POSTREPLICATION REPAIR OF ULTRAVIOLET DAMAGE TO DNA, DNA-CHAIN ELONGATION, AND EFFECTS OF METABOLIC INHIBITORS IN MOUSE L CELLS

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ABSTRACT Alkaline sucrose sedimentation studies of DNA from mouse L cells have demonstrated the following effects of several inhibitors of nucleic acid and protein synthesis on postreplication repair of ultraviolet (UV) damage to their DNA. The DNA newly synthesized by a 2 h [^{3}H]thymidine (dThd) label following 254 nm UV irradiation of 20 J/m^2 is made in smaller segments of the number average mol wt (Mn) of $\sim 10 \times 10^6$ than the control of $\sim 40 \times 10^6$. The presence of caffeine at a concentration of 2 mM during the labeling of the irradiated cells reduces the Mn value to 5.8×10^6, which is nearly comparable to, but somewhat larger than the expected distance between dimers in parental DNA. Afterwards, such an interrupted DNA made in the irradiated cells is completely repaired to the present maximum Mn value of 40×10^6 in the consecutive 4 h chase in unlabeled dThd. The presence of the nucleic acid inhibitor, either 2 mM hydroxyurea, 50 μM arabinofuranosyl cytosine, 2 mM excess dThd or 5 μg/ml of actinomycin D (AMD) during 2- to 24-h chase periods after a 2 h postirradiation label prevents the repair to various extents, while 2 mM caffeine completely inhibits it. In the unirradiated cells, these agents except excess dThd and caffeine also interfere severely with normal elongation of nascent DNA made by a 3 min pulse label, but do not appreciably induce single chain breaks of either newly synthesized or parental DNA. The inhibition of the repair by AMD suggests that *de novo* elongation of DNA to close the gaps in new DNA made in the irradiated cells requires at least a template-dependent DNA polymerase. In contrast, 100 μg/ml of cycloheximide allows to complete the gap-filling repair, while it simply reduces the rates of chain growth for the repair and normal replication. Therefore, the similar sensitivity of gap-filling repair and normal replication towards the above inhibitors indicates that a preexisting DNA polymerizing system appears to be responsible and to play a common role without new protein synthesis, as far as the repair at early time after UV is concerned.

INTRODUCTION

Mammalian cells perform excision repair for UV-induced pyrimidine dimers in their DNA at different efficiencies (Cleaver, 1968, 1971; Regan et al., 1968; Painter and Cleaver, 1969). Extremely UV-sensitive, typical human xeroderma pigmentosum (XP)

cells lack the excision ability (Cleaver, 1968, 1971; Setlow et al., 1969; Regan et al., 1971). Certain culture lines of rodent origins possess the greatly reduced ability of dimer excision (Trosko et al., 1965; Klimek, 1966; Horikawa et al., 1968), although they manifest a little amount of repair or unscheduled synthesis (Painter and Cleaver, 1969; Fujiwara and Kondo, 1974 *b*).

Mouse, hamster, and human cells including XP have been shown to have another ability of postreplication repair for UV damage to their DNA (Cleaver and Thomas, 1969; Meyn and Humphrey, 1971; Lehmann, 1972 *a*; Lehmann and Kirk-Bell, 1972; Buhl et al., 1972 *b*, 1973, 1974; Fujiwara and Kondo, 1974 *a*, *b*). DNA newly synthesized within the first hour by UV-irradiated mammalian cells is of lower mol wt than that by nonirradiated cells, suggesting that the dimers interrupt DNA synthesis and the synthesis resumes beyond the dimers, leaving the gaps in new strands probably opposite the dimers remaining in parental DNA. This process is very similar to that in *Escherichia coli* (Rupp and Howard-Flanders, 1968; Smith and Meun, 1970). In mammalian cells, unlike in *Escherichia coli,* such gaps in nascent DNA strands are afterwards filled by a process involving *de novo* synthesis (Lehmann, 1972 *a*; Buhl et al., 1972 *b*; Fujiwara and Kondo, 1974 *a*, *b*). However, Chiu and Rauth (1972) have obtained a different result with mouse L cells which indicates that DNA synthesized shortly after UV irradiation is made in normal size or much longer segments than the interdimer spacings of parental DNA. Further, at long times after UV irradiation, Chinese hamster CHO (Meyn and Humphrey, 1971), mouse L5178Y (Lehman and Kirk-Bell, 1972) and human excisionless XP cells (Buhl et al., 1973) restore the ability to synthesize DNA in nearly the same size as unirradiated cells, although a large percentage of dimers remain in the parental DNA. Regarding these points, a possibility that the dimers are not responsible for the interruption of normal replication and the formation of daughter-strand gaps has been excluded by an adequate experiment by Buhl et al. (1974) which demonstrates that in the rat kangaroo kidney cells with photoreactivating enzyme such lesions are photoreactivable dimers. The recovery mechanism occurring during long time after exposure is not yet known.

In addition, our current knowledge is little about the inhibitors of the *de novo* synthesis type of postreplication repair and their modes of action. The only two facts so far known are: (*1*) caffeine and its analog theophilline inhibit the reconstruction of the interrupted DNA (Cleaver and Thomas, 1969; Fujiwara and Kondo, 1972; Lehmann and Kirk-Bell, 1972; Trosko and Chu, 1973; Buhl and Regan, 1974) presumably by interacting with denatured regions of the DNA (Domon et al., 1970), and (*2*) hydroxyurea also prevents postreplication repair (Lehmann, 1972*a*; Buhl et al., 1972*a*) as a result of the inhibition of DNA elongation (Coyle and Strauss, 1970; Scudiero and Strauss, 1974). Regarding normal chain growth of DNA, *Escherichia coli* cells involve the synthesis of Okazaki pieces and the efficient filling-in by the DNA polymerase I of naturally occurring gaps between the pieces (Okazaki et al., 1971). Similarly, discontinuous replication takes place in mammalian cells (Schandl and Taylor, 1969; Fujiwara, 1972 *a*). Such a mechanism of normal chain elongation is assumed to be partly relevant with that of *de novo* gap-filling in the irradiated cells.

This report is concerned with effects of inhibitors of nucleic acid and protein synthesis on the elongation of nascent DNA in unirradiated mouse L cells and on post-replication repair of the interrupted DNA synthesized shortly after UV irradiation.

MATERIALS AND METHODS

Cells and Culture

The cell line used was clone L5 derived from mouse L929 cells (Terasima at al., 1968). The cells were normally grown in Falcon plastic Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) with synthetic, dThd[1]-free F10HI medium (Ham, 1963) supplemented with 10% calf serum at 37°C in a water-saturated atmosphere of 5% CO_2 in air (Fujiwara, 1967). The doubling time was about 20 h. As described previously (Fujiwara, 1972*b*), exponentially growing cells were partially synchronized as to DNA synthesis by a 16 h induction with 1 μM FdUrd plus 50 μM Urd (the treated cells were termed FdUrd-L5 cells), and subsequently DNA synthesis was induced by addition of 1–50 μM dThd for desired lengths of time as will be described below.

Pulse-labeling and Chase of Unirradiated Cells

To label nascent short pieces of replicating DNA, nonirradiated FdUrd-L5 cells were pulse-labeled for the first 3 min with 5 μCi/ml (1 μM) of [methyl-^{3}H]dThd (sp act 5 Ci/mmol, The Radiochemical Centre, Amersham, England) in the presence of FdUrd plus Urd. Then, the labeled cells were washed twice with prewarmed medium and chased in a 50-fold excess of unlabeled dThd, 50 μM, for additional 30 and 120 min to allow the elongation of nascent DNA. In the other experiments identical to the labeling of UV-irradiated cells (see below), FdUrd-L5 cells were labeled for the first 2 h and then chased for 4 h in nonradioactive dThd in the similar manner.

UV Irradiation, Labeling, and Chase for Detection of Postreplication Repair

As described previously (Fujiwara, 1972*b*), FdUrd-L5 cells were exposed to a single fluence of 0 or 20 J/m^2 of predominantly 254 nm UV at an incident intensity of 0.7 J/m^2 · s from a Toshiba low-pressure mercury germicidal 15 W lamp. Immediately, the cells were incubated in 5 μCi/ml of [^{3}H]dThd in the presence of FdUrd plus Urd, and labeled during the initial 2 h of the [^{3}H]dThd-rescued DNA synthesis period. At this time, alkaline sucrose sedimentation revealed that the 2 h-labeled DNA from the irradiated cells was of lower molecular weight (Fujiwara, 1972*b*; see Fig. 4*b*). In order to study the kinetics of postreplication repair of the interrupted DNA, the irradiated, labeled cells were washed twice and reincubated in 50 μM nonradioactive dThd in the presence of FdUrd plus Urd for additional 2, 4, and 24 h.

Treatments with Various Compounds

Effects of various inhibitors of nucleic acid and protein synthesis on normal and repair elongations of DNA chains were studied in the following ways. The unirradiated 3 min-, and 2 h-labeled, and the UV-irradiated 2 h-labeled FdUrd-L5 cells, as described above, were treated

[1] The abbreviations used are: dThd, thymidine; FdUrd, 5-fluoro-2'deoxyuridine; Urd, uridine; BrdUrd, 5-bromo-2'-deoxyuridine; araC, 1-β-D-arabinofuranosyl cytosine; araCTP, araC triphosphate; HU, hydroxyurea; CHX, cycloheximide; AMD, actinomycin D; EDTA, disodium ethylenediaminetetraacetate; UV, ultraviolet light; Mw, weight average molecular weight; Mn, number average molecular weight; XP, xeroderma pigmentosum; PPO, 2,5-diphenyloxazole; POPOP, 2, 2'-*p*-phenylene-bis(5-phenyloxazole).

with the following compounds during chase periods of 30–120 min, 4 h, and 2–24 h, respectively. Unless otherwise specified, final concentrations of the compounds were: HU (Sigma Chemical Co., St. Louis, Mo.), 2 mM; araC (Upjohn Co., Kalamazoo, Mich.), 50 μM; excess dThd (Calbiochem, Los Angeles, Calif.), 2 mM; caffeine (Calbiochem), 2 mM; AMD (Schwarz/Mann, Orangeburg, N.Y.), 5 μg/ml; CHX (Nakarai Chemical Co., Kyoto, Japan), 100 μg/ml.

In addition, to see whether or not these agents induce single strand breaks of preexisting DNA, the cells in log-phase were prelabeled with 0.05 μCi/ml of [2-^{14}C]dThd (sp act 58.3 mCi/mmol) for 48 h, washed, and reincubated for several hours in growth medium prior to the treatments with each inhibitor for 2, 4, and 16 h.

Alkaline Sucrose Sedimentation and Measurement of Radioactivity

The cellular DNAs from all the experiments described above were analyzed for alkaline sucrose sedimentation properties in an SW39L head of a Beckman Model L ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) according to our previous method (Fujiwara, 1972 *a*, *b*). The methods for lysing cells and centrifuging DNA are briefly outlined in the legend to Fig. 1. Acid-insoluble radioactivity of individual sucrose fractions (10 drops/fraction) collected from the bottoms of the tubes was measured in PPO-POPOP-toluene (5 g:0.05 g:1,000 ml) using a Packard Model 3330 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The values of Mw and Mn were determined from sedimentation profiles, as described previously (Fujiwara, 1972 *b*).

RESULTS

Effects of Metabolic Inhibitors and Caffeine on Chain Elongation of Nascent DNA and on Preexisting DNA in Unirradiated Cells

FdUrd-L5 cells were pulse-labeled with [^{3}H]dThd for the first 3 min and then chased in unlabeled dThd in the presence or absence of each inhibitor for additional 30 and 120 min. Fig. 1 *a* shows the control alkali-sucrose profiles of DNA from the untreated cells. Nascent DNA immediately after a 3 min pulse label consists of the main fraction of short pieces ($\leq$ 4 S) and additional intermediates of 19 and 35 S. The subsequent chase in unlabeled dThd allows to elongate nascent DNA, and within 120 min the label is integrated into a single DNA copy (76.5 S) of the present maximum Mw value of $\sim 80 \times 10^6$ (range: $30–200 \times 10^6$). This elongation process was documented in detail in the previous report (Fujiwara, 1972 *a*).

Fig. 1 *b* displays that a 30 min chase of the pulse-labeled cells in the presence of 2 mM HU depresses the elongation of DNA chains and pools them at a 35 S position of growing intermediates, as agreed with the result of Coyle and Strauss (1970), while the control DNA is elongated to 61 S (Fig. 1 *a*). The inhibition by HU at this concentration is not complete because by 120 min of chase in its presence a part of DNA is elongated from the 35 S pool to the 76.5 S unit (Fig. 1 *b*). Fig. 2 shows that 2 mM caffeine significantly slows down the rate of DNA-chain growth at 30 min, but strands return to their normal lengths by 120 min. In addition, during chase periods in the presence of caffeine, short pieces of DNA as much as 10–20% of the total radioactivity are still left unjoined on top of the gradient (Fig. 2). The similar caffeine effects have been reported in mouse L5178Y (Lehmann, 1972 *b*) and human cells (Buhl and Regan, 1974). Therefore, it appears that the reversible interaction of caffeine with denatured

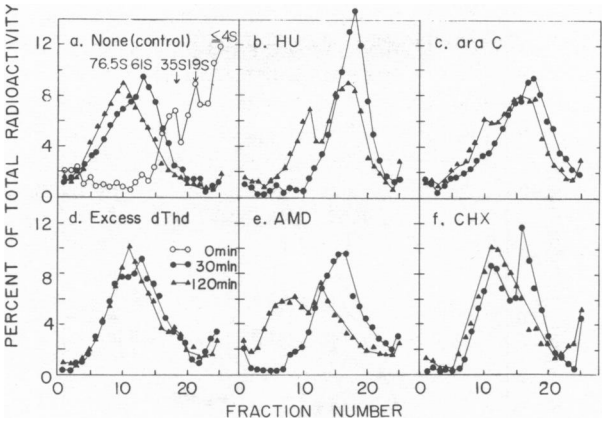

FIGURE 1 Effects of various compounds on the elongation and joining of replicating DNA in unirradiated mouse L5 cells. The FdUrd-L5 cells were pulse labeled with 5 μCi/ml of [^{3}H]dThd for the first 3 min and chased for additional 30 and 120 min in 50 μM unlabeled dThd with or without compounds. Final concentrations used are: HU, 2 mM; araC, 50 μM; excess dThd, 2 mM; AMD, 5 μg/ml; CHX, 100 μg/ml. The treated cells were lysed in 0.5 ml of 0.25% sodium dodecylsulfate-0.015 M EDTA-0.15 M sodium bicarbonate, pH 8.0, and digested for 4 h at 37°C after an addition of 0.4 ml of 5 mg/ml preheated Pronase (Kaken Chemical Co., Tokyo). The lysate was finally alkalinized with 0.1 ml of 3 N NaOH and a 0.2 ml aliquot ($\approx$ 5,000 lysed cells/0.2 ml; < 0.1 μg DNA) was layered on top of a 4.8 ml linear gradient of 5 to 20% (wt/vol) alkaline sucrose solution containing 0.8 M NaCl, 0.2 M NaOH, 0.01 M EDTA, and 0.025 M sodium *p*-aminosalicylate, pH 12.5. The material was centrifuged at 35,000 rpm for 2 h at 20°C in an SW39L head of Spinco Model L Ultracentrifuge (Spinco Div., Beckman Instruments, Palo Alto, Calif.) using the labeled T4D DNA for reference. After run, 10-drop fractions from the bottoms of the tubes were collected onto 2.5 cm Whatman No. 3MM filter paper discs. Acid-insoluble radioactivity was measured.

regions of replication forks results in the simple reduction in the rate of chain growth and a partial premature termination of synthesis.

The results with the other compounds newly tested in this experiment are shown in Figs. 1 *c–f*. The DNA profiles after araC (Fig. 1 *c*) and AMD treatments (Fig. 1 *e*) appear to be basically similar to those after the HU treatments as described above (Fig. 1 *b*). However, the concentration of araC used causes a rather fixed skewness of radioactivity distributions to high molecular weight from the 35 S peak, but it inhibits chain elongation more effectively (Fig. 1 *c*) than HU (Fig. 1 *b*) and AMD (Fig. 1 *e*). On the other hand, the effects of CHX on the sedimentation rate are phenomenologically similar to those of caffeine, since CHX simply reduces the rate of chain growth by 30 min, but allows the completion of growing strands in a sufficiently long time of 120 min (Fig. 1 *f*). Incidentally, CHX at this concentration is known to inhibit more than 95% of protein synthesis (Gautschi et al., 1973). As shown in Fig. 1 *d*, however, nascent DNA is normally elongated in the presence of excess dThd. Accordingly, these results suggest that available, perhaps preexisting DNA polymerases and ligase are required for continuing the growth of pulse-labeled, small nascent pieces once the synthesis has been initiated in vivo.

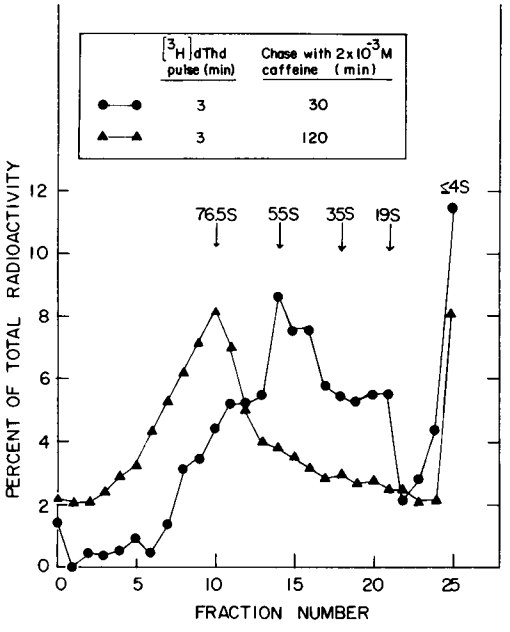

FIGURE 2 Effects of 2 mM caffeine on replicating DNA in unirradiated mouse L5 cells.

Table I shows the effects of the compounds on preexisting DNA. In these experiments, [^{14}C]dThd-prelabeled cells were treated with the compounds for various times, and their DNAs were analyzed by sedimenting in alkaline sucrose. All Mn values detected are more or less similar to those of control, since they range from 34 to 40 $\times$ 10^6 within 16 h of the treatments until the cell death due to unbalanced growth is assumed to begin. This indicates that neither of the inhibitors induces single strand breaks of parental DNA to a significant extent. Therefore, the actions of HU, araC, and AMD under the present conditions are not on the existing DNA, but rather on

TABLE I
EFFECTS OF VARIOUS COMPOUNDS ON THE PRELABELED DNA

Compounds	Mn* $\times$ 10^{-6}			
	0 h	2 h	4 h	16 h
None	40	40	40	40
HU, 2 mM	—	39	39	—
AraC, 50 μM	—	40	40	36
dThd, 2 mM	—	40	40	40
AMD, 5 μg/ml	—	38	37	34
CHX, 100 μg/ml	—	40	41	38
Caffeine, 2 mM	—	40	40	40

*Mn was estimated from the formula, $Mn = \Sigma fi/\Sigma(fi/Mi)$, where fi is the fraction of the total radioactivity in the ith fraction and Mi is the molecular weight in that fraction.

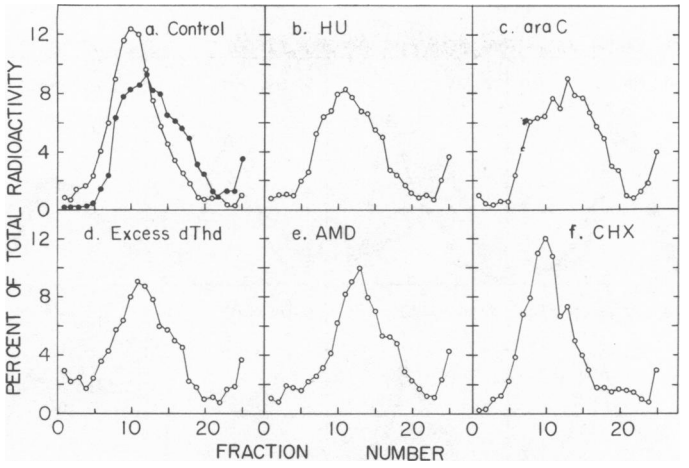

FIGURE 3 Effects of metabolic inhibitors on the DNA newly synthesized for 2 h in unirradiated mouse L5 cells. The FdUrd-L5 cells were labeled for 2 h, washed, and reincubated for further 4 h in medium containing each inhibitor indicated. ●——●, immediately after 2 h labeling; ○——○, labeled for 2 h and incubated for additional 4 h.

newly synthesized, nascent DNA in such a manner that small nascent fragments cannot fully join.

Effects of the Compounds on DNA Labeled for 2 h in Unirradiated Cells

Fig. 3 displays alkaline sucrose profiles of DNAs from the unirradiated FdUrd-L5 cells that were labeled during the first 2 h with [^{3}H]dThd alone and then chased for additional 4 h in unlabeled dThd in the presence or absence of each inhibitor. First of all, the sedimentation pattern of the DNA from CHX-treated cells (Fig. 3*f*) is very similar to that of control (Fig. 3*a*, open circle), indicating that CHX has again no effect on newly synthesized DNA. The profiles from the cells treated with either HU (Fig. 3*b*), excess dThd (Fig. 3*d*) or AMD (Fig. 3*e*) during the 4 h chase incubation exhibit no marked difference from that of the 2 h-labeled, nonchased DNA (Fig. 3*a*, closed circle), implying that these agents "freeze" the 2 h-labeled DNA. However, the profile from araC-treated cells shows a little shift back towards lower molecular weight (Fig. 3*c*). Therefore, araC appears to induce few breaks in newly synthesized DNA, although its major effect is the inhibition of normal chain growth (Fig. 1*c*).

Kinetics of Postreplication Repair and Effects of Various Compounds on the Repair

These studies were carried out in the following ways. FdUrd-L5 cells were labeled during the first 2 h after UV irradiation with 0 or 20 J/m^2, and then chased in unlabeled dThd for desired periods up to 24 h. Fig. 4*b* shows the typical repair kinetics [mere repetition of our previous work (Fujiwara, 1972*b*)]. The DNA newly synthesized 2 h after UV irradiation is made in smaller segments, of a Mn value of 10.6 × 10^6, than that (~40 × 10^6) of control (Figs. 4*a*, *b* and Table II). Although caffeine retards

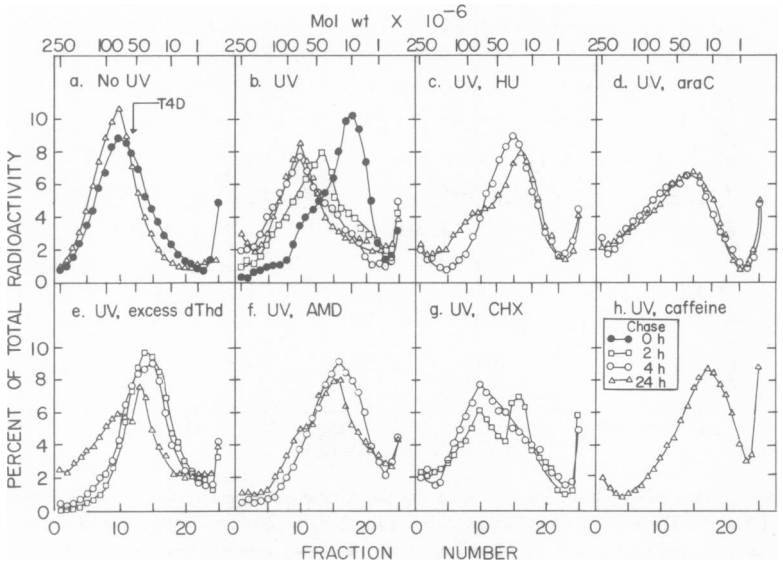

FIGURE 4 Effects of metabolic inhibitors and caffeine on the repair of the interrupted DNA newly synthesized by UV-irradiated mouse L5 cells. The FdUrd-L5 cells were exposed to 0 (*a*) or 20 J/m^2 (*b–h*) of 254 nm UV, labeled for 2 h with 5 μCi/ml of [^{3}H]dThd, and chased for additional 2, 4, and 24 h, as indicated, in the presence or absence of the inhibitors. The other procedures and final concentrations used are the same as described in the legend to Fig. 1.

TABLE II

EFFECTS OF VARIOUS COMPOUNDS ON POSTREPLICATION REPAIR OF THE INTERRUPTED DNA SYNTHESIZED BY MOUSE L5 CELLS AFTER UV IRRADIATION OF 20 J/M^2

Treatments‡	Mn* × 10^{-6}			
	0 h	2 h	4 h	24 h
DNA from unirradiated cells				
None	40.0	—	—	45.0
DNA from the irradiated cells				
None	10.2§	19.2	35.1	38.0
HU, 2 mM	—	—	15.7	15.8
AraC, 50 μM	—	—	20.1	20.2
Excess dThd, 2 mM	—	14.1	17.0	30.5
AMD, 5 μg/ml	—	—	14.5	16.0
CHX, 100 μg/ml	—	17.2	33.0	—
Caffeine, 2 mM	5.8 ‖	—	—	7.1

*Mn values from fractions 5 to 22 in Fig. 4 were calculated as described in Table I.

‡The compounds listed were continuously present with 50 μM nonradioactive dThd during the chase periods indicated.

§Mn of the DNA obtained immediately after a 2 h post-UV labeling.

‖ Data from Fujiwara (1972*b*). Caffeine was present during the 2 h post-UV labeling.

the elongation of shorter segments (Fig. 2), its presence for sufficiently long times results in much smaller Mn values of 5.8 to 7.1 $\times 10^6$ (Table II), which are somewhat larger than the interdimer spacing of parental DNA at 20 J/m^2 (Fujiwara, 1972*b*; Lehmann, 1972*a*). At short times after UV irradiation, therefore, the present L5 cells are suggested to produce the gaps in new DNA presumably opposite the dimers in parental DNA. The interrupted DNA becomes larger with time as the result of *de novo* gap-filling and ultimately the repair is completed in 4 h (Fig. 4*b*; Table II).

On the basis of the repair kinetics in alkali-sucrose, effects of various compounds on repair were studied. Figs. 4*c–h* display the results after treatment for 2, 4, and 24 h of the dThd chase. Caffeine (Fig. 4*h*) and HU (Fig. 4*c*) typically prevent the repair of interruptions in new DNA made after irradiation, as reconciled with the previous data (Cleaver and Thomas, 1969; Fujiwara and Kondo, 1972; Lehmann, 1972*a*; Buhl et al., 1972*a*). Among the inhibitors of DNA synthesis newly tested, araC (Fig. 4*d*) and excess dThd (Fig. 4*e*) partially inhibit the repair as shown in the profiles, while a breakthrough is that Mn of the DNA after 24 h-treatment with excess dThd greatly increases to about 30 $\times 10^6$ (Table II; Fig. 4*e*). AraC does not cause such a progressive repair at long time, because Mn values calculated from Fig. 4*d* remain constant at $\sim 20 \times 10^6$ over the whole period (Table II). Therefore, it is possible that araC becomes an effective inhibitor of the *de novo* repair elongation after it is converted to araCTP as a DNA polymerase inhibitor (Momparler, 1972). Similarly, a final concentration of AMD as high as 5 μg/ml prevents the repair (Fig. 4*f*), although a partial increase in Mn from 10 $\times 10^6$ at 0 h to 16 $\times 10^6$ at 24 h is observed (Table II). AMD at this concentration also inhibits normal DNA synthesis by about 80% (Fujiwara and Ohashi, unpublished data). These effects vary with the concentration of AMD, and for instance 0.1 and 0.5 μg/ml do not alter the cell's ability to repair (data not shown). In contrast, CHX even at 100 μg/ml permits to accomplish the repair of the lesions in daughter strands in 4 h, although the sedimentation rate is temporarily disturbed by 2 h (Fig. 4*g*).

These results here imply that an in vivo DNA polymerizing system which preexists in the cell may perform the gap-filling repair in UV-irradiated mouse L cells.

DISCUSSION

The results presented in Fig. 4*b* and Table II indicate that DNA newly synthesized 2 h after UV irradiation in FdUrd-synchronized mouse L5 cells is made in somewhat larger segments than the expected distance between dimers in parental DNA. This suggests that the size of the new segments is approximately equal to the average distance between 1 to 2 dimers. In contrast, Chiu and Rauth (1972) have shown that exponentially growing mouse L60T cells synthesize DNA in nearly normal size after low UV doses or in much larger size than the distance between dimers after high UV doses. The lesion responsible for interrupting DNA synthesis is shown to be the dimer using photoreactivation of UV-irradiated rat kangaroo cells (Buhl et al., 1974). Therefore, the reason for this discrepancy between the two lines of L cells is not clear, but L60T

cells could have a more efficient repair system (Chiu and Rauth, 1972) than the present L5 cells, or some different method for bypassing. In this respect, mammalian cells can recover the ability to synthesize DNA in normal-sized segments at long times after UV irradiation (Meyn and Humphrey, 1971; Lehmann and Kirk-Bell, 1972; Buhl et al., 1973, 1974). This phenomenon suggests that the dimer-containing regions may be modified in yet unknown manner so that DNA synthesis occurs normally (Buhl et al., 1973).

The present alkaline sucrose studies on postreplication repair at early time after UV irradiation demonstrate that all the nucleic acid synthesis inhibitors tested prevent the repair to various extents in the irradiated L5 cells (Fig. 4; Table II). Similarly, these agents except excess dThd depress the elongation of nascent DNA made by a 3 min pulse in unirradiated cells (Fig. 1), but do not appreciably induce single chain breaks of preexisting DNA (Table I). In addition, these agents "freeze" the newly synthesized DNA as shown in Fig. 3. In this respect, nalidixic acid allows a partial increase in size of 9–10 S Okazaki pieces during an additional incubation, but not further over 30–40 S, without inflicting any effect on sedimentation properties of the preexisting DNA in *Escherichia coli* (Pisetsky et al., 1972). Further, they also found that newly synthesized, integrated DNA ($\geq$ 60 S) was degraded to a 30–40 S form when exposed to naldixic acid. In the present experiments, araC, unlike the others, causes a very little shift-back of sedimentation profile from 76.5 S to 61 S of newly synthesized DNA in unirradiated cells (Fig. 3*c*), suggesting that it could induce few breaks in newly synthesized DNA alone. However, araC inhibits the repair greatly and results in keeping the sedimentation profiles constant for long time up to 24 h (Fig. 4*d*). Accordingly, it is possible that the profiles observed for the araC inhibition of the repair is not only due to its induction of breaks in newly synthesized DNA. On the other hand, CHX causes no such inhibitions, while it temporarily retards the rates of normal (Weintraub and Holtzer, 1972; Gautschi and Kern, 1973; Fig. 1*f*) and repair elongations (Fig. 4*g*). As described above, the similar sensitivity of both gap-filling and normal replication to various inhibitors suggests that perhaps preexisting DNA polymerases and ligase play a common role. Further, 5 μg/ml of AMD partially abolishes normal and repair elongations (Figs. 1*e*, 4*f*; Table II), indicating that AMD-sensitive, template-requiring DNA polymerases are responsible for both processes. This fact may not favor an idea that the end addition (go-around-dimer bypass) alone performs the whole process of gap-filling in length of $\sim 10^3$ nucleotides, since terminal nucleotidyl transferase requires no template in vitro (Bollum and Setlow, 1963) and exists in only calf thymus. The precise mechanism of polymerization of the strands opposite the dimers is not yet clarified by the present experiments.

Moreover, araCTP inhibits DNA polymerases in mammalian cells (Furth and Cohen, 1968; Momparler, 1972). In addition, it is incorporated into DNA to a very limited extent, so that the arabinofuranosyl moiety at the 3'-OH end of the primer chain acts as a chain terminator (Momparler, 1972). This mode of araC action is favorable to explain the constant skewness of the sedimentation profiles in Figs. 1*c* and

4 *d*, showing that a limited leaky elongation for repair and normal replication appears to occur until araC becomes effective.

Finally, remarks are made concerning a large difference between the responses of excision repair and postreplication repair to the agents used in this work. A high concentration of AMD inhibits both types of repair for UV damage to DNA (Cleaver, 1969; Fig. 4 *e*). The other compounds such as HU, araC, excess dThd, CHX, and caffeine have been shown to have little or no effects on dimer excision and repair synthesis in mammalian cells (Cleaver, 1969, 1971; Cleaver et al., 1972; Regan et al., 1968; Gautschi et al., 1973). In this regard, the size of filled-in gaps after dimer excision ranges from 30 to 100 nucleotides (Edenberg and Hanawalt, 1972; Cleaver et al., 1972), being an order of magnitude smaller than that, $\sim 10^3$ nucleotides, of postreplication gaps in newly synthesized DNA after UV irradiation (Lehmann, 1972 *a*; Buhl et al., 1972 *b*). The completion of repair synthesis for the former excision gaps will be more rapid than that of the *de novo* gap-filling of the latter, so that repair synthesis is less sensitive to the inhibitors. However, the region in the gap-filling process in DNA newly synthesized after UV contains the dimer, and such a synthesis seems very similar to semiconservative replication (Figs. 1 and 4). Therefore, the high sensitivity of postreplication repair to HU, araC, excess dThd, and AMD, unlike of repair synthesis, may result from the greater size of the particular gaps and the insertion of the greater number of nucleotides into them. HU, araC, and excess dThd cause the reduction in supplies of deoxyribonucleotides which eventually leads to the inhibition of DNA synthesis. A paradoxical finding that excess dThd permits the elongation of replicating DNA in unirradiated cells (Fig. 1 *d*), but it interferes with the repair of the gaps made in new strands of the irradiated cell DNA (Fig. 4 *e*) may be understandable on the basis of the excess dThd-reduced supplies of precursors. Finally, caffeine in excess results in the complete inhibition of postreplication repair in its presence (Fig. 4 *h*), but the reversible inhibition of normal chain growth (Fig. 2), suggesting that it binds in increased proportion not only with larger denatured regions (Domon et al., 1970; Lehmann, 1972 *b*), but also with those containing the dimers.

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