiated cells, we have determined both the total RNA in the nuclei of MPC-11 at 40 min after irradiation (in the presence of actinomycin D) and the accumulation of [^{3}H]uridine in hnRNA during a 20-min pulse from 20 to 40 min after irradiation (Table II). The total RNA per nucleus and the incorporation of [^{3}H]uridine decrease with increasing UV dose. The RNA present in the nuclei at 40 min after irradiation is the sum of the RNA synthesized before irradiation and not decayed by this time and the RNA synthesized after irradiation. Both RNAs, we assume, are turned over with

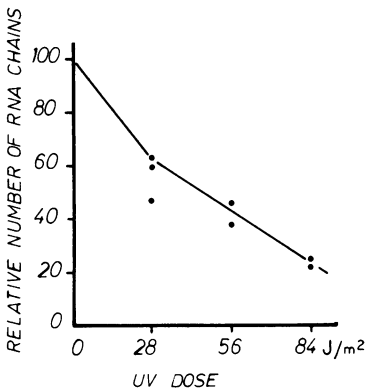

FIGURE 8

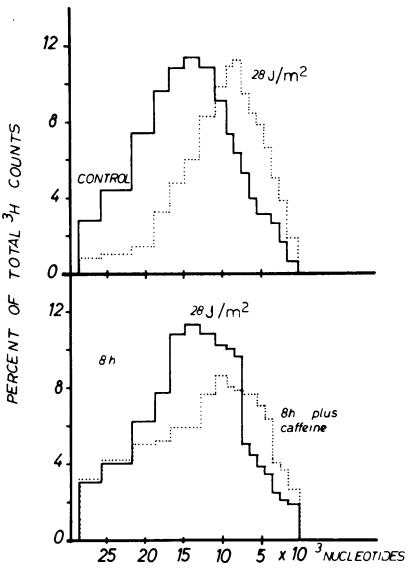

FIGURE 9

FIGURE 8 Relative number of hnRNA chains synthesized in MPC-11 during a 20-min pulse-labeling period as a function of UV dose to the cells. The gel profiles of tritium-labeled hnRNA from Figs. 1–3 and of one or more duplicate experiments at each UV dose (28, 56, and 84 J/m^2) were partitioned into approximately 20 molecular weight classes from 3.3×10^5 to 1×10^8 daltons. The total number of hnRNA chains in the gel from control cells is set at 100 and used as a standard to calculate the relative number of hnRNA chains synthesized in the UV-irradiated cells. The number of counts in each class was converted to RNA chain number according to:

$$\left(\begin{array}{l}\text{Relative number}\\ \text{of RNA chains)}i,j,\end{array}\right. = \frac{(\text{cpm})i,j}{(\text{mol wt class})} \times \frac{(^{14}\text{C cpm in 28S})\ \text{control}}{(^{14}\text{C cpm in 28S})\ i},$$

where i is the index for a particular gel and dose, and j is the molecular weight class index (3.3×10^5, 6.6×10^5, 9.9×10^5, etc.). Multiple points for a given UV dose represent RNA chain numbers observed in different sets of experiments.

FIGURE 9 Histograms of gel profiles of hnRNA synthesized before and after irradiation in the presence and absence of caffeine. [^{14}C]uridine prelabeled cells were UV-irradiated (28 J/m^2) and incubated with and without (10^{-3}M) caffeine under 5% CO_2. Samples (2 ml) were withdrawn, actinomycin D-treated, and pulse-labeled with [^{3}H]uridine, and the RNA was isolated by the CsCl precipitation method and electrophoresed on 1.8% acrylamide/0.5% agarose gels. The RNA gel profiles were partitioned into various molecular weight classes and corrected for losses in RNA isolation with the aid of the internal ^{14}C ribosomal RNA markers. ^{3}H incorporation in hnRNA from 1.5×10^3 to 2.9×10^4 nucleotides (approximately 20 size classes) was summed up and the percent of these total counts in various size classes was plotted. Upper panel: hnRNA synthesized in MPC-11 before and shortly after irradiation in the absence of caffeine. Lower panel: hnRNA synthesized in irradiated cells after 8 h of postirradiation incubation with and without caffeine.

TABLE II

RELATIVE AMOUNTS OF RNA IN NUCLEI OF MPC-11 AT 40 MIN AFTER UV IRRADIATION AND [^{3}H]URIDINE UPTAKE INTO hnRNA

UV dose	Observed relative amount of RNA		Relative [^{3}H]uridine uptake		Calculated relative amount of RNA
J/m^2					
0	1.00*	1.00‡	1.00*	1.00‡	1.00§
28	0.80	0.76	0.58	0.62	0.74
56	0.57	0.60	0.35	0.29	0.55
84	0.44	0.44	0.19	0.18	0.45

*and ‡ denote independent experiments.

§Calculated with assumption that no ribosomal RNA precursors are present in the nuclei after 40 min of actinomycin D treatment and using half-lives of 25 min for hnRNA and rates of hnRNA synthesis as measured by [^{3}H]uridine uptake.

a half-life of 25 min (Brandhorst and McConkey, 1974). At t = 40 min, 0.33 of the RNA synthesized before irradiation is still in the nucleus and an additional amount of RNA, determined by the new rate of synthesis and by turnover after UV irradiation, will have accumulated. Assuming that the [^{3}H]uridine uptake into hnRNA is an accurate measure of RNA synthesis rates, we can calculate the amount of RNA accumulated after UV irradiation according to $(RNA)_t = (a/b)(1 - e^{-bt})$, where $(RNA)_t$ is the amount of RNA at time t, a is the rate of RNA synthesis and b is the rate of decay by first-order kinetics (Brandhorst and McConkey, 1974; Hercules et al., 1976). Table II shows that the actually observed amount of RNA in the nuclei and the amount calculated with the assumption that uptake of [^{3}H]uridine is an accurate reflection of RNA synthesis are in very good agreement. Therefore, little, if any, change in specific activity of hnRNA synthesized after various doses of UV irradiation could have occurred and the RNA chain number determinations presented in Fig. 8 should be fairly accurate. Also, uridine uptake and phosphorylation were shown to be unaffected in L cells by UV doses up to 100 J/m^2 (Hackett, 1974). In addition to the reduction in RNA chain number as an immediate result of UV irradiation, postirradiation incubation leads to further reduction in total radioactivity incorporated into RNA, while at the same time, the normal size distribution of hnRNA is restored. Reduction of label uptake into RNA with prolonged times of postirradiation incubation is at least in part due to a complete loss of RNA synthesis in a fraction of cells, as we have observed in autoradiographic studies (data not shown).

Repair Inhibition by Caffeine

As a rule, murine cell lines are capable of postreplication repair and display low activities of excision repair (Klimek, 1966; Rasmussen and Painter, 1966; Painter and Cleaver, 1969). To identify the repair mechanism responsible for the slow restoration of full-length hnRNA transcripts in UV-irradiated MPC-11, we studied the transcripts in cells incubated in the presence of 10^{-3}M caffeine. Caffeine was shown to inhibit host cell reactivation of UV and X-ray damage in bacteriophage DNA (Sauerbier,

1964*a*,*b*), bacterial DNA (Metzger, 1964), and postreplication repair in mammalian cells (Buhl and Regan, 1974; Buhl et al., 1973). It was found to have no effect on excision repair in human cells (Regan et al., 1968; Cleaver, 1969).

We incubated control cells and UV-irradiated cells in the presence of 10^{-3}M caffeine for various times, inhibited rRNA synthesis by actinomycin D, and labeled the hnRNA by a 20-min incorporation of [^{3}H]uridine. RNA was isolated and resolved by size on agarose/acrylamide gels. We observed that caffeine at 10^{-3} M has no effect on hnRNA size in control cells and no effect on size of hnRNA synthesized immediately after UV irradiation (unpublished observation).

However, caffeine inhibits to a considerable extent the restoration of full-length transcripts during postirradiation incubation (Fig. 9). These data suggest that postreplication repair is responsible for restoring the template activity of DNA for hnRNA transcription. We noticed some recovery of hnRNA transcript size in 28 J/m^2 irradiated cells between 0 and 2 h of incubation with caffeine. This could be due to incomplete inhibition of postreplication repair or to low levels of excision repair. The same analysis of hnRNA size in 84 J/m^2 irradiated cells was performed and restoration of full-length hnRNA transcription was completely inhibited during a 12-h postirradiation incubation (unpublished observation).

Absence of Pyrimidine Dimer Excision in MPC-11

To confirm the conclusion that postreplication repair is mainly responsible for restoration of synthesis of full-length hnRNA transcripts in UV-irradiated MPC-11, we measured the extent of the pyrimidine dimer excision in 84 J/m^2 irradiated cells. DNA was labeled with [*methyl*-^{3}H]thymidine (1.0 μCi/ml) for about 24 h in exponentially growing cells. Exogenous [^{3}H]thymidine was removed and the cells were then UV-irradiated with 84 J/m^2 and incubated in growth medium in the dark. The ratios of radioactivity in pyrimidine dimers over radioactivity in thymine were determined in the TCA-soluble and insoluble fractions after 0, 2, and 8 h or postirradiation incubation. Table III shows that there is no preferential loss of pyrimidine dimers from the TCA-

TABLE III

DISTRIBUTION OF [^{3}H]THYMINE AND THYMINE DIMERS IN DNA FROM UV-IRRADIATED MPC-11 CELLS

UV exposure	Time after exposure	Acid-insoluble fraction			Acid-soluble fraction		
		^{3}H in thymine-pyrimidine dimers ($\hat{TP}$)	^{3}H in thymine (T)	^{3}H in $\hat{TP}$/T	^{3}H in thymine-pyrimidine dimers ($\hat{TP}$)	^{3}H in thymine (T)	^{3}H in $\hat{TP}$/T
J/m^2	*h*			%			%
84	0	13×10^3	92×10^5	0.14	60	27×10^3	0.22
	2	10×10^3	86×10^5	0.12	18	20×10^3	0.09
	8	9.5×10^3	69×10^5	0.14	18	18×10^3	0.10

insoluble fraction within 8 h of incubation, nor any increase in the $\hat{T}P/T$ ratio in the soluble fraction.

DNA Size Analysis in UV Irradiated MPC-11

To correlate in time and amount the previously observed restoration of synthesis of full-length transcripts with the repair events on the DNA level, we determined the size of parental DNA (DNA synthesized before UV irradiation) and of the daughter DNA (DNA synthesized after UV irradiation) by alkaline sucrose gradient centrifugation. Parental DNA was labeled with [*methyl*-^{14}C]thymidine for 30 h, extraneous [^{14}C]-thymidine was removed, and the cells were UV-irradiated with 28 J/m^2 effective dose. Immediately after irradiation, the cells and a nonirradiated control culture were labeled for 30 min with [*methyl*-^{3}H]thymidine; extraneous label was washed out and samples removed for alkaline sucrose gradient analysis at 0, 2, 4, 6, 8, and 12 h. The results are shown in Fig. 10. Daughter DNA strands are short in UV-irradiated cells during the first 30 min of synthesis and they increase in size during postirradiation

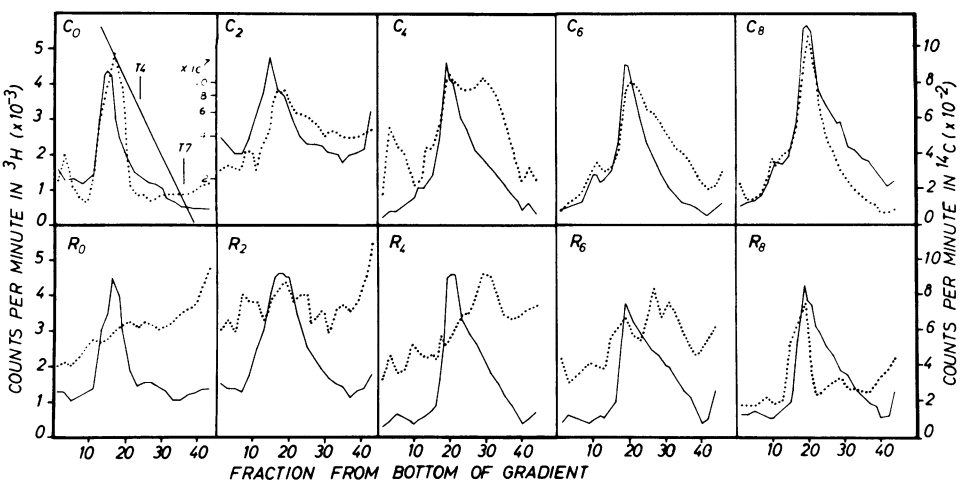

FIGURE 10 Sedimentation profiles of MPC-11 parental and daughter DNA strands in alkaline sucrose gradients immediately after UV irradiation at 28 J/m^2 and after various times of post-irradiation incubation. Exponentially growing cells were prelabeled with [^{14}]thymidine (0.5 μCi/ml) for 30 h, thoroughly washed, and UV-irradiated with 28 J/m^2 effective dose at a concentration of 3 × 10^6 cells/ml. Control and UV-irradiated cells were pulse-labeled with [^{3}H]thymidine (50 μCi/ml) for 30 min. Radioactive thymidine was washed out and the cells were incubated in fresh medium at 37°C under 5% CO_2. At the requisite times, approximately 6 × 10^4 cells were lysed on top of the gradient for 20 min at 25°C, and the denatured DNA was sedimented for 3 h at 23,000 rpm in a SW27 rotor. The gradients were fractionated and the radioactivity determined in a toluene-based scintillator. Continuous lines: ^{14}C-labeled parental DNA. Dotted lines: ^{3}H-labeled daughter DNA. Panels C_0 through C_8: DNA from unirradiated cells at 0 through 8 h after labeling with [^{3}H]thymidine. Panels R_0 through R_8: DNA from 28 J/m^2 irradiated cells isolated at 0, 2, 4, 6, and 8 h of postirradiation incubation. The positions of bacteriophage T4 and T7 markers are indicated in frame C_0. The straight line in panel C_0 gives the log molecular weight of DNA based on the sedimentation of T4 and T7 markers.

incubation, to reach the size of parental strands after 8 h. In contrast, parental DNA does not change its size as a consequence of UV irradiation and postirradiation incubation. Daughter DNA strands in unirradiated MPC-11 display the same size as parental strands at all times after the 30-min labeling period.

DISCUSSION

UV irradiation of several rodent cell lines and of excision repair-deficient xeroderma pigmentosum (XP) cells causes production of DNA daughter strands considerably shorter than those observed in unirradiated control cells (Cleaver and Thomas, 1969; Rupp et al., 1969; Buhl et al., 1972*b*). Postreplication repair increases the size of daughter strands by filling single strand gaps (Lehmann, 1972) in the course of several hours (Edenberg and Hanawalt, 1973) and ultimately all of the DNA appears to be semiconservatively replicated (shown for Chinese hamster ovary [CHO] cells, Meyn et al., 1976).

In addition to the length increase by postreplication repair of pulse labeled daughter strands (pulse chase type experiments), CHO cells (Meyn and Humphrey, 1971) mouse L 5178Y cells (Lehmann and Kirk-Bell, 1972), and XP cells (Buhl et al., 1973) recover the ability to synthesize long DNA daughter strands without gaps after several hours of postirradiation incubation without removing an appreciable number of UV-induced thymine dimers. The ability to synthesize long daughter strands during short-term [^{3}H]thymidine pulses at several hours after irradiation could have been recovered through radiation-induced SOS repair (see Witkin, 1976).

We have investigated the template properties of DNA in asynchronously growing MPC-11 cells for in vivo hnRNA synthesis as a function of UV dose and time of postirradiation incubation. Since no thymine-containing pyrimidine dimers are removed from the DNA of MPC-11 during 8 h of postirradiation (Table III), the template properties of DNA can be restored only by removal of UV photoproducts other than pyrimidine dimers, or by providing new templates through the synthesis of DNA daughter strands, or by sister chromatid exchange of undamaged DNA segments. We observe a UV dose-dependent reduction in hnRNA transcript size and a gradual recovery of synthesis of longer transcripts with time of postirradiation incubation (Figs. 1–3). Quantitative evaluation of the loss of hnRNA transcripts shows that one transcription-terminating UV lesion is introduced per 1,000 DNA base pairs at an effective dose of 120 J/m^2 (calculated from the data of Table I), in agreement with earlier observations (Hackett and Sauerbier, 1975; Giorno and Sauerbier, 1976). At 120 J/m^2, approximately 2–2.5 pyrimidine dimers are formed per 1,000 DNA base pairs (e.g., Wulff, 1963). Thus, it is possible that every pyrimidine dimer in the transcribed DNA strand serves to terminate transcription (see also Michalke and Bremer, 1969, and Ali et al., 1976).

After 8–12 h of postirradiation incubation, the hnRNA size distribution approaches the distribution observed in unirradiated cells. Since pyrimidine dimers are not eliminated from parental DNA, the active template for long hnRNA synthesis is likely to be the newly synthesized daughter DNA strands.

The kinetics of repair are not easily established because of imprecisions in our experimental observations and because of the asynchrony of the cells. However, we notice that cells receiving 28 J/m^2 effective UV dose do not show a faster recovery per transcription-terminating UV damage than those with 84 J/m^2 effective dose. This could indicate that removal of damage does not occur with zero-order kinetics; although the facts that we are working with asychronously growing cells and that the recovery time is in the order of one cell division make even this statement tenuous. We wish to point out, nevertheless, that the data on damage removal are compatible with first-order kinetics.

While synthesis of full-length transcripts is restored by postirradiation incubation, the average amount of hnRNA synthesized per cell decreases, which could indicate that some cells in the UV-irradiated population cease to participate in hnRNA synthesis. This possibility was tested by autoradiography of MPC-11 cells [^{3}H]-uridine-labeled at various times of postirradiation incubation. It was found that label incorporation is progressively reduced in all cells with increasing times of postirradiation incubation and that an increasing fraction of the cells cease to incorporate label.

Size analysis of parental DNA labeled with [*methyl*-^{14}C]thymidine before irradiation with 28 J/m^2 and of daughter DNA, [*methyl*-^{3}H]thymidine labeled for 30 min immediately after irradiation, reveals the following: The size of parental DNA is not noticeably shortened by the 28 J/m^2 irradiation, yet the size of daughter DNA is considerably shorter and more heterogeneous than that of control cells without irradiation (Fig. 10). Because of the heterogeneity in size of daughter DNA in the UV-irradiated cells, quantitative correlations between DNA size and pyrimidine dimer spacing on parental DNA cannot be made. Qualitatively, panels R_2 through R_8 show a progressive increase in size of the daughter DNA strands, which are completely grown after 8 h of postirradiation incubation. This time scale of recovery is similar to the one for the recovery of full-length hnRNA transcripts (Fig. 5). From this correlation in time and from the fact that pyrimidine dimers are not removed from parental DNA strands, it becomes likely that gap filling and ligation of the daughter strands (postreplication repair) are the steps responsible for restoring synthesis of full-length hnRNA transcripts. This suggestion is supported by the observation that caffeine, an inhibitor of postreplication repair, inhibits the restoration of synthesis of full-length hnRNA transcripts in UV-irradiated MPC-11 cells.

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