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LESIONS INDUCED IN DNA BY ULTRAVIOLET LIGHT ARE REPAIRED AT THE NUCLEAR CAGE

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SUMMARY

In mammalian cells, *S*-phase DNA synthesis occurs at sites fixed to a sub-nuclear structure, the nuclear matrix or cage. This is an ordered network of non-histone proteins, which maintains its essential morphology even in the absence of DNA. We show here that unscheduled DNA synthesis following exposure of HeLa cells to ultraviolet light also takes place at this sub-structure. We also show that ultraviolet irradiation grossly reorganizes nuclear DNA, arresting *S*-phase synthesis at the cage and leaving the residual synthesis highly localized.

INTRODUCTION

Nuclear DNA is organized into a series of loops by attachment to a nuclear sub-structure, the matrix or cage (Cook & Brazell, 1975, 1978; Cook, Brazell & Jost, 1976; Igo-Kemenes & Zachau, 1978; Paulson & Laemmli, 1977). This is an ordered network of non-histone proteins, which maintains its essential morphology even in the absence of DNA (for a review, see Hancock, 1982). It is also the site of *S*-phase DNA synthesis, so that sequences usually found out in the loops must first become more closely associated with the cage before they are replicated (Dijkwell, Mullenders & Wanka, 1979; Pardoll, Vogelstein & Coffey, 1980; McCready *et al.* 1980). We now investigate whether lesions induced by ultraviolet light, which are presumably introduced randomly around the loops, are repaired at the site of the lesion (i.e. out in the loop) or whether they, too, require prior attachment to the cage.

The association of *S*-phase synthesis with the cage was demonstrated in two ways (McCready *et al.* 1980). Living cells were lysed in Triton X-100 and 2 M-NaCl to release nucleoids containing histone-free DNA looped and attached to cages (Cook & Brazell, 1975; Cook, Brazell & Jost, 1976). As the DNA is supercoiled it must be intact (Cook & Brazell, 1975, 1976). The first approach was biochemical and involved progressively detaching DNA from cages with nucleases; pulse-labelled DNA resisted detachment. The second involved electron microscopic autoradiography. If nucleoids are floated on an aqueous surface, their DNA spreads out from the cage into a discrete skirt (Fig. 1): autoradiography shows that pulse-labelled DNA is almost exclusively associated with the cage. We chose to study the site of repair using the latter method since individual nucleoids made from cells engaged in unscheduled DNA synthesis (UDS) can then be distinguished from their much more heavily labelled counterparts in *S* phase.

MATERIALS AND METHODS

Ultraviolet (u.v.) irradiation and labelling

Medium (minimum essential medium supplemented with 10% new-born calf serum) was removed from HeLa cells growing logarithmically in Petri dishes, the cells were irradiated at 37 °C using a germicidal tube (Cook & Brazell, 1976) and warm medium was added. After 30 min at 37 °C, [*methyl*-1′,2′-^{3}H]thymidine (100 Ci/mmol, 100 μCi/ml) was added for 2·5 or 5 min, then removed by rinsing the monolayer five times with ice-cold phosphate-buffered saline supplemented with 0·5 mM-EDTA and 2·5 mM-thymidine. Cells were detached with a rubber policeman, washed three times in the ice-cold saline and lysed immediately.

Isolation of nucleoids

Cells were lysed in 0·5% Triton, 2 M-NaCl and nucleoids isolated as described by Cook *et al.* (1976).

Electron microscopy and autoradiography

Nucleoids were spread and autoradiographs prepared as described by McCready *et al.* (1980). All spreads seen were photographed and grains counted; those with >10 grains were selected (normally >90%) and grains over each cage expressed as a percentage of the total over skirt and cage. Spreads were easily categorized as *S* or non-*S*, e.g. in Fig. 4D averages of >500 and 38 (range 0–130) grains lay over *S*-phase and non-*S* spreads, respectively. In Fig. 4C incorporation was lower, with a less clear-cut distinction between the two categories; therefore, we classed the 75% of spreads with the lowest grain counts as being non-*S* since 25% of cells are normally in *S*.

RESULTS

HeLa cells were u.v.-irradiated (15 or 40 J/m^2), grown for 30 min, pulse-labelled (2·5 or 5 min) with [^{3}H]thymidine, nucleoids were isolated and spread, and autoradiographs were prepared. Silver grains were counted over each spread (i.e. cage and skirt) and the proportion over the cage was calculated. The total number of grains/spread was used to categorize spreads as derivatives of *S* phase or non-*S* phase cells. Representative autoradiographs are given in Figs 2 and 3 and histograms of label distributions in Fig. 4. Four observations can be made, as follows.

u.v. irradiation redistributes DNA

First, u.v. irradiation affects the distribution of label in randomly labelled nucleoids (24 h pulse; Fig. 4A). 35–55% (average 45%) of grains appear over the cage in nucleoids from unirradiated cells, with little variation from one spread to another. If nucleoids are isolated from randomly labelled cells, which have then been u.v.-irradiated, fewer grains are found over the cage (i.e. average 29%) and many more over the skirt; there is considerable variation from one nucleoid to another (range 5–50%). This may reflect either the gross decondensation of chromatin induced by u.v. light *in vivo* (Schor, Johnson & Waldren, 1975) or extensive nicking in DNA undergoing repair. We know, however, that nicking nucleoid DNA with sufficient γ-rays to release all supercoiling does not significantly alter the distribution of spread DNA (unpublished results).

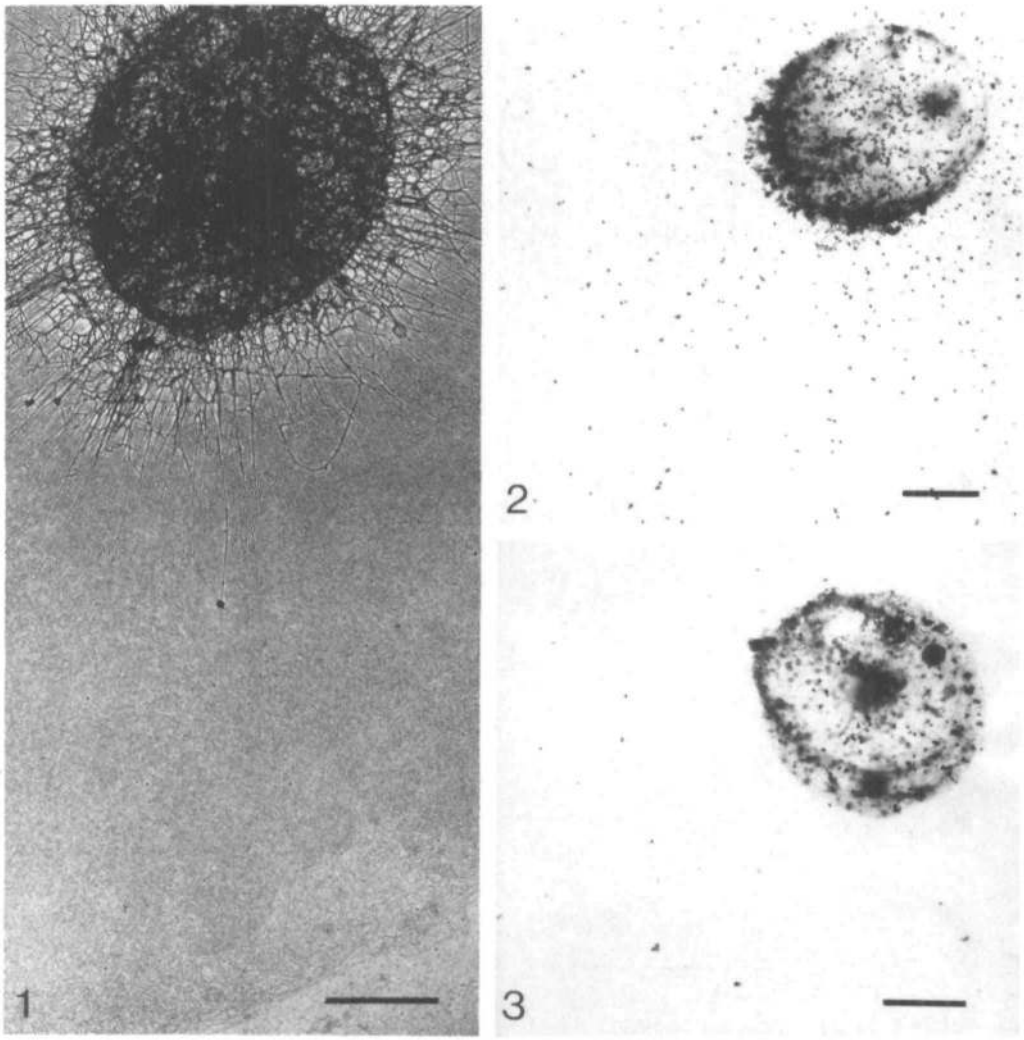

Fig. 1. Electron micrograph of a nucleoid spread, stained and shadowed to show the central cage and attached DNA skirt, which extends almost to the edge of the field. Bar, 5 μm.

Fig. 2. EM autoradiograph of a spread from an irradiated (15 J/m^2) cell, labelled for 24 h as in Fig. 4A. The grain distribution reflects the DNA distribution. The preparation is shadowed but not stained, to avoid obscuring grains, so no skirt is seen at this magnification. Bar, 5 μm.

Fig. 3. EM autoradiograph of a spread from an irradiated (15 J/m^2) non-*S*-phase cell, labelled for 5 min as in Fig. 4D; 80 % of 98 grains in this spread lie over the cage. Bar, 5 μm.

Unscheduled DNA synthesis occurs at the cage

The second, more important, observation is that after u.v. irradiation a considerable amount of pulse-label is clearly associated with the cage (Fig. 4C–F); the distribution of label differs significantly from total DNA distributions in both unirradiated and irradiated controls (i.e. Fig. 4A). For example, in Fig. 4C cells were

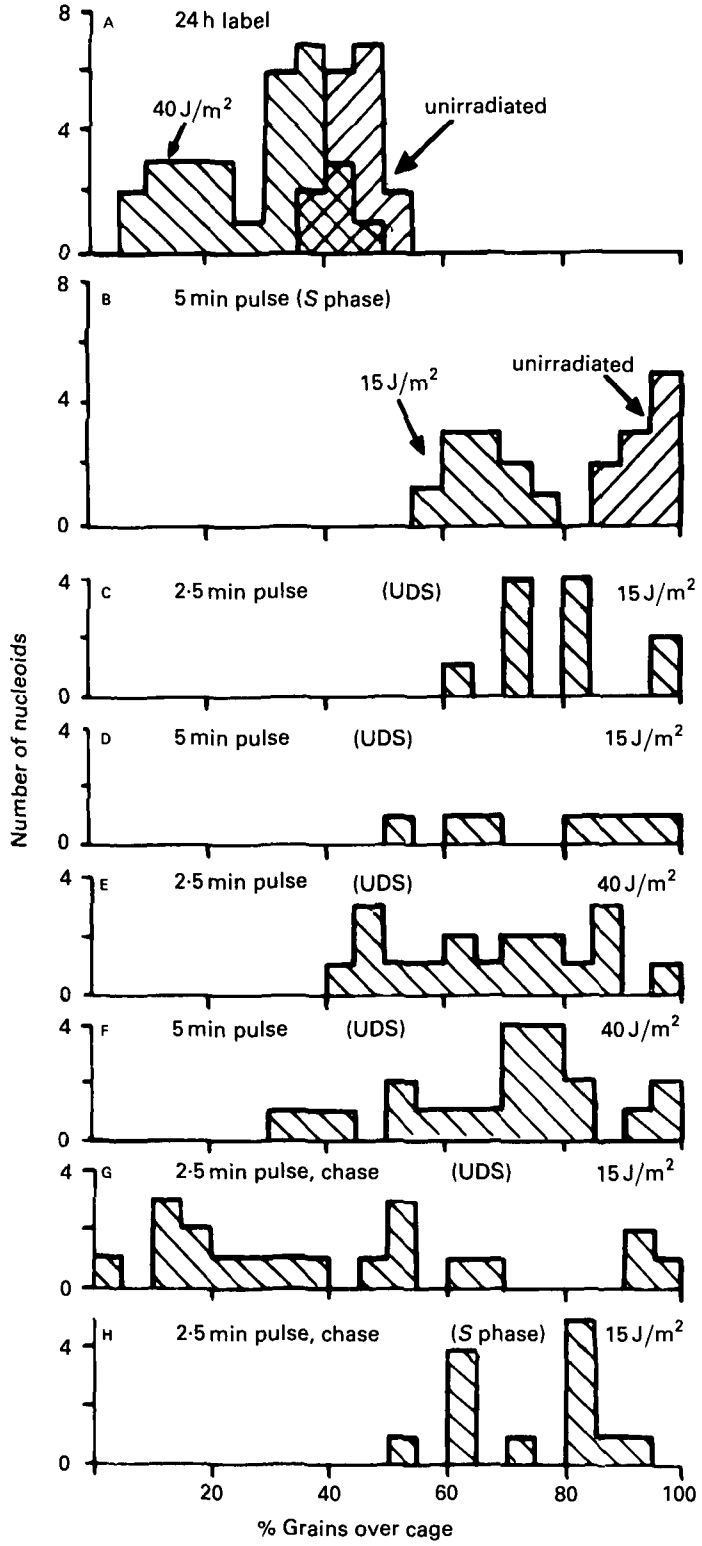
A
24 h label
40 J/m²
unirradiated
B
5 min pulse (S phase)
15 J/m²
unirradiated
C
2·5 min pulse
(UDS)
15 J/m²
D
5 min pulse
(UDS)
15 J/m²
E
2·5 min pulse
(UDS)
40 J/m²
F
5 min pulse
(UDS)
40 J/m²
G
2·5 min pulse, chase
(UDS)
15 J/m²
H
2·5 min pulse, chase
(S phase)
15 J/m²
Number of nucleoids
% Grains over cage
0
4
8
20
40
60
80
100

Fig. 4

irradiated (15 J/m^2) and 30 min later labelled for 2·5 min with [^{3}H]thymidine. Eleven of the spreads had more than 10 grains and were from cells engaged in UDS (see Materials and Methods); in all, >60 % grains were over the cage, quite unlike the control distributions in Fig. 4A where *no* spread had >55 % grains over the cage. Essentially similar results were obtained with the other doses and labelling times (Fig. 4C–F). We conclude, therefore, that UDS occurs at the cage or internal matrix. This implies either that lesions are repaired as chromosome loops are fed through a fixed enzyme complex or that lesions in loops are attached before repair.

In principle, pulse-label in repaired sites at the cage should be chased away to yield the control distribution of total DNA found in Fig. 4A; however, the results are not so clear cut (Fig. 4G). Label is chased away in some cases but not others. Possibly, the combined effects of u.v. damage and radiolabelling inhibit repair or leave some cells so debilitated that they cannot repair completely.

Mullenders, van Zeeland & Natarajan (1983) have used the biochemical approach and were unable to show any association of UDS with the matrix. They inhibited *S*-phase synthesis with cytosine arabinoside and hydroxyurea, and measured incorporation during 10-min pulses. However, our experience shows that such a pulse is too long to reveal any significant association of label with the sub-structure; attachment of DNA for repair is more transient than the attachment during *S*-phase synthesis (compare Fig. 4B with D,F). For example, the *average* proportion of grains over the cage after a 10-min pulse was similar to the average given by the unirradiated distribution in Fig. 4A, even though the distributions were somewhat different (unpublished results). In this context it should be remembered that only 30–100 nucleotides around each lesion are repaired (Cleaver, 1981), that nucleotides are polymerized by various polymerases at rates >1000/min (Kornberg, 1974) and that the time taken to repair each lesion *completely* – not just nucleotide incorporation – is 3–10 min (Erixon & Ahnstrom, 1979).

u.v. light arrests S-*phase synthesis*

Our third observation concerns the effects of u.v. light on *S*-phase synthesis. When unirradiated nucleoids are pulsed for 5 min, >90 % of label lies over cages (Fig. 4B) and all can be chased into skirts, confirming earlier results (i.e. from the unirradiated distribution in Fig. 4B into its counterpart in Fig. 4A). Following irradiation, more

Fig. 4. Histograms of grain distributions over nucleoid spreads. A. 24 h label(0·05 μCi/ml), then ± irradiation, incubate at 37 °C for 30 or 90 min (incubation for 30 or 90 min gave identical distributions so the results have been pooled); the unirradiated distribution reflects the total DNA distribution that is redistributed by irradiation. B. ± Irradiation (15 J/m^2), then 5 min pulse, *S*-phase spreads; *S*-phase incorporation is cage-associated and somewhat redistributed by irradiation. C–F. Irradiation (C,D, 15; E,F, 40 J/m^2), then 2·5 (C,E) or 5 min pulse (D,F), non-*S* spreads; label is associated with cages. G. 15 J/m^2 irradiation, then 2·5 min pulse followed by 57·5 min chase, non-*S* spreads; some label remains associated with cages. H. *S*-phase spreads from G; label remains associated with cages. The total number of grains (cage:skirt) in each experiment were: A, −u.v. (4222:4840), +u.v. (5066:11284); B, −u.v. (7106:440), +u.v. (7504:3952); C, (174:48); D, (475:132); E, (750:380); F, (1705:1008); G, (1140:581); H, (10345:3828).

label is found in skirts, suggesting that u.v. irradiation redistributes some label incorporated at the cage and what pulse-label there is at cages cannot be so effectively chased away (Fig. 4H); the majority remains cage-associated, presumably because *S*-phase replication forks are arrested at u.v.-induced lesions. Therefore, this u.v. effect resembles the behaviour of *S*-phase nucleoids after treatment with inhibitors of DNA synthesis (McCready *et al.* 1980).

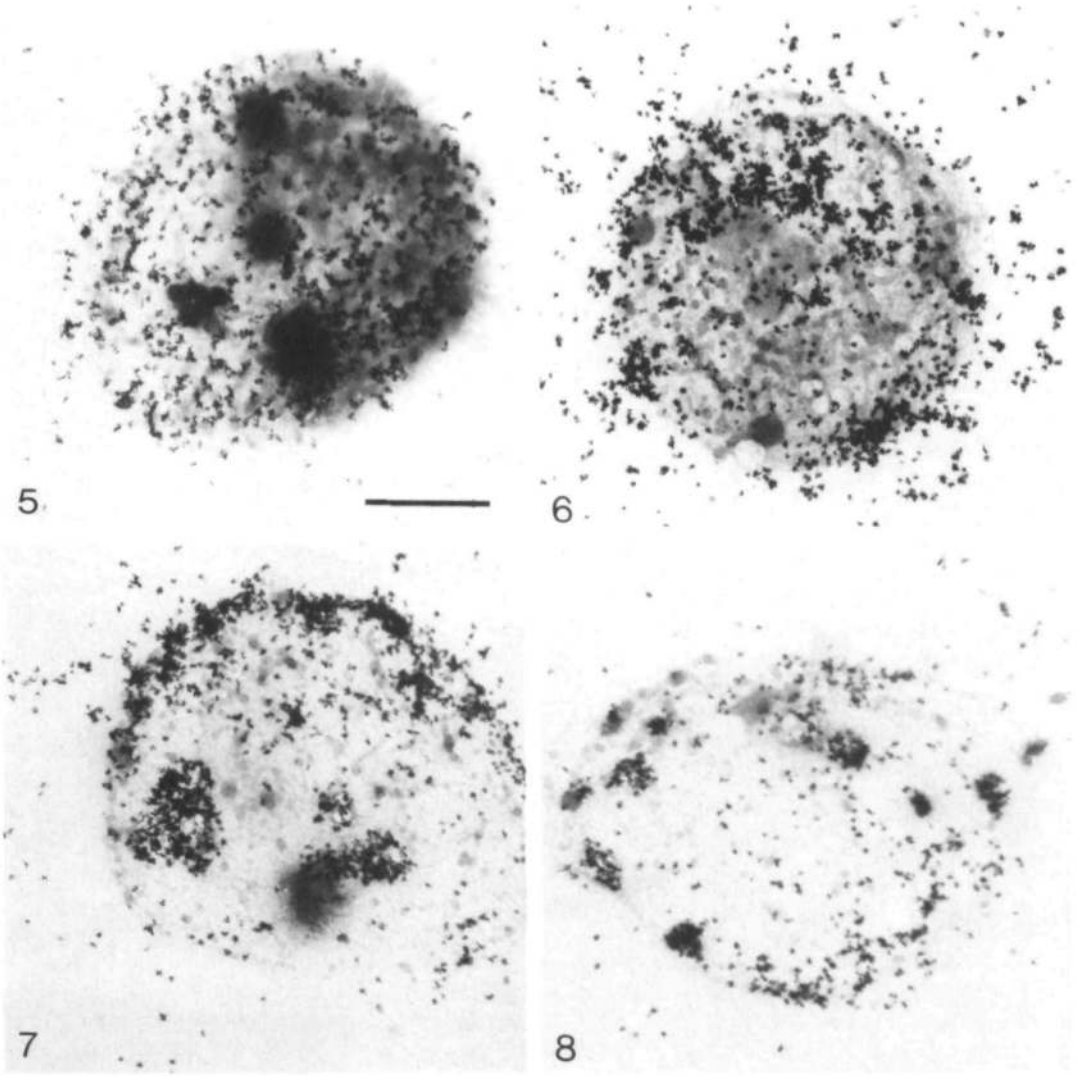

Fig. 5. EM autoradiograph of a nucleoid spread from an unirradiated *S*-phase cell labelled for 5 min, showing typical even distribution of grains over the cage. Bar, 5 μm. Figs 5–8 are at the same magnification.

Figs 6, 7. EM autoradiograph of a nucleoid spread from an irradiated (15 J/m^2) cell labelled for 5 min, showing characteristic clustering of grains over localized areas of cage.

Fig. 8. EM autoradiograph of a nucleoid spread from an irradiated (15 J/m^2) cell labelled for 2·5 min then chased for 57·5 min, showing many grains remain clustered.

S-phase synthesis after u.v. irradiation is localized

Fourth, following irradiation, the pulse-label in *S*-phase nucleoids is not randomly distributed throughout the cage as it is in normal *S*-phase (Fig. 5), but concentrated in local centres (Figs 6, 7). These concentrations remain even after a chase as in Fig. 4H (Fig. 8). This implies that polymerizing centres are clustered within the nucleus.

DISCUSSION

In common with the approach used by many others we have used doses of u.v. light that are, in the biological context, extremely high. For example, 15 J/m^2 reduces viability to 2·7 % and incorporation (2·5 or 5 min pulse), which is mostly *S*-phase, to 30 % of normal; 40 J/m^2 almost eliminates viability and reduces incorporation to 12 %. Therefore, it remains possible that the effects we see are the response of dying cells rather than the normal repair processes of living cells. In future experiments we think it feasible to use lower doses (e.g. 5 J/m^2) without extending the autoradiographic exposure times much beyond 3 months. (In addition, these experiments can be conducted using the light microscope.)

Repair of damage induced by u.v. light involves cutting one strand of the DNA duplex ('incision'), removal of the principal photoproduct, the thymine dimer ('excision'), synthesis of DNA complementary to the unaffected strand and 'ligation' of the final phosphodiester bond to restore the intact duplex (Cleaver, 1974). We assume in the following discussion that lesions are induced randomly in the loops, and that a lesion's initial position in a loop does not influence its rate of repair. (These assumptions would prove faulty if, for example, lesions were preferentially induced in transcribing sequences, which we know are closely associated with cages (Jackson, McCready & Cook, 1981), or if lesions in such transcribed regions were repaired before those in outlying non-transcribed regions.) If pulse-label were incorporated at repairing sites out in the loop, we would expect the resulting grain distribution to reflect the distribution of DNA. Clearly, it does not: grains are found predominantly over the cage. Bearing in mind the reservations and assumptions expressed above, all our evidence is most simply interpreted in terms of cage-associated repair. Therefore, two additional steps must be added to the repair pathway outlined above: the lesion or the resulting nick or gap must attach to the cage before repair synthesis and the repaired site must detach subsequently. We can only speculate as to the precise timing of attachment and detachment. However, we do know that repair relaxes supercoiling in the loops (Cook & Brazell, 1976), so that when nicks and/or gaps are present they cannot be attached in a manner that prevents such relaxation. It will be of interest to see whether deficient repair in various pathological states results from a failure of a lesion to associate correctly with, and dissociate from, the cage. In addition, this approach can be used to investigate the sites of repair of other types of lesion.

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