J. Cell Sci. 57, 261–275 (1982)
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CELL-CYCLE KINETICS AND ULTRAVIOLET LIGHT SURVIVAL IN UV-1, A CHINESE HAMSTER OVARY CELL MUTANT DEFECTIVE IN POST-REPLICATION RECOVERY

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SUMMARY

UV-1, an ultraviolet-sensitive mutant of CHO-K1, is abnormally slow to recover from the inhibition of DNA synthesis caused by u.v. irradiation. When synchronized UV-1 cells are irradiated in G_1, their movement into S phase is unaltered, but thymidine incorporation is depressed (compared with that in the parent cell similarly treated). When irradiated in S phase, again incorporation is more depressed, and S phase suffers a greater delay in UV-1 than in the parent cell. UV-1 and its parent have similar capacities for excision repair of u.v.-induced damage inflicted in G_1, and so enter S phase with similar amounts of unrepaired damage. The single-cell survival was measured after irradiation at different points in the cell cycle. The mutant and parent cells have similar values of D_0 (mean lethal dose) except in mitosis, when the parent cell shows markedly greater resistance to u.v. irradiation. D_q (quasi-threshold dose) is fairly constant for the parent cell, but in UV-1 it falls to a minimum in S phase. The responses of UV-1 to u.v. irradiation are generally consistent with its known defect in the process of post-replication recovery, i.e. the ability to join up the abnormally small DNA fragments synthesized on a u.v.-damaged template.

INTRODUCTION

UV-1 is a mutant of the Chinese hamster cell, CHO-K1, which was isolated by nylon-cloth replica-plating on the basis of its enhanced sensitivity to killing by ultraviolet (u.v.) light (Stamato & Waldren, 1977). u.v.-sensitive mutants are commonly defective in some aspect of the repair of u.v.-induced DNA damage (Thompson *et al.* 1980; Schultz, Trosko & Chang, 1981), and we found (Stamato, Hinkle, Collins & Waldren, 1981) that, although UV-1 has a normal capacity for excision repair, it is defective in 'post-replication recovery'. The DNA synthesized by UV-1 after u.v. irradiation (replicative rather than repair synthesis) has the same initial size as the DNA made by the parent cell; however, the conversion of nascent DNA to high molecular weight DNA is fourfold slower in UV-1 (Stamato *et al.* 1981). This deficiency in post-replication recovery is associated with a diminished rate of mutagenesis, suggesting that post-replication recovery in normal cells is an error-prone process, which may be responsible for the production of mutations (Stamato *et al.* 1981).

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The purpose of this report is to test the prediction that a defect in u.v. response specific to *S* phase will have effects on cell survival after u.v. irradiation that are related to the position of cells in the cell cycle at the time of irradiation. Using cells collected by metaphase arrest and released synchronously into interphase, we have irradiated UV-1 and its parent cell at intervals during the cycle, and measured the single-cell plating efficiency. We have also examined the effect of u.v. irradiation at different times in the cycle on the overall rate of DNA synthesis and on the transit of cells through *S* phase, and we have compared rates of enzymic incision at DNA damage sites in synchronized cells. Our findings substantiate the characterization of the lesion in UV-1, and throw some light on the relationship in normal cells between repair, survival, and the cell cycle.

MATERIALS AND METHODS

Cell culture and synchronization

The cell lines studied were 772-56 (a glycine- and proline-requiring mutant derived from CHO-K1) and UV-1, isolated from 772-56 as a u.v.-sensitive mutant (Stamato & Waldren, 1977). They were cultured in F12 medium (Ham, 1965), without hypoxanthine or thymidine, and with 5% serum (3:1, newborn calf:foetal calf serum). They normally grew in plastic dishes, but for synchronization they were cultured as monolayers in rotating glass bottles. An 8-h incubation with $2{\cdot}5 \times 10^{-3}$ M-thymidine was followed by 10 h in fresh medium, with colcemid (0·05 μg/ml) present during the final 4 h. Mitotic cells (purity at least 90%) were collected in suspension by gentle agitation of the bottles, centrifuged, washed twice with warm (37 °C) phosphate-buffered saline (PBS), and either used at once in experiments examining mitotic cells, or centrifuged, resuspended in growth medium, and plated out in dishes to progress to the required stage of the cell cycle.

u.v. irradiation

In all but survival experiments (see below), cells were irradiated *in situ* in dishes, after removing growth medium. The source was a germicidal lamp emitting mainly at 254 nm and the exposure rate was either 0·4 or 1·0 $Jm^{-2}s^{-1}$. Medium was then replaced. Control cells were mock-irradiated.

Monitoring DNA synthesis

The time-course of DNA synthesis in random or synchronous cultures was determined by pulsing samples of cells at intervals with [*methyl*-^{3}H]thymidine (Radiochemical Centre, Amersham) as described in the figure legends. After 30 min incubation, labelled medium was removed, the cells washed in cold saline (0·9% NaCl) and either: (1) fixed with Carnoy's fixative (3:1, v/v, methanol:glacial acetic acid), extracted several times with cold (4 °C) 5% (w/v) trichloroacetic acid, and prepared for autoradiography as described (Collins & Johnson, 1979); or (2) lysed in 1 ml 0·5 M-NaOH, acidified with 1 ml 20% (w/v) trichloroacetic acid, and macromolecules collected on Whatman GF/C glass-fibre filters for scintillation counting of ^{3}H incorporated into DNA.

Measuring excision repair-related DNA break accumulation

Cells in rotating bottles were incubated for 24 h with [^{3}H]thymidine (55 Ci/mmol, 0·1 μCi/ml) to prelabel DNA, and then synchronized as above. Mitotic cells were plated out at 10^5 per 35-mm dish. They were irradiated with u.v. (10 Jm^{-2}) during the early part of the cell cycle, and incubated with DNA synthesis inhibitors hydroxyurea (HU; 10^{-2} M) and 1-β-D-arabinofuranosylcytosine (araC; 10^{-4} M) (both from Sigma), as described in Results.

After incubation, cells were washed with cold saline, and 1 ml alkaline sucrose (5 % sucrose, 0·01 M-EDTA, 0·3 M-NaCl, 0·15 M-NaOH) was added to each dish. Alkaline lysis continued for 20 min at 4 °C, and was ended by addition of 0·2 ml 1 M-KH_2PO_4. DNA was sheared by passing through a 25-gauge needle, the sample diluted to 20 ml with 0·04 % sodium dodecyl sulphate in water, and the DNA separated by hydroxyapatite chromatography into native and denatured fractions (Collins, 1977). The extent of alkaline denaturation of pre-labelled DNA depends on the frequency of DNA breaks introduced by repair enzymes at sites of u.v. damage. Results are shown in terms of DNA breaks per 10^9 daltons, according to a calibration of the lysis solution using cells X-irradiated to produce known break frequencies (Squires, Johnson & Collins, 1982).

Measuring u.v. survival

Mitotic or interphase cells (trypsinized at 3-hourly intervals from cultures set up earlier with mitotic cells) in suspension in warm PBS were exposed to u.v. light from a germicidal lamp emitting at 0·2 $Jm^{-2}s^{-1}$ (lower doses) or 1 $Jm^{-2}s^{-1}$ (higher doses). Samples were removed after appropriate exposure times to give u.v. doses of 0, 2, 5, 10 or 20 Jm^{-2}. These samples were inoculated at known cell densities in F12 medium in triplicate 35-mm dishes (quadruplicate for unirradiated samples), and incubated for about a week before fixing, staining and scoring colonies.

Determination of ribonucleotide and deoxyribonucleotide pool sizes

Cells were grown to high density (but sub-confluent) in 100 mm plastic dishes, washed twice with warm saline and frozen on a block of solid CO_2. Ice-cold 2 M-perchloric acid (100 μl) was added and the frozen layer was scraped off and transferred to a test tube. Concentrations of ribonucleotides and deoxyribonucleotides were determined by high-performance liquid chromatography on Partisil-10 SAX columns (Whatman, Inc.) using a Waters HPLC system. The method, modified from that of Hartwick & Brown (1975), will be described elsewhere (Patterson, Collins, Laas & Waldren, unpublished).

RESULTS

DNA synthesis following ultraviolet irradiation

To examine the effect of u.v. light on the overall level of DNA synthesis, we irradiated unsynchronized cultures of 772-56 (the parental cell) and UV-1 with 2 or 10 Jm^{-2} and incubated them with [^{3}H]thymidine at intervals over the next 16 h. Incorporation of ^{3}H into DNA (expressed as a percentage of the incorporation in control, unirradiated cells) is shown in Fig. 1. ([^{3}H]thymidine was supplied together with all four DNA precursor nucleosides, unlabelled, at 10^{-5} M, to eliminate the dependence of incorporation on endogenous pool size, which might be affected by u.v. irradiation.) DNA synthesis in 772-56 cells is slightly depressed by irradiation with 2 Jm^{-2}, but recovers fully by 7 h after irradiation; after 10 Jm^{-2}, the depression is more severe and incorporation recovers only to 60 % of the control level. In UV-1, the depression of DNA synthesis is greater, at either dose, and recovery occurs to a lesser extent than in 772-56.

DNA synthesis following u.v. irradiation of synchronized cells

In an unsynchronized population, cells receive u.v. at different points in their proliferation cycle. It is impossible to determine from Fig. 1 whether the reduced level of DNA synthesis after irradiation represents simply a reduction in ongoing synthesis, or whether, for example, cells no longer pass from G_1 into S phase, thus reducing the

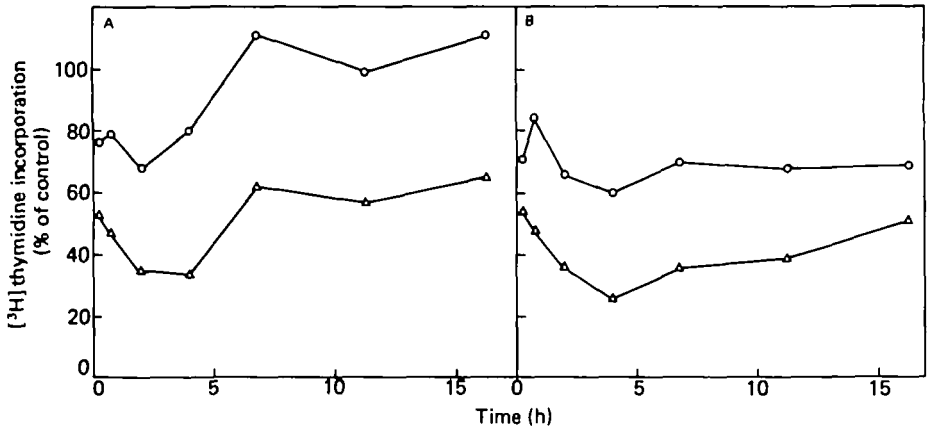

Fig. 1. DNA synthesis after u.v. irradiation of unsynchronized cells: A. 772–56, B, UV-1. Equal numbers of cells plated in 35 mm dishes the previous day were u.v.-irradiated with 2 Jm^{-2} (○) or 10 Jm^{-2} (△), or unirradiated; they were incubated with deoxyadenosine, deoxycytidine, deoxyguanosine and thymidine at 10^{-5} M each. At intervals, [^{3}H]thymidine (21 Ci/mmol, 0·5 μCi/ml) was added to dishes, and incorporation into DNA during a 30 min incubation was measured. Results (mean incorporation from duplicate dishes) are shown relative to incorporation by unirradiated cultures.

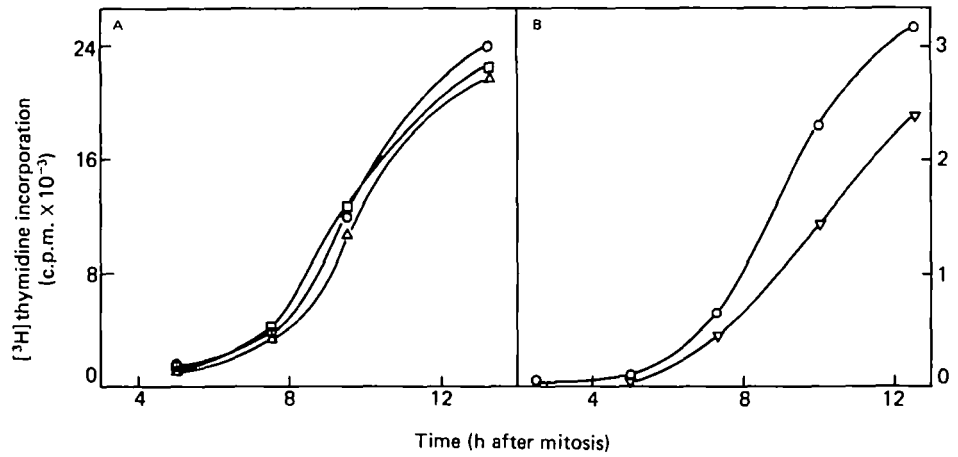

Fig. 2. DNA synthesis after u.v. irradiation of 772–56 in G_1. A and B represent separate experiments. In each, equal numbers of cells, synchronized at mitosis, were plated in 35 mm dishes and incubated at intervals with [^{3}H]thymidine (55 Ci/mmol, 0·2 μCi/ml) for 30 min. Incorporation of ^{3}H into acid-insoluble material is plotted against the time of the midpoint of the labelling period. Without irradiation (○); irradiated with u.v. (2 Jm^{-2}) at 1·5 h (△) or 3 h (□) after mitosis; or with 10 Jm^{-2} at 1·5 h after mitosis (▽).

number of cells in S phase. We therefore synchronized cells by mitotic selection, and examined the effect of u.v. irradiation in G_1 or S phase on the overall level of DNA synthesis and on the progress of cells into and out of S phase.

Looking first at 772-56 (Figs. 2, 3), irradiation with 2 Jm^{-2} either at 1·5 or at 3 h after mitosis has virtually no effect on subsequent [^{3}H]thymidine incorporation

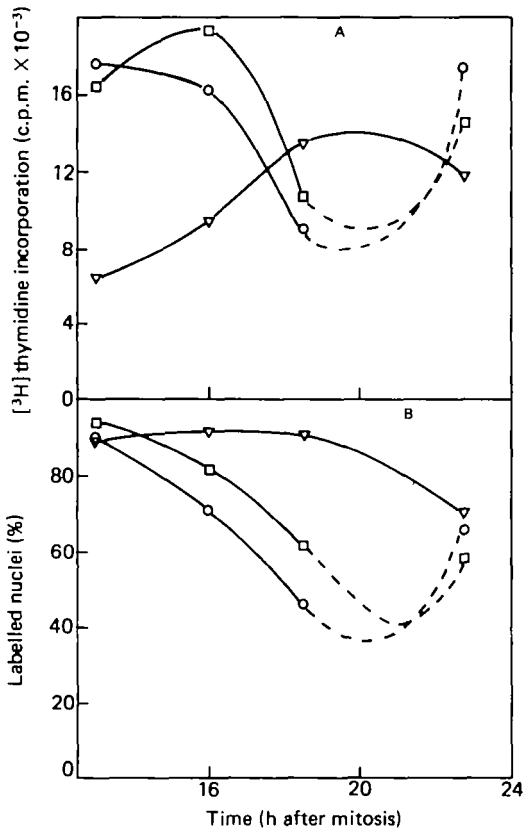

Fig. 3. DNA synthesis after u.v. irradiation of 772-56 in *S* phase. Equal numbers of cells, synchronized at mitosis, were plated in 35 mm dishes and incubated at intervals with [^{3}H]thymidine (55 Ci/mmol, 0·2 μCi/ml) for 30 min. Incorporation of ^{3}H into acid-insoluble material (A), or the % of labelled nuclei, representing *S* phase cells, detected autoradiographically (B), is plotted against the time of the midpoint of the labelling period. Even after irradiation, *S* phase cells in autoradiographs were heavily labelled, and there was no possibility of confusion with cells undertaking repair DNA synthesis, since the latter was not detectable under these labelling conditions. Without irradiation (○); u.v.-irradiated at 10·5 h after release from mitosis, with 2 Jm^{-2} (□) or 10 Jm^{-2} (▽). Broken lines indicate the probability that cells labelled at the final time point represent the beginning of the wave of DNA synthesis in the subsequent cell cycle.

(Fig. 2A). A higher u.v. dose (10 Jm^{-2}) does depress incorporation, by 25–30% (Fig. 2B). However, the entry of cells into *S* phase is unaffected by these doses of u.v. in G_1, with 50% of nuclei labelled by [^{3}H]thymidine at about 7 h after mitosis (autoradiographic data, not shown). If a dose of 2 Jm^{-2} is administered at a time when almost all cells are in *S* phase, incorporation of [^{3}H]thymidine from 2·5 h later is little different from incorporation in unirradiated cells, declining as the cells leave *S* phase and rising again as the cells enter the next cycle (Fig. 3A); Fig. 3B gives the corresponding proportions of cells in *S* phase, and indicates that this dose delays exit from *S* phase by about 1·5 h. After 10 Jm^{-2}, incorporation of [^{3}H]thymidine is low for several hours before

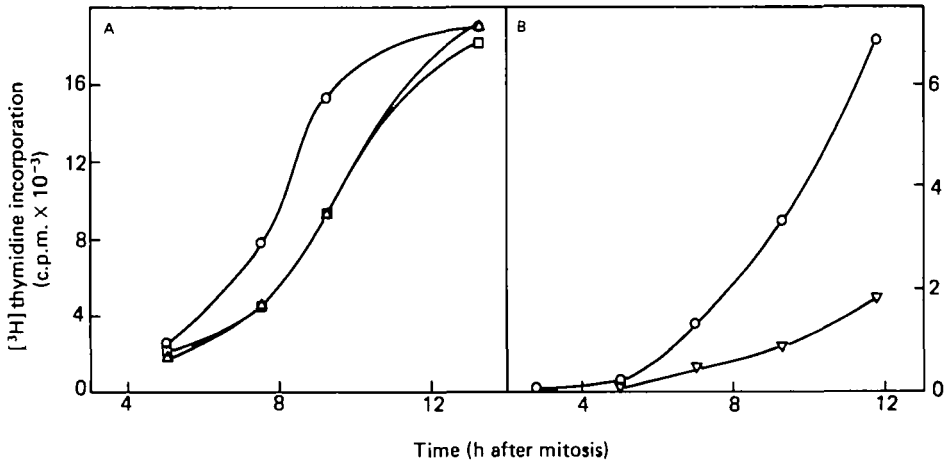

Fig. 4. DNA synthesis after u.v. irradiation of UV-1 in G_1. Details as for Fig. 2.

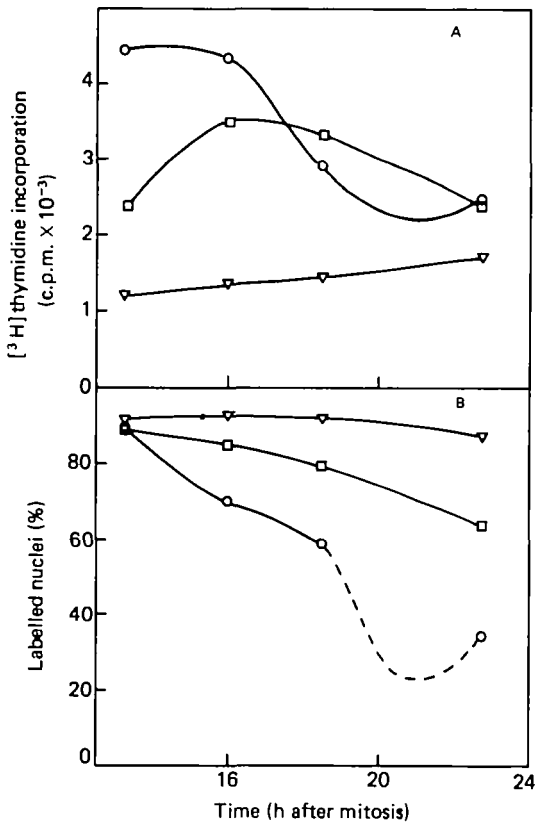

Fig. 5. DNA synthesis after u.v. irradiation of UV-1 in *S* phase. Details as for Fig. 3.

recovering to near the level seen in unirradiated cells at the peak of *S* phase (Fig. 3A), and the delay in exit from *S* phase is 6–7 h (Fig. 3B).

Similar experiments were carried out with UV-1 (Figs. 4, 5). A u.v. dose of 2 Jm^{-2} in G_1 (at either 1·5 or 3 h) depresses subsequent [^{3}H]thymidine incorporation (Fig. 4A) to about the same extent as 10 Jm^{-2} administered to 772-56 (Fig. 2B), and incorporation in UV-1 after 10 Jm^{-2} in G_1 is reduced to a third or less of the level in unirradiated cells (Fig. 4B). However, in spite of these severe effects of irradiation, entry of the population into *S* phase is unaltered, even at the higher u.v. dose (autoradiographic data, not shown). When UV-1 is irradiated with 2 Jm^{-2} in *S* phase, incorporation is initially depressed, recovers, and then falls again following the pattern of the unirradiated control cells (Fig. 5A); exit of cells from *S* phase is delayed by this dose by at least 4 h (Fig. 5B). A dose of 10 Jm^{-2} in *S* phase causes a long-lasting depression of [^{3}H]thymidine incorporation (Fig. 5A), and irradiated cells have hardly begun to leave *S* phase by 23 h, at which time the unirradiated population is apparently entering *S* phase in the next cell cycle (Fig. 5B). Thus *S*-phase irradiation of UV-1 has a far more severe effect than similar irradiation of the parent cell.

Capacity for excision repair

It is possible to assay the rate of incision by repair endonuclease at u.v. damage sites in DNA, by blocking DNA polymerization and resealing of repair patches with DNA synthesis inhibitors HU and araC (Collins & Johnson, 1981), so that the single-strand DNA breaks resulting from incision accumulate. In cells previously incubated with [^{3}H]thymidine to label the DNA, the breaks are conveniently measured by alkaline lysis followed by hydroxyapatite chromatography (Ahnström & Edvardsson, 1974; Collins, 1977); the breaks act as unwinding points for alkaline denaturation and the resulting single-stranded DNA is separated from double-stranded DNA on hydroxyapatite. The proportion of single-stranded DNA increases with the frequency of breaks, and can be calibrated against the known frequency of breaks introduced by X-irradiation. Figs. 6 and 7 show that, for both 772-56 and UV-1, DNA breaks accumulate during incubation with HU and araC immediately after u.v. irradiation (triangles). If cells are preincubated with HU and araC before irradiation, the breaks accumulated during subsequent incubation are in most cases slightly increased (circles). We have previously reported (Collins & Johnson, 1981; Collins, Jones & Waldren, 1982) that preincubation enhances break accumulation, presumably by maximizing inhibition of repair DNA polymerization. There is little difference between 772-56 and UV-1 in the rate of DNA break accumulation after irradiation early in G_1 (Figs. 6A, 7A) though when cells are irradiated 2·5 h later (Figs. 6B, 7B), accumulation appears to cease at an earlier time in 772-56 than in UV-1. Figs. 6B and 7B also show the DNA breaks accumulated when HU and araC are not added immediately after irradiation but after a 2·5 h delay (broken lines). There is clearly substantial incision activity in both cell types, i.e. they are still engaged in DNA repair some hours after administering u.v. Preincubation with inhibitors is obviously not possible in this sort of experiment, and so the DNA break frequency is likely to give an underestimate of the true incision activity.

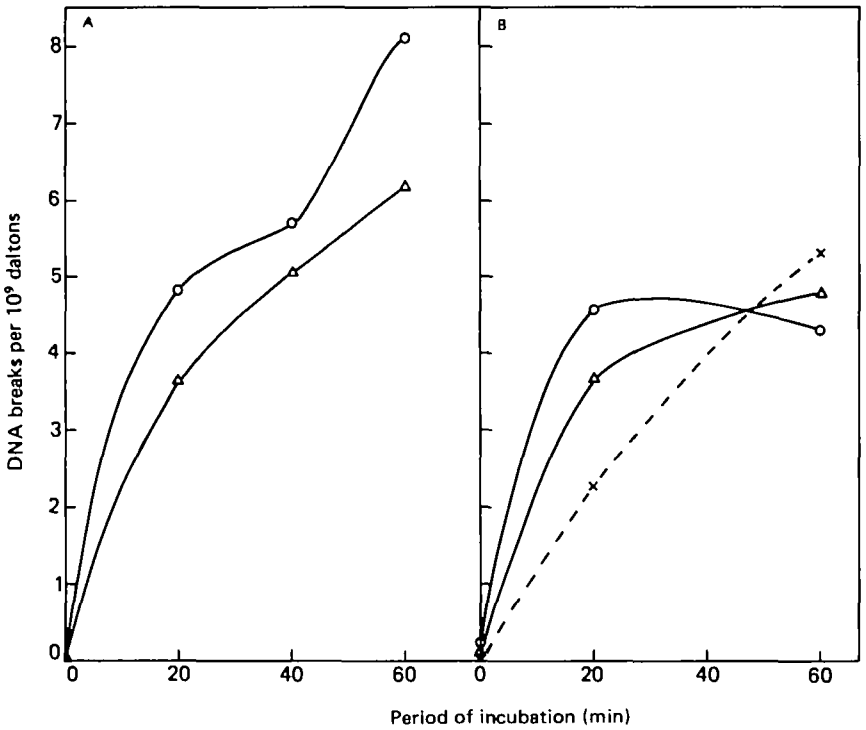

Fig. 6. u.v.-dependent DNA break accumulation in 772-56 cells. A. Synchronized prelabelled cells were u.v.-irradiated (10 Jm^{-2}) at 2·25 h after release from mitosis. (△) Incubated with HU and araC for the periods shown after irradiation. (○) Incubated with HU and araC for 30 min before irradiation as well as for the periods shown after irradiation. B. (△, ○) As in A except that irradiation was at 4·75 h after release from mitosis. (×) Incubation with HU and araC started at 4·75 h, but these cells were irradiated at 2·25 h. DNA breaks (resulting from enzymic incision at damage sites) were measured by alkaline lysis and hydroxyapatite chromatography.

Single-cell survival

Mitotic cells were released to progress through the cell cycle. At 3-hourly intervals samples were irradiated with various doses of u.v. and plated out as single cells to assay for colony-forming ability as a measure of survival. The survival curves for 772-56 and UV-1 are shown in Fig. 8, and the parameters D_0 (mean lethal dose), D_q (quasi-threshold dose) and n (extrapolation number), taken from these curves, are shown in Table 1. There is a clear dependence of survival on position in the cell cycle, for both cell types; at the higher doses, mitotic cells are most resistant to killing, G_1–early S cells are most sensitive, and by late S a recovery of resistance is seen. The overall levels of survival reflect the different u.v.-sensitivities of random populations of the two cell types (Stamato & Waldren, 1977). Referring to the doses used in the experiments already described, a dose of 2 Jm^{-2} permits a high level of survival (relative to unirradiated cells) at all stages for each cell type, whereas 10 Jm^{-2} kills fewer than 50% of 772-56 but more than 50% of UV-1 at all stages.

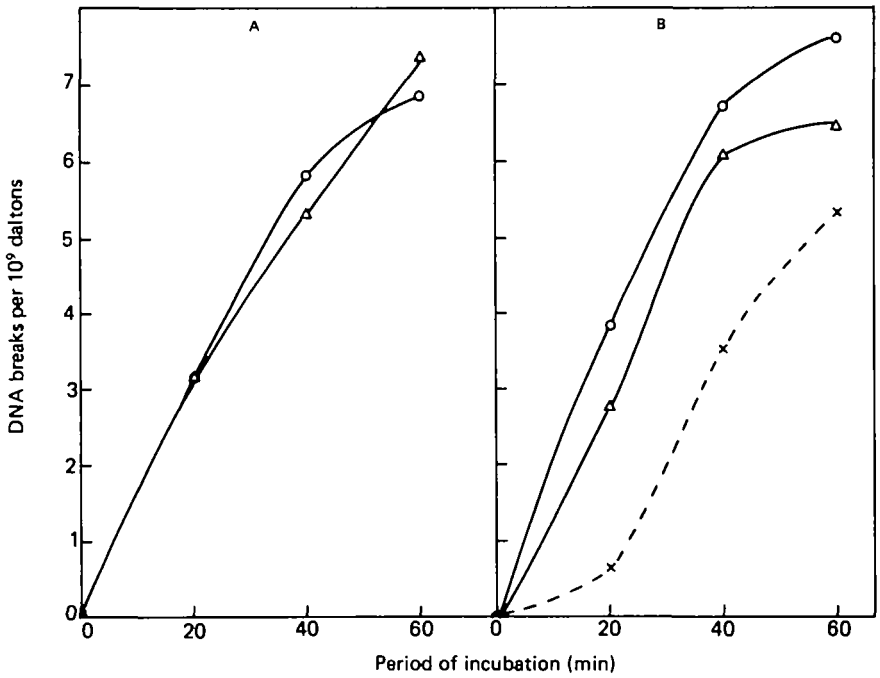

Fig. 7. u.v.-dependent DNA break accumulation in UV-1. Details as for Fig. 6.

Table 1. *Ultraviolet light survival parameters for 772-56 and UV-1 cells: obtained by extrapolating the straight lines from the high dose data of Fig. 8*

		Time after release from mitosis (h)					
		0	3	6	9	12	15
772-56	D_0 (Jm^{-2})	10·0	2·7	2·4	2·5	3·8	4·3
	D_q (Jm^{-2})	7·3	8·2	8·8	8·8	7·8	8·1
	n	2·1	21·0	39·0	34·0	7·8	6·6
UV-1	D_0 (Jm^{-2})	4·0	2·0	2·7	3·1	3·2	3·4
	D_q (Jm^{-2})	6·0	7·7	4·4	3·7	4·0	4·3
	n	4·5	43·0	5·1	3·3	3·5	3·5

DNA precursor pool size

As the abnormality in UV-1 is connected with the process of replicative DNA synthesis, we examined whether the concentrations of DNA precursors in the cellular pool differed between the two cell types, since a defect in precursor metabolism might conceivably affect the response of the replicative machinery to u.v. damage in the template DNA. Table 2 indicates that 772-56 and UV-1 contain virtually the same concentrations of each of the ribonucleotides and deoxyribonucleotides measured. (Since these determinations were made using unsynchronized populations, it remains possible that subtle cell-cycle-related differences do exist.)

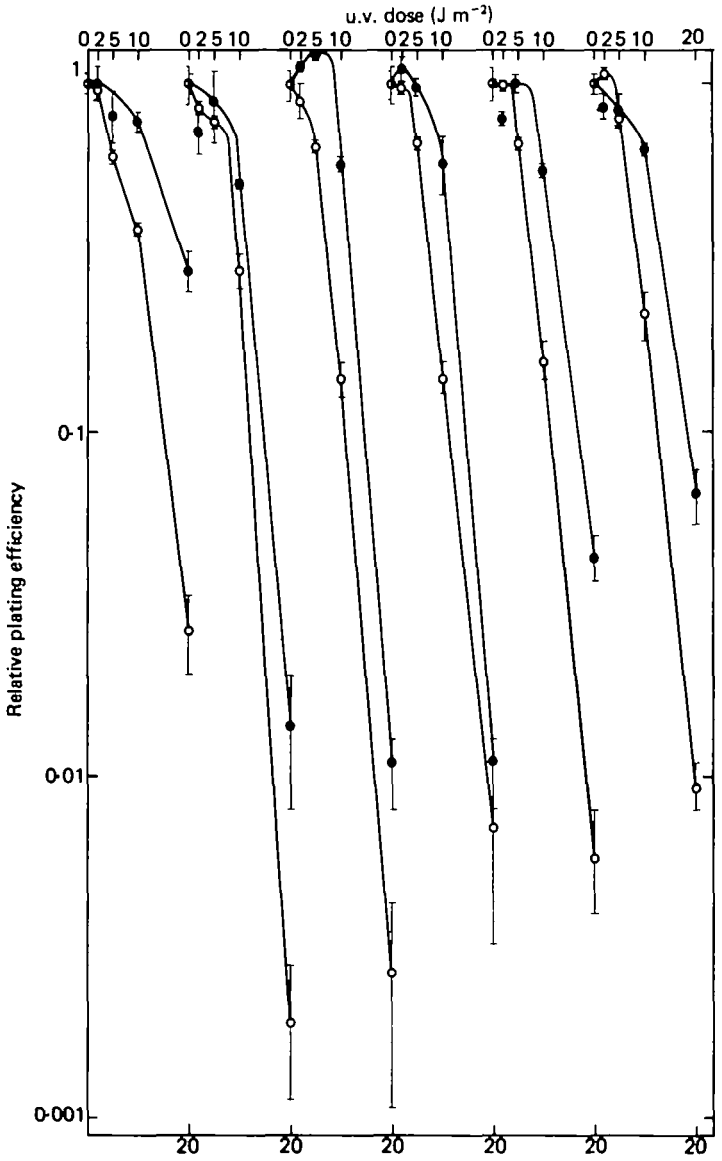

Fig. 8. Single-cell survival after u.v. irradiation of synchronized cells. Samples from synchronous populations of 772-56 (●) or UV-1 (○) were u.v.-irradiated at 3-hourly intervals from mitosis (shown in series from left to right) and plated out to form colonies. Survival is expressed relative to the survival of unirradiated cells. Bars indicate S.E. of mean. In a parallel population of 772-56, 50 % of cells had entered *S* phase by 6·6 h and a maximum of 74 % were in *S* phase at 12 h. In the case of UV-1, 50 % had entered *S* phase by 6·2 h and a maximum of 79 % were in *S* phase at 12 h.

Table 2. *Nucleic acid precursor pool sizes in* 772-56 *and UV-1 cells*

	772-56	UV-1
Ribonucleotides	Concentration (nmol/mg protein)	
ADP	2·30	2·00
GDP	0·61	0·56
ATP	22·6	27·2
CTP	1·52	1·28
GTP	4·96	6·48
UTP	3·48	3·32
Deoxyribonucleotides	Concentration (pmol/mg protein)	
dATP	58	69
dCTP	54	50
dGTP	10	15
dTTP	52	53

DISCUSSION

We report here various aspects of the cellular response to u.v. irradiation, examined in synchronized populations of the u.v.-sensitive mutant, UV-1, and its parent, 772-56.

The inhibitory effect of u.v. irradiation on DNA synthesis in mammalian cells is well documented. In HeLa cells (Djordjevic & Tolmach, 1967; Edenberg, 1976), normal human fibroblasts (Rudé & Friedberg, 1977), mouse L cells (Domon & Rauth, 1968) and Chinese hamster cells (Meyn, Hewitt, Thomson & Humphrey, 1976; Doniger, 1978) the incorporation of labelled precursor into DNA is depressed for a few hours after irradiation with doses of 10 Jm^{-2} or less. It then, typically, recovers (Edenberg, 1976; Rudé & Friedberg, 1977). Our results with asynchronous 772-56 cells (Fig. 1 A) agree with these earlier reports. In addition to normal human cells, Rudé & Friedberg (1977) examined cells from patients with xeroderma pigmentosum (XP), a disease characterized by marked u.v. sensitivity and by defects in DNA repair. Given 5 Jm^{-2} irradiation, cells of XP variant (defective in post-replication recovery, i.e. the ability to cope with DNA damage present during *S* phase DNA replication) recovered from the inhibition of DNA synthesis more slowly than did normal cells; and cells of XP(A) (with a severe deficiency in excision repair) showed no recovery in 24 h. (However, given u.v. doses representing equal degrees of cell killing in the three strains, all showed similar kinetics of inhibition and recovery.) In the case of the u.v.-sensitive mutant UV-1 (Fig. 1 B), the level of inhibition of DNA synthesis that is reached soon after irradiation is slightly greater than that seen in 772-56, but recovery is very much slower.

The effect of u.v. on DNA replication has also been studied at the molecular level, using techniques such as DNA fibre autoradiography, bromodeoxyuridine incorporation and CsCl gradient centrifugation. Edenberg (1976) found that the mean length of newly synthesized DNA in HeLa cells increased with time early after u.v. irradiation, but then levelled off; the kinetics and dose dependence of the process fitted the

hypothesis that replication forks tend to stop movement when they reach a site of damage in DNA (presumably a cyclobutane pyrimidine dimer, the predominant lesion caused by u.v. irradiation). Re-initiation of synthesis was not seen to occur within 90 min of irradiation. However, Meyn *et al.* (1976) reported that, in CHO cells, doses of up to 10 Jm^{-2} did not prevent eventual complete replication; i.e. dimers do not represent a permanent block of DNA synthesis. In hamster cells (Doniger, 1978), 25 min after u.v. irradiation (5 Jm^{-2}) 50% of replicons were no longer functioning, while in those that were the rate of replication fork movement was unchanged; i.e. u.v. had an 'all-or-nothing' effect on replication rate. A higher dose (10 Jm^{-2}) did begin to reduce the rate of fork movement as well. Whereas the size of replicated fragments after u.v. was found by Edenberg (1976) to approximate to the distance between dimers, Doniger (1978) reported that these fragments were long enough to contain at least four dimers, but that the size of nascent DNA analysed by alkaline sucrose gradient sedimentation was equivalent to the inter-dimer distance. The implication of this study is that replication may be blocked by a dimer, or it may proceed past a dimer leaving a gap in the newly synthesized strand. Whether the smaller size of DNA made after u.v. irradiation is the result of blocked replication (i.e. premature termination) or gapped synthesis, or both, is the subject of current debate (e.g. see Park & Cleaver, 1979; Lehmann & Karran, 1981). Whatever the details of the inhibition of DNA replication by u.v., cells can overcome it; as well as the recovery of thymidine incorporation mentioned above, the small nascent DNA fragments eventually increase to the size of bulk DNA, and this process is commonly referred to as post-replication repair (PRR) or daughter-strand repair. We prefer the term post-replication recovery (Stamato *et al.* 1981), since it is not clear whether any special repair system is involved. It is in post-replication recovery that the UV-1 defect lies; UV-1 elongates the nascent DNA fragments after u.v. irradiation more slowly than the parent cell (Stamato *et al.* 1981).

The more severe inhibition of thymidine incorporation seen in synchronized UV-1 cells, and the greater delay in progress through *S* phase, compared with 772-56, are not necessary consequences of the slow elongation of nascent DNA, since this latter process is one of gap-filling and ligation, and itself involves negligible precursor incorporation. Possibly the delay in completion of a particular cluster of replication units prevents initiation of replication in clusters later in the sequential process of replication.

As is reported above, u.v. irradiation in G_1 has no effect on the time or extent of subsequent entry into *S* phase. This is in agreement with reports for human HeLa cells (Djordjevic & Tolmach, 1967) and mouse L cells (Domon & Rauth, 1968), though Bootsma & Humphrey (1968) reported that entry of human kidney T cells into *S* phase was accelerated by 2–3 h by u.v. irradiation in G_1. The prolongation of *S* phase by irradiation during that phase, seen in Figs. 3 and 5, was also inferred in the earlier studies. In the cell-cycle repsonse to u.v., UV-1 differs from 772-56 quantitatively (showing greater depression of incorporation and longer delays) but not qualitatively.

The greater depression of DNA synthesis in UV-1 after G_1 irradiation is unlikely to result from the presence of more unrepaired damage in comparison with the parent

cell. The rates of excision repair, as indicated by the capacity to accumulate DNA breaks (i.e. incisions) when repair synthesis is inhibited, are similar in asynchronous 772-56 and UV-1 cells (Stamato *et al.* 1981), and we show here that they are similar also in early G_1 (Figs. 6A, 7A). The apparent difference between the two cells when irradiated in late G_1 (Figs. 6B, 7B), with UV-1 showing more breaks than 772-56 at long times of incubation, may be real, but is probably not significant. The data obtained on incubating cells with inhibitors 2·5 h after irradiation are clear evidence that DNA damage does remain at this time, and that both cells are able to deal with it. The u.v. dose given to these cells (10 Jm^{-2}) produces about 120 dimers per 10^9 daltons (Collins, Downes & Johnson, 1980). The rate of DNA break accumulation in Figs. 6A and 7A is about 0·15 breaks per 10^9 daltons per min. If incision had continued at this rate during the 2·5 h interval, only about a fifth of the dimers would have been repaired. The conclusions from these results are: first, that cells (normal or mutant) damaged in G_1 enter S phase at the normal time in spite of the presence of substantial unrepaired damage; and, second, that the residual damage is not inaccessible or 'protected' but is being actively processed by repair enzymes. Thus, cells in G_1 do not seem to possess any means to hold back replication of DNA until damage has been removed.

In the 772-56 cell line, as in other hamster lines (Han & Sinclair, 1969; Burg, Collins & Johnson, 1977), mitosis is the phase most resistant to u.v. killing; and late G_1 – early S phase cells are most sensitive, when survival is expressed in terms of D_0 (Table 1). D_q is relatively constant through the cycle. UV-1 shows a similar pattern in terms of D_0, except that mitotic cells are more sensitive than is the case with 772-56. There is a marked difference between the two cell lines in D_q, which in UV-1 fluctuates from a value similar to that for 772-56 in mitosis and early G_1, to a value only half of that for 772-56 in late G_1 and S phase. In one interpretation of single-cell survival curves (Hahn & Little, 1972), the exponential region is regarded as indicating the accumulation with dose of potentially lethal damage. It is not clear why D_0, which is a function of this region of the curve, should vary between the two cell types at mitosis but not at other times. D_q, i.e. the quasi-threshold, or shoulder of the curve, is thought to represent the capacity of the cell to tolerate damage, which is therefore regarded as sublethal; the tolerance is presumed to be due to DNA repair. It is tempting, then, to explain the low D_q value for UV-1 in mid-interphase as being the direct result of the deficiency in a particular S-phase-linked mode of repair, i.e. post-replication recovery. However, certain complicating factors arise. First, both excision repair and PRR need to be considered. Whereas excision restores the damaged DNA to its original form, PRR is strictly a means of coping with damage so that replication can proceed; the damage (dimers) remains in the replicated parental DNA and is presumably later excised. The likely effect of a capacity for PRR on D_q is hard to predict. Second, an amount of damage inflicted in G_1 equivalent in dimers to the D_q dose would persist largely unrepaired well into S phase (see the discussion of incision above) and would cause disruption of DNA synthesis (see Figs. 2 and 4); yet for UV-1 D_q is at its highest for irradiation at this point, i.e. cells are well able to tolerate this damage as sublethal. Third, the prolongation of S phase as a result of u.v. irradiation,

more pronounced in UV-1 than in 772-56, may be a contributory factor in cell death, but it also provides an extended period of prereplication for part of the genome, during which continuing excision would be expected to remove a significant amount of otherwise lethal damage (i.e. to extend the shoulder of the curve).

This paper represents a preliminary attempt to correlate perturbations of the cell cycle by DNA damage with a known defect in cellular repair capacity. It is hoped that similar studies with cells showing different repair defects will follow, and that the interrelations between DNA damage, repair, the cell cycle and cell survival will become less obscure.

This work was supported by the Cancer Research Campaign, and by grants MOD 15–10, CA-20810 and HD-02080, and NIEHS (ES-0155). Preliminary work was done while A.C. was the recipient of an American Cancer Society – Eleanor Roosevelt – International Cancer Fellowship awarded by the International Union Against Cancer.

We thank Kate Barber, Denise Snead and Bill Laas for technical assistance, and Drs R. T. Johnson and S. Squires for helpful discussions.

This is contribution 380 from the Eleanor Roosevelt Institute for Cancer Research and the Florence Sabin Laboratories for Genetic and Developmental Medicine, and the Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Centre, Denver, Colorado.

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(*Received* 5 *March* 1982)