DNA REPAIR AFTER ULTRAVIOLET IRRADIATION OF ICR 2A FROG CELLS

PYRIMIDINE DIMERS ARE LONG ACTING BLOCKS TO NASCENT DNA SYNTHESIS

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ABSTRACT The ability of ICR 2A frog cells to repair DNA damage induced by ultraviolet irradiation was examined. These cells are capable of photoreactivation but are nearly totally deficient in excision repair. They have the ability to convert the small molecular weight DNA made after irradiation into large molecules but do not show an enhancement in this process when the UV dose is delivered in two separate exposures separated by a 3- or 24-h incubation. Total DNA synthesis is depressed and low molecular weight DNA continues to be synthesized during pulse-labeling as long as 48 h after irradiation. The effects of pyrimidine dimer removal through exposure of UV irradiated cells to photoreactivating light indicate that dimers act as the critical lesions blocking DNA synthesis.

INTRODUCTION

In a variety of animal cells the DNA synthesized initially after ultraviolet (UV) irradiation is of a low molecular weight compared to DNA synthesized in unirradiated cells. If the cells are allowed to incubate for several hours after the irradiation and then pulse-labeled, the size of the newly synthesized DNA approaches that made in unirradiated cells (Meyn and Humphrey, 1971; Buhl et al., 1973). The recovery in size of normal nascent DNA synthesis is not to be confused with the conversion of low molecular weight DNA produced initially after irradiation into a high molecular weight size observed in a pulse-chase experiment (Cleaver and Thomas, 1969; Buhl et al., 1972). This converison will be referred to as postreplication repair (PRR). Although it is unclear what importance the recovery of normal nascent DNA synthesis has for cellular survival, it is of interest to determine what role, if any, this recovery process may play in the enhancement of PRR found with cells that have been treated with a particular UV dose delivered in two exposures separated by an incubation period compared to the rate of PRR in cells exposed to the same UV dose delivered in a single irradiation (D'Ambrosio and Setlow, 1976; D'Ambrosio et al., 1978). This paper reports the results of experiments carried out to answer these questions using the ICR 2A cell line which was derived from the frog *Rana pipiens* (Freed and Mezger-Freed, 1970 a and b). This cell line proved useful in these studies as it was discovered in preliminary experiments that these cells did not show a recovery of normal nascent DNA synthesis so that comparisons could be made with other animal cells that carry out this activity. In addition, the ICR 2A cells were found to be nearly totally deficient in excision repair so that studies examining DNA synthesis in UV

irradiated cells could be carried out over long periods of time without the complicating factor of loss of dimers from DNA.

The results of experiments described in this paper suggest that the recovery of normal nascent DNA synthesis may play an important role in cellular survival and may also be associated with the enhancement in PRR observed in a split dose experiment. An additional aspect of the ICR 2A cells employed in these experiments was their proficiency in photoreactivation (Rosenstein and Ohlsson-Wilhelm, 1979; Freed et al., 1979). Because of the specificity of photoreactivating enzyme (DNA photolyase, E.C. 4.1.99.3) for the repair of pyrimidine dimers in DNA (Setlow et al., 1965; Setlow, 1966) it was possible to show that dimers acted as the blocks preventing recovery of normal nascent DNA synthesis.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Cultures of the haploid frog cell line ICR 2A were grown in humidified incubators at 24°C in modified Liebowitz medium (MLM, Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% fetal calf serum (Flow Laboratories, Rockville, M.D.). Under these conditions the cells had an ~20% plating efficiency and a population doubling time of 48 h. The cells were the generous gift of Dr. J. Freed and Dr. L. Mezger-Freed, Institute for Cancer Research, Fox Chase, Pa.

Irradiation Conditions and Determination of Survival

Either 500 or 5,000 cells were plated in 60-mm plastic dishes (Corning Glass Works, Corning, N.Y.). Before UV irradiation the cells were washed twice with phosphate buffered saline (PBS, 8.0 g NaCl, 0.2 g KCl, 1.5 g Na_2HPO_4, and 0.2 g KH_2PO_4 in 1.5 liter of distilled H_2O) which was removed before the irradiation. The source of 254-nm UV radiation was three GE G8T5 germicidal bulbs. When photoreactivating light (PRL) was used the cells were covered by 2 ml of PBS and exposed for 60 min to two 15-W GE black lights at a distance of 4 cm to obtain maximum photoreactivation. The light was passed through 6 mm of lead glass which acted as a heat barrier and also a filter to remove wavelengths <310 nm (Jagger, 1967). Under these conditions the temperature of the PBS remained at 23°–27°C. After 2 wk of growth the cells were washed twice with a 0.55% NaCl solution and methanol added for 5 min. The methanol was poured off and a Giemsa solution (Harleco, Gibbstown, N.J.) was added for 1 min and then diluted tenfold. After 10 min the cells were washed twice with distilled H_2O and allowed to dry. Groups of 32 or more cells were counted as colonies.

Measurement of Endonuclease-sensitive Sites

5×10^5 cells were plated in a 60-mm dish and incubated in medium containing either [^{3}H]thymidine (dThd) at 0.3 μCi/ml (6.7 Ci/mmol) or [^{14}C]dThd at 0.06 μCi/ml (50 Ci/mol; New England Nuclear, Boston, Mass.). After incubation for 3 d the ^{3}H-labeled cells were irradiated with 0 or 7.5 J/m^2 of UV. These cultures were then either incubated in the dark or exposed to PRL. The ^{14}C-labeled cells were collected immediately after treatment with 7.5 J/m^2 of UV. The cultures were then washed with an ice-cold EDTA-salt solution (Setlow et al., 1969), removed from the plate and for each treatment the ^{3}H-labeled and ^{14}C-labeled cells were mixed together. They were washed with ice-cold PBS and lysed in 0.5 ml of lysing solution (0.02 M Tris-HCl, pH 8, 0.04 M NaCl, 0.002 M EDTA) containing 0.24% Sarkosyl (Ciba Geigy Corp., Ardsley, N.Y.). 45 μg of Pronase (Calbiochem, La Jolla, Calif.) was added and the proteins digested at 45°C for 10 min and at 37°C for an additional 50 min. The DNA was extracted with 0.5 ml of phenol equilibrated with endonuclease buffer (0.02 M Tris-HCl, pH 8, 0.04 M NaCl, 0.02 M EDTA) by gentle rotation for 1 h. The aqueous layer containing the DNA was removed, extracted twice with an equal volume of ether, and dialyzed overnight at 4°C against the endonuclease buffer. 100 μl of the DNA solution was incubated at 37°C for 30 min with 10 μl of a crude extract from *Micrococcus luteus*, which contains an endonuclease activity that produces breaks in DNA containing

pyrimidine dimers (Paterson, 1978). The extract was the gift of Dr. G. Kantor, Wright State University, Dayton, Ohio. The mixture was placed in a lysis layer (0.2 ml of 0.5 M NaOH, 5 mM EDTA) on top of an alkaline sucrose gradient (5–20% sucrose, 0.5 M NaCl) and the DNA centrifuged at 50,000 rpm for 90 min in an SW 60 rotor of a Beckman L5-50 ultracentrifuge. Fractions were collected from the bottom of the gradient and the amount of acid insoluble radioactive material was determined in a liquid scintillation counter.

The number average molecular weight (M_n) was calculated (Regan et al., 1971) from the distribution of the radioactivity and the number of breaks per dalton of DNA was equal to $1/M_n$. The difference between the number of breaks per dalton in the ^{14}C-labeled DNA and the ^{3}H-labeled DNA represents the number of endonuclease sensitive sites removed from the DNA by the paritcular treatment. Because a double label was used it was possible to avoid errors arising from variations in the sizes of DNA extracted from the samples.

Bromodeoxyuridine Photolysis Technique

2.5×10^5 cells were plated per 60-mm dish and incubated for 3 d with either 0.3 μCi/ml of [^{3}H]dThd (6.7 Ci/mmol) or 0.06 μCi/ml [^{14}C]dThd (50 Ci/mol). After exposure to 7.5 J/m^2 of UV the ^{3}H-labeled cells were incubated for 24 h in fresh medium containing 0.1 mM bromodeoxyuridine (BrdUrd, Sigma Chemical Co., St. Louis, Mo.) and 2.0 mM hydroxyurea (Sigma) and the ^{14}C-labeled cells incubated in 0.1 mM dThd and 2.0 mM hydroxyurea. The cells were then washed with the EDTA salt solution and exposed to 2,000 R of x-rays to facilitate strand separation in the alkaline sucrose sedimentation (Elkind and Kamper, 1970). The ^{3}H and ^{14}C labeled cells were harvested together and suspended at 10^6 cells/ml in the EDTA salt solution. They were then exposed to 313-nm radiation produced by a water cooled Phillips SP 500-W high pressure mercury arc lamp used with a Bausch and Lomb grating monochromater. The slits were set at 4 mm (full-width, half-maximum band pass: 6.4 nm) and the light passed through a thin sheet of Mylar to screen out wavelengths shorther than 305 nm (Jagger, 1967) and focused onto a quartz microcuvette containing 0.2 ml of cells in an ice chilled holder. The dose rate (70 W/m^2) was measured using a dosimeter composed of a Beckman 12055 Photocell and a Keithley 160B Digital Multiplier (Keithley Instruments, Inc., Cleveland, Ohio). The dosimeter was calibrated against a YSI-Kettering (Yellow Springs, Ohio) model 65 Radiometer. After irradiation, 5×10^4 cells were placed in a lysis layer (0.3 ml of 1 M NaOH, 10 mM EDTA) on top of an alkaline sucrose gradient (5–20%, 2 M NaCl). After 30 min the DNA was centrifuged at 30,000 rpm for 130 min in an SW 50.1 rotor of a Beckman L5-50 ultracentrifuge. Fractions were collected from the bottom of the gradient and the amount of acid insoluble radioactive material was determined.

M_w was calculated from the distribution of radioactivity and M_n was determined from M_w on the assumption that the breaks formed in the DNA were distributed randomly so that $M_n = M_w/2$ (Daniels and Alberty, 1966). M_w rather than M_n was calculated from the radioactive profile because M_n is very sensitive to fluctuations in the amount of DNA near the top of the gradient and with our centrifugation conditions the DNA exposed to high 313-nm doses did not sediment far into the gradient. The number of breaks per dalton of DNA is equal to the reciprocal of M_n and the difference between reciprocals, $\Delta(1/M_n) = (1/M_n)_{\text{BrdUrd}} - (1/M_n)_{\text{dThd}}$ represents the number of breaks produced in the DNA through photolysis. A best fit line was then determined for these points and the patch size and the number of repaired sites calculated according to the method of Regan and Setlow (1974).

Analysis of DNA Synthesis after UV Irradiation

2.5×10^5 cells were plated in a 60-mm dish and incubated with 0.06 μCi/ml of [^{14}C]dThd (50 Ci/mol) for 3 d to label parental DNA. The radioactive medium was replaced with nonradioactive medium for 1 h and the cells UV irradiated. After this the cultures were incubated an additional 1.5–48 h and 10μCi/ml [^{3}H]dThd (50 Ci/mmol) added to the medium. After 30 min fresh medium was put on the cells containing unlabeled dThd (10^{-5} M) for 0–24 h. The cells were washed with the EDTA-salt solution, exposed to 2,000 R of x-rays, suspended at 10^6 cells/ml, and 50 μl placed in a lysis layer (0.3 ml of 1 M NaOH, 10 mM EDTA) on top of an alkaline sucrose gradient. After 30 min the DNA was centrifuged at 30,000 rpm for 130 min in an SW 50.1 rotor of a Beckman L5-50 unltracentrifuge. The

fractions were collected from the bottom of the gradient and the amount of acid insoluble radioactive material determined.

To measure total DNA synthesis, the same labeling procedure was followed but the cells were removed from the plate, resuspended in 0.25 ml of lysing solution, and Sarkosyl added to 0.24% to lyse the cells. The amount of acid insoluble radioactive material was determined and the ratio of ^{3}H-to ^{14}C-counts was calculated. The ^{14}C label acted as an internal control and its inclusion made it possible to avoid the problem that the number of cells tested varied from one sample to another. The ^{14}C label was not diluted during the incubation before pulse labeling because the UV irradiation inhibited cell division.

RESULTS

Photoreactivation

ICR 2A cells were UV irradiated and then either placed in the dark or exposed to PRL. After 2 wk of growth the colonies were stained with Giemsa and counted. The plating efficiency of the unirradiated control was 19% and this value was used for 100% survival. Cells not exposed to PRL were very UV sensitive, (Fig. 1) with a D_0 calculated to be 1.2 J/m^2, but cells which received the PRL treatment showed a much higher level of survival with a photoreactivable sector (PR sector, Jagger, 1967) of 0.69. This increase in survival was not the result of photoprotection (Jagger, 1967) because survival of cells exposed to PRL before the UV irradiation was apprxoimately the same as for cells which only received UV (Table I). The increase in survival appears to have resulted from an enzyme mediated reaction because UV irradiated cells exposed to PRL while at 4°C showed a much lower survival than cells exposed to PRL at 24°C (Table I).

To determine the fraction of pyrimidine dimers removed by exposure to PRL the same procedure was carried out on cells containing labeled DNA. The DNA was extracted from these cells and incubated with an extract from *M. luteus*, which possesses an endonuclease

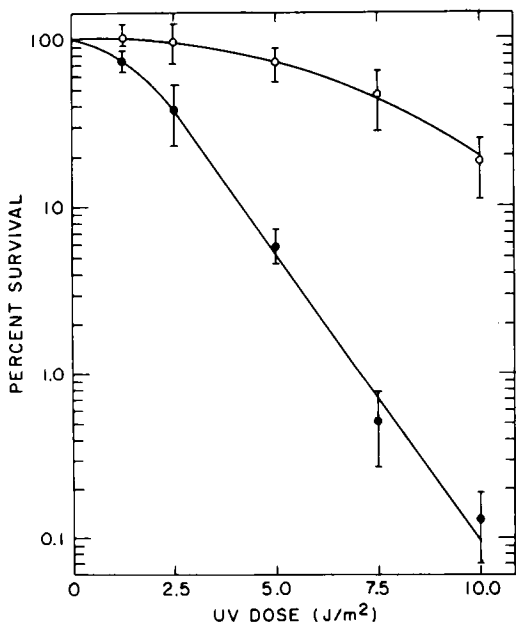

FIGURE 1 Survival after UV irradiation ± photoreactivation. No PRL (●), 60-mm PRL (○).

TABLE I
EXPOSURE TO PRL EITHER BEFORE UV IRRADIATION OR AT 4°C AFTER UV IRRADIATION

UV dose	PRL	Temp during PRL exposure	Surviving fraction*
(J/m^2)		(°C)	
0	–	24	1.0
0	–	4	0.83
7.5	–	24	0.0073
7.5	–	4	0.0062
7.5	+	24	0.51
7.5	+	4	0.032
7.5	+‡	24	0.0047

*The plating efficiency for untreated cells was 18%.
‡PRL was carried out before the UV irradiation.

activity that produces nicks in dimer containing DNA (Paterson, 1978). The treated DNA was sedimented on alkaline sucrose gradients and the radioactive profiles shown in Fig. 2. The M_n of the DNA from cells exposed to UV was much lower than that from unirradiated cells while DNA from both irradiated and unirradiated cells not treated with the *M. luteus* extract were approximately the same size. This indicates that the decrease in molecular weight resulted from the action of the endonuclease activity on dimer containing DNA. Endonuclease treated DNA from cells exposed to UV but followed by PRL was almost the same size as DNA from unirradiated cells, indicating most of the dimers were removed (Fig. 2). The number of breaks per 10^8 *D* of DNA from unirradiated cells, UV irradiated cells, and UV irradiated cells exposed to PRL was 11, 30, and 15, respectively. Hence, ~80% of the dimers were removed by exposure to PRL. Because the fraction of dimers removed was higher than the PR sector for survival, nondimer lesions induced by UV irradiation may have played a small role leading to cell death.

The specific activity of the ICR 2A photoreactivating enzyme was measured with an in vitro assay based on the absorption of nuclease-resistant dimer containing DNA to DEAE-substituted filter disks (Sutherland and Chamberlin, 1973). The specific activity was determined to be 50–100 pmol DNA repaired per milligram of protein in extract per hour of exposure to PRL (W. Farland, personal communication). This value is comparable to the specific activity of photoreactivating enzyme in a crude extract from the yeast *Saccharomyces cerevisiae.*

Excision Repair

The ability of the ICR 2A cells to carry out excision repair was examined by exposing cells to 7.5 J/m^2 of UV followed either by immediate lysis or a 24-h incubation before lysis. The DNA was extracted from the cells and treated with the *M. luteus* extract but very little difference was detected between the radioactive profiles (Fig. 3) with $\Delta(1/M_n)$ equal to $1/10^8$ *D*. Since the initial number of sites was $19/10^8$ *D*, only 5% of the dimers was removed by excision repair. The ICR 2A cells were also tested using the bromodeoxyuridine photolysis technique (Regan et al, 1971; Rosenstein et al., 1980), which is a more sensitive assay for detecting low levels of excision repair. Using this technique it was found (Fig 4.) that ~1 repaired region/10^8 *D* of DNA was observed in cells incubated for 24 h after exposure to 7.5

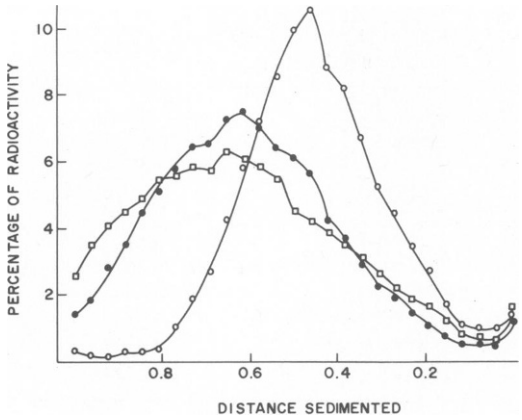

FIGURE 2

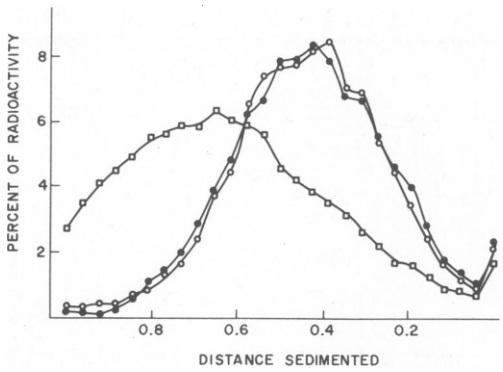

FIGURE 3

FIGURE 2 Removal of endonuclease sensitive sites by photoreactivation. Alkaline sucrose gradient profiles of DNA extracted from unirradiated cells (□), cells exposed to 7.5 J/m^2 UV (○), or cells exposed to 7.5 J/m^2 UV followed by PRL for 60 min (●). In each case the DNA was treated with the *M. luteus* extract and sedimented at 50,000 rpm for 90 min.

FIGURE 3 Removal of endonuclease sensitive sites by excision repair. Alkaline sucrose gradient profiles of DNA extracted from unirradiated cells (□), from cells exposed to 7.5 J/m^2 UV and immediately lysed (○), or cells exposed to 7.5 J/m^2 and lysed after a 24-h incubation (●). In each case the DNA was treated with the *M. luteus* extract and sedimented at 50,000 rpm for 90 min.

J/m^2 of UV, in agreement with the value obtained from the endonuclease-sensitive site assay. The patch size was calculated to be 130 nucleotides.

DNA Synthesis in UV Irradiated Cells

^{14}C-labeled cells were exposed to 5 J/m^2 of UV, incubated 1.5 h, pulse-labeled for 30 min with [^{3}H]dThd, and placed in fresh medium containing 10^{-5} M dThd for 0–24 h. The 1.5-h incubation after the UV irradiation was necessary because pulse-labeling at earlier times

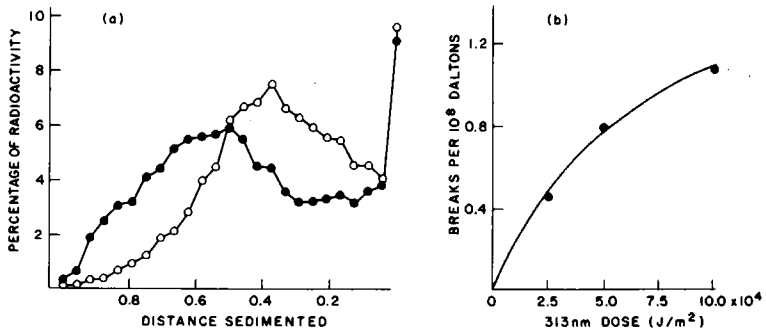

FIGURE 4 Photolysis of BrdUrd incorporated into parental DNA through excision repair. (*a*) Alkaline sucrose gradient profiles of DNA from cells exposed to 7.5 J/m^2 UV and chased for 24 h in medium containing 10^{-5} M BrdUrd (○) or 10^{-5} M dThd (●). The cells were exposed to 10^5 J/m^2 of 313-nm radiation and the DNA sedimented at 30,000 rpm for 130 min. (*b*) Dose response curve for cells exposed to 313-nm radiation. The number of breaks per 10^8 daltons was calculated from the difference between the reciprocals of the number average molecular weights for DNA from BrdUrd and dThd chased cells.

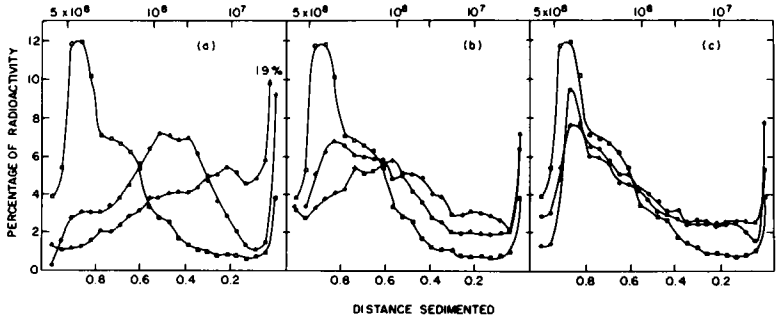

FIGURE 5 Postreplication repair. Alkaline sucrose gradient profiles of DNA from unirradiated cells (●) or cells exposed to 5 J/m² UV (○) and pulse labeled with [³H]dThd for 30 min. The cells were chased for (*a*) 0 h, (*b*) 3 h, or (*c*) 24 h, and the DNA sedimented at 30,000 rpm for 130 min. Parental [¹⁴C]dThd labeled DNA (□) was included in each gradient.

would result in end addition of the label to replicons already growing before irradiation and would therefore obscure the effect of the UV. After incubation the cells were lysed on top of alkaline sucrose gradients and the DNA sedimented. The size of the DNA made initially in the irradiated cells was smaller than that made in unirradiated cells (Fig. 5 *a*). When the cells were chased for 3 h there was a conversion of the pulse labeled DNA to a higher molecular weight (Fig. 5 *b*) and after 24 h the pulse-labeled DNA was converted nearly to that of parental size (Fig. 5 *c*). Thus, ICR 2A cells carry out PRR.

To investigate whether a split dose treatment would enhance the rate of PRR, cells were irradiated either with 7.5 J/m² or 2.5 J/m² of UV, followed by a 3-h incubation and exposure to an additional 5.0 J/m². 1.5 h later the cells were pulse-labeled for 30 min with [³H]dThd

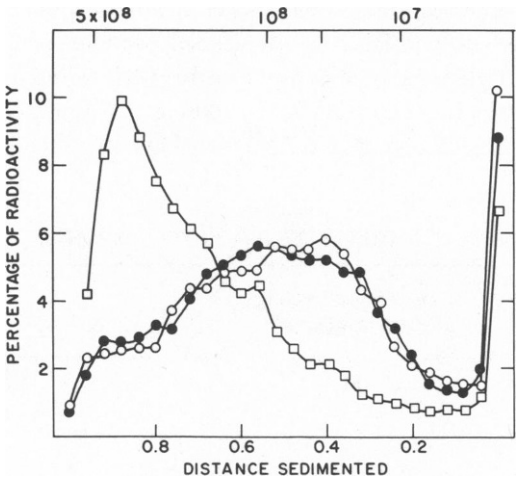

FIGURE 6 Postreplication repair after a split dose treatment. Alkaline sucrose gradient profiles of cells exposed to 7.5 J/m² UV and then pulse-labeled for 30 min with [³H]dThd (●) or exposed to 2.5 J/m² UV, incubated for 3 h and exposed to an additional 5.0 J/m² UV and pulse-labeled (○). In each case the cells were chased for 3 h after pulse-labeling and the DNA sedimented at 30,000 rpm for 130 min. Parental [¹⁴C]dThd labeled DNA (□) was included in each gradient.

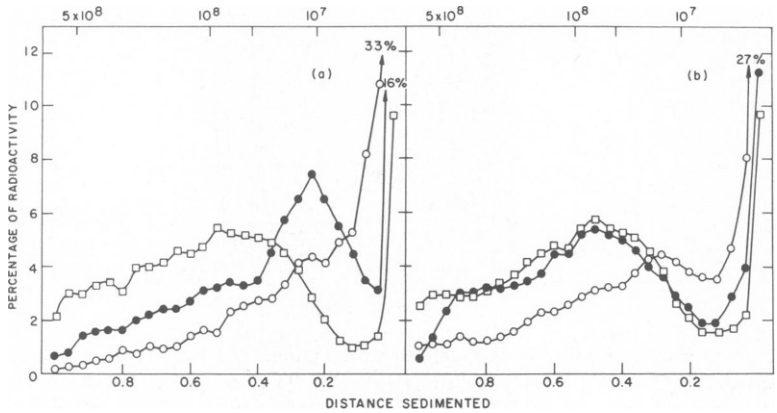

FIGURE 7 Nascent DNA synthesis in cells exposed to UV ± PRL. Alkaline sucrose gradient profiles of DNA from (*a*) unirradiated cells (□) or cells exposed to 7.5 J/m² UV and then pulse-labeled for 30 min at 1.5 h (●) or 48 h (○) after irradiation; (*b*) cells exposed to 1.5 J/m² UV (□), 7.5 J/m² UV (○), or 7.5 J/m² UV followed by PRL for 60 min (●) and pulse-labeled for 30 min. In all cases the DNA was sedimented at 30,000 rpm for 130 min.

and incubated for 3 h in fresh medium containing unlabeled dThd. The size of the DNA made in both cases was approximately the same (Fig. 6) so that no enhancement was observed. Similar results were obtained with a 24-h interval between the two doses.

Nascent DNA synthesis long after UV irradiation was examined by exposing ICR 2A cells to 7.5 J/m² of UV and pulse-labeling 1.5 or 48 h after the irradiation. The DNA made after 48 h of incubation was still much smaller than that synthesized in unirradiated cells (Fig. 7 *a*), indicating that the ICR 2A cells do not show an ability to recover normal nascent DNA synthesis even at long times after irradiation. In addition, the amount of label incorporated into DNA remained depressed several days after irradiation (Table II). To determine if pyrimidine dimers were blocks to DNA synthesis, cells were exposed to 7.5 J/m² of UV and either incubated in the dark or exposed to a treatment, PRL for 60 min, that removes 80% of the dimers. A third culture was exposed to a UV dose of 1.5 J/m², which produced the same number of dimers as in the cells exposed to 7.5 J/m² of UV and PRL. AFter a 24-h incubation

TABLE II
DNA SYNTHESIS AT VARIOUS TIMES AFTER EXPOSURE TO UV ± PRL

UV dose	Time of pulse-labeling after UV irradiation*	$^3H/^{14}C$	
		No PRL	PRL‡
(*J/m²*)	(*h*)		
0	—	2.60	1.46
7.5	1.5	0.45	0.73
7.5	24	0.36	0.86
7.5	48	0.31	0.97
7.5	72	0.30	0.92

*10 μCi/ml (^{3}H)dThd was added to cells that had incubated in (^{14}C)dThd for 3 d before irradiation. After 30 min the cells were lysed and the amount of acid insoluble radioactive material determined.
‡The PRL treatment was carried out immediately before the pulse-labeling.

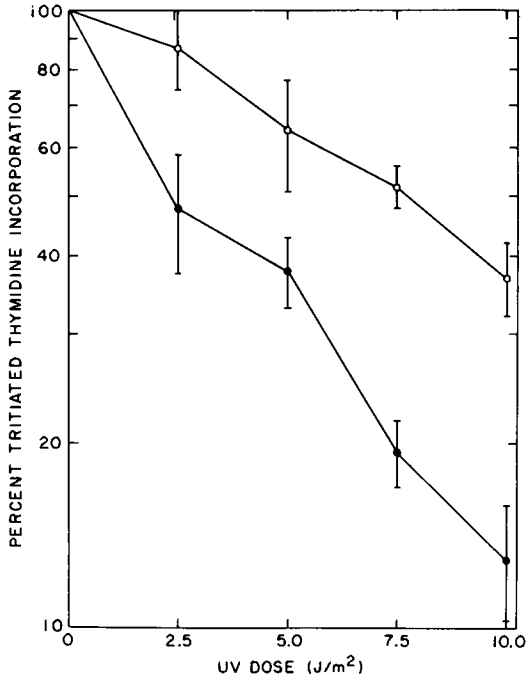

FIGURE 8 Total DNA synthesis in cells exposed to UV ± PRL. Cells were grown for 3 d in medium containing [^{14}C]dThd and then exposed to UV alone (●) or UV followed by PRL for 60 min (○). After a 24-h incubation [^{3}H]dThd was added to the medium and the cells lysed after 30 min. The number of counts in TCA precipitable material was determined and the ratio of $^{3}H/^{14}C$ was calculated. This ratio was 14.5 for untreated cells and 13.6 for cells exposed to PRL alone. These values were set at 100% for the no PRL and PRL treatments, respectively.

the cultures were pulse-labeled for 30 min. The cells exposed to 7.5 J/m^2 of UV produced small molecular weight DNA, while the DNA synthesized in cells exposed to 1.5 J/m^2 of UV or 7.5 J/m^2 of UV followed by PRL was of a higher molecular weight (Fig. 7 *b*). These results indicate that pyrimidine dimers act as blocks to nascent DNA synthesis for at least 48 h after UV irradiation. The amount of total DNA synthesis also increased when the cells were exposed to PRL (Table II and Fig. 8) with a PR sector for DNA synthesis of 0.58. This value was lower than the fraction of dimers removed (0.80) and the PR sector for survival (0.69) which indicates that the role of nondimer lesions causing inhibition of DNA synthesis is minor but may be of greater importance than its role in cell killing. These results are similar to those obtained with chick embryo fibroblasts (Lehmann and Stevens, 1975).

DISCUSSION

The results reported in this paper indicate that pyrimidine dimers induced in the DNA of ICR 2A frog cells by UV irradiation act as blocks to DNA synthesis for long times. 48 h after irradiation the size of the DNA made during a pulse-label was smaller and total DNA synthesis was depressed as compared to unirradiated cells. But when the cells were exposed to PRL after UV irradiation the size of the nascent DNA synthesized was nearly the same as in

unirradiated cells and there was an increase in total DNA synthesis. Because photoreactivating enzyme is specific for the monomerization of pyrimidine dimers (Setlow et al., 1965; Setlow, 1966), we conclude that dimers act as the major blocks to nascent DNA synthesis. The inability of the ICR 2A cells to recover normal nascent DNA synthesis is in contrast to other animal cells tested which recover the ability to synthesize DNA of the same molecular weight as that in unirradiated cells even though they may not have recovered from a depression in total DNA synthesis (Meyn and Humphrey, 1971; Buhl et al., 1973; Rude and Friedberg, 1977). This recovery is not due to the removal of dimers by excision repair because the recovery has been observed in cells incapable of removing dimers through excision repair. This inability to recover normal nascent DNA synthesis may be an important reason for the sensitivity of the ICR 2A cells to UV irradiation (D_0 = 1.2 J/m^2) as compared to V79 cells (D_0 = 5 J/m^2) and other cell lines that are defective in excision repair. The greater resistance of the V79 cells suggest that the ability to recover normal nascent DNA synthesis several hours after UV irradiation plays an important role in cellular survival. It is interesting to note that UV irradiated ICR 2A cells were capable of converting the small molecular weight DNA made during a pulse-label into a large molecular weight size after a chase so that this process (PRR) must take place by a different mechanism from the steps involved in recovery of normal nascent DNA synthesis. The ICR 2A cells also did not show the enhancement of PRR after a split dose treatment that was observed in both Chinese hamster and human cells (D'Ambrosio and Setlow, 1976; D'Ambrosio et al., 1978). That the ICR 2A cells exhibit neither the enhancement of PRR nor the recovery of normal nascent DNA synthesis, whereas Chinese hamster and human cells carry out both of these activities, suggests that these two processes are related and may be part of some inducible repair system. Alternatively, the enhancement of PRR may be an artifact resulting from the ability of the cells to produce large molecular weight DNA by the time the second dose is delivered in a split dose treatment (Painter, 1978). Thus the inability of the ICR 2A cells to carry out normal nascent DNA synthesis several hours after UV irradiation may be ultimately reflected in a lack of enhancement of PRR.

The ICR 2A cells are highly proficient in photoreactivation as represented by a loss of pyrimidine dimers in UV irradiated cells exposed to PRL and an increase in cell survival as measured by colony forming ability. This cell line should therefore serve well not only in experiments examining the role of the pyrimidine dimer in blocking DNA replication but also in studies testing the importance of dimers as lesions responsible for cell killing, mutagenesis, and transformation.

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