Transient conformation changes in chromatin during excision repair of ultraviolet damage to DNA

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ABSTRACT

DNA labeled for 15 minutes during UV induced repair synthesis is two-fold more sensitive to micrococcal nuclease than the bulk nuclear DNA. As the length of the labeling period increases from 15 minutes to 4 hours the nuclease sensitivity of repair labeled DNA approaches that of bulk chromatin. Pulse-chase experiments indicate that the nuclease sensitivity of the repaired DNA labeled during a brief pulse decreases with a half-life of about 15 minutes. In contrast to previous interpretations, we consider these results to mean that immediately after synthesis, chromatin labeled during repair has a conformation which renders it more susceptible to nuclease digestion than the bulk chromatin. With time these repaired regions are assembled into a nucleosome structure with normal nuclease sensitivity.

INTRODUCTION

The repair of DNA damage in eukaryotic cells is a multi-step process involving 1) recognition of DNA damage, 2) enzymatic removal of damaged sites and 3) resynthesis of the damaged strand using the complementary strand (1,2). How chromatin structure influences these different steps in DNA repair is an important question, which is only beginning to be investigated. Recent work has suggested that chromatin structure can influence DNA repair in several different ways. Variations in reactivity of chromatin subfractions with alkylating agents have been shown (3-5). Nucleosome structure influences the removal of 3-methyladenine by a purified glycosylase (6), and protein nucleic acid interactions have been shown to mask pyrimidine dimers from UV endonucleases (7-8). Preferential localization of DNA repair synthesis in the linker portion of the nucleosome has
been suggested for both alkylating agents and UV (9-13). The above work clearly indicates that chromatin structure has an important influence on many steps of DNA repair. The purpose of these experiments was to examine the nuclease accessibility of UV induced DNA repair synthesis in CV-1 cells, and re-examine previous interpretations that sites of damage in linker regions may be preferentially repaired.

MATERIALS AND METHODS

Cell Culture. Monkey kidney cells (CV-1) were grown in Eagle's minimum essential medium supplemented with 15% fetal calf serum. One day after subculturing the cells were grown in medium containing 0.01 μCi/ml of 14C thymidine (dThd) for 2-3 days. The medium was changed, and the cells were allowed to grow to confluence in unlabeled medium.

Irradiation and Labeling Conditions. Confluent cultures were pretreated for 2 hours prior to UV-irradiation with 2 mM hydroxyurea (HU). The medium was then removed, and the cells were irradiated with 254 nm UV light at an incident dose of 1.3 J/m^2/S. Immediately after irradiation the cultures were labeled for 15 minutes or longer with medium containing 10 μCi/ml of 3H-Thd and 2 mM HU. For the pulse chase experiments the radioactive medium was removed, and replaced with medium containing 2 mM HU, 10^-4 M thymidine and 10^-5 M deoxycytidine. For the experiments in which the labeling time was either 2 hours or 4 hours after irradiation, the medium was changed 2 hours prior to labeling and replaced with medium containing 2 mM HU. In all cases the cells were collected by scraping the dishes, washed with saline, and pelleted by centrifugation. The cellular pellets were frozen in liquid N_2 and stored at -80°.

Nuclei Isolation. The frozen cellular pellet from 3-6 100 mm^2 dishes was suspended in 10 mM Tris, 10 mM NaCl, 1 mM MgCl_2 pH 7.4 (STM buffer) for 10 minutes. The cells were dounce homogenized, and the nuclei were collected by centrifugation at 1,500 rpm for 10 minutes in a IEC model PR-J centrifuge. The nuclei were washed with STM 1% Triton X-100 and centrifuged. The nuclear pellet was again suspended in
STM buffer and centrifuged. The nuclear pellet was suspended in 1.0 ml STM buffer, and the nuclei concentration was determined by hemocytometer counting.

**Nuclease Digestion.** Approximately 1 x 10^6 nuclei were added to STM buffer containing 0.2 mM CaCl_2. Micrococcal nuclease 18,530 units/mg in STM buffer was added to give a final concentration of 0.5 μg/ml. The nuclei were digested for varying lengths of time. The enzymatic digestions were stopped by the addition of an equal volume of 1N perchloric acid and 1.4 M NaCl. The samples were centrifuged at 10,000 rpm for 10 minutes, and the acid soluble supernate was collected. The acid insoluble pellet was dissolved in 2.0 ml 1N NaOH. For liquid scintillation counting 0.5 ml of the acid soluble supernate was added to 1 ml H_2O plus 10 ml Aquasol. 0.5 ml of the alkali solubilized pellet was added to a mixture of 0.5 ml H_2O, 0.5 ml 1N glacial acetic acid plus 10 ml Aquasol. Counting efficiencies, and ^14C spillover into the ^3H channel were determined by the use of internal standards, and were corrected for.

**Isolation and Gel Electrophoresis of DNA Fragments.** Nuclei were isolated from repair labeled CV-1 cells, and were suspended in STM CaCl_2 buffer at a concentration of approximately 1.3 x 10^7 nuclei/ml. They were digested with 10 μg/ml of micrococcal nuclease for 15 minutes at 37°. The enzyme digestions were terminated by adding 0.2 volumes of 50 mM EDTA. The resulting DNA fragments were then purified as previously described (10).

For the determination of the radioactive profile, the samples (90-125 μg of DNA) were electrophoresed on a 5.0% polyacrylamide slab gel (10 x 0.3 cm) for 2 hours at 10 volts/cm. The gels were sliced into 1.1-mm sections and placed in scintillation vials containing 1.0 ml of NCS/H_2O (9:1) (Amersham). The slices were kept for 18 hours at 37°. Ten milliliters of toluene containing Omnifluor (New England Nuclear) were added, and the radioactivity was determined by liquid scintillation counting. Corrections were made for ^14C spillover into the ^3H window by the use of internal standards. In all cases an acrylamide methylene-
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bisacrylamide ratio of 19:1 was used. The electrophoresis buffer used was the Tris-borate-EDTA system described by Peacock and Dingmann (14). The size of the DNA fragments from micrococcal nuclease treated CV-1 nuclei were estimated using the restriction fragments of φX174 replicative form treated with Hae III restriction enzyme (Bethesda Research Laboratories), and the restriction fragments of PM2 DNA made by treating PM2 DNA (Boehringer Mannheim) with Hae III (Bethesda Research Lab). The size of the PM2 DNA fragments produced when studied by 3.5% or 5.0% polyacrylamide gel electrophoresis were in close agreement with the values reported by Kovacic and Van Holde (15).

RESULTS

Nuclei isolated from UV-irradiated CV-1 cells which had been labeled for 15 minutes with ^3H-Thd immediately after UV irradiation were digested by micrococcal nuclease (Fig. 1). The repair labeled DNA was more susceptible to micrococcal

![Figure 1](image-url)

Figure 1. Micrococcal nuclease digestion for varying lengths of time of nuclei isolated from UV irradiated (13 J/m^2) CV-1 cells which were labeled with ^3H-Thd for 15 minutes immediately after UV irradiation. •—••—• digestion of ^3H repair labeled chromatin O—O—O digestion of ^14C bulk chromatin.
nuclease digestion than the bulk DNA at early times of digestion. When 25% of the bulk DNA had been converted to an acid soluble form 54% of the repair label was acid soluble.

Similar digestion results were also obtained for nuclei isolated from CV-1 cells which were labeled with $^3$H-Thd for 15 minutes at either 2 hours or 4 hours after UV-irradiation (Fig. 2).

As the length of the labeling period was extended from 15 minutes to 4 hours there was a dramatic change in the nuclease sensitivity of the repair label (Fig. 2). After 4 hours of continuous labeling the repair label had the same nuclease sensitivity as the bulk labeled DNA. But it is evident that the repair label in cells labeled for 15

![Figure 2](image_url)

**Figure 2.** Ratios of (Percent $^3$H acid soluble/Percent $^{14}$C acid soluble) at the time of digestion when 20-25% of the $^{14}$C label has been converted to an acid soluble form. 
$\text{O-O-O}$ ratios derived from micrococcal nuclease digestion of UV-irradiated ($13 \text{ J/m}^2$) CV-1 cells which were labeled for 15 minutes with $^3$H-Thd either immediately, 2 hours or 4 hours after UV. $\bullet-\bullet-\bullet$ ratios derived from micrococcal nuclease digestion of UV-irradiated CV-1 cells which were continuously labeled for varying lengths of time. Bars show standard deviation of the mean values.
minutes at 4 hours after UV irradiation had the same two-fold increase in nuclease sensitivity as nuclei labeled immediately after UV (Fig. 2).

In order to investigate if there was a decrease with time in the nuclease sensitivity of DNA labeled for 15 minutes during repair, we performed several pulse chase experiments. Nuclei were isolated from UV irradiated CV-1 cells which had been labeled for 15 minutes, and then chased in non-radioactive medium for varying lengths of time. As the length of the chase period increased the nuclease sensitivity of the repaired DNA approached that of the bulk DNA (Fig. 3). By 4 hours the repaired and bulk DNA had the same nuclease sensitivity (Fig. 3).

The decline in nuclease sensitivity with time is linear on a semilogarithmic plot (Fig. 4) which suggests that this

![Figure 3](image_url)

**Figure 3.** Ratios of (Percent $^3$H acid soluble/Percent $^{14}$C acid soluble) at the time of digestion when 20-25% of the $^{14}$C label has been converted to an acid soluble form. Closed circles are the ratios derived from micrococcal nuclease digested nuclei isolated from UV irradiated CV-1 cells which were pulse labeled for 15 minutes with $^3$H-Md, and then chased for varying lengths of time in cold medium. Bars show standard deviation of the mean values.
Figure 4. Semilog plot of the change in nuclease sensitivity of the repair label with chase time. The percent nuclease sensitivity was calculated using the formula

\[
\text{Ratio time (X)} = \frac{\text{Ratio 15 minute label}}{\text{Ratio 15 minute label}} \times 100 \text{ from the results in figure 3.}
\]

change in nuclease sensitivity is a first order process with an approximate half-life of 15 minutes.

These results (Fig. 3) cannot be accounted for by continued repair synthesis during the chase, because the incorporation of label from endogenous pools was insufficient to make significant changes in nuclease sensitivity. The incorporation of \(^3\)H-Thd as determined from the \(^3\)H/\(^14\)C ratios is non-linear at early times. The \(^3\)H/\(^14\)C ratio after a 30 minute labeling period is 6 times that of a 15 minute labeling period. A 15 minute labeling period followed by a 15 minute chase, however, only results in a 2-fold increase in the \(^3\)H/\(^14\)C ratio, so this situation is equivalent to continuous labeling for much less than 30 minutes. Therefore the change in nuclease sensitivity of the repair label that occurs during the chase must be due to a change in the chromatin conformation and not a consequence of a labeled pool.
It should be pointed out that even with a short labeling time of 15 minutes not all of the repair label is susceptible to micrococcal nuclease digestion (Fig. 1). Even after 30 minutes of digestion, approximately 30% of the radioactivity incorporated by repair is resistant to nuclease digestion. The repair label which was resistant to micrococcal nuclease digestion, was analyzed by polyacrylamide gel electrophoresis. Both the $^3$H and $^{14}$C labeled DNA fragments have similar distributions in the gel (Fig. 5) and the size of the DNA fragments was estimated to be 155, 145, 130, 105, 96, 78, 62 and 50 base pairs. Similar values have been previously reported (16-18).

**DISCUSSION**

Our results on the nuclease sensitivity of UV induced DNA repair synthesis in nuclei isolated from CV-1 cells indicate that at short labeling periods the repair label is over two-fold more susceptible to nuclease digestion than the

**Figure 5.** $^3$H and $^{14}$C radioactive profiles of 5.0% polyacrylamide gels of DNA fragments produced by micrococcal nuclease digestion of repair labeled nuclei. The nuclei were isolated from CV-1 cells, labeled for 15 minutes immediately after UV. Open circles $^3$H-Thd, closed circles $^{14}$C-Thd. The arrows in the figure show the position of migration and the size in base pairs of restriction fragments of φX174 replicative form treated with Hae III. The direction of migration is from left to right.
bulk DNA. With longer labeling periods the nuclease sensitivity of the repair label approaches that of the bulk DNA. This increased nuclease sensitivity immediately after brief labeling periods could indicate either a transient structural conformation of the repair label which makes it more susceptible to nuclease digestion than the bulk DNA, or preferential localization of the label in the linker region of nucleosomes (11,12,13,19,20). We now consider that the former interpretation is more likely to be correct.

The repair label attains the normal nuclease sensitivity of the bulk DNA, with a half-life of about 15 minutes indicating that with time the repair label is assembled into a conformation which has the normal protein-nucleic acid interactions. This decrease in nuclease sensitivity could be due to either a reassembly of repaired DNA to native chromatin conformations which is supported by our analysis of repair labeled DNA fragments, or a rapid sliding of DNA along nucleosomes to randomize the label (19,20). Recent work suggests that nucleosome sliding occurs only at elevated salt conditions, and thus is unlikely to account for the change in nuclease sensitivity of the repair label (21,22).

Our interpretation that UV-induced repair synthesis is not preferentially located in the linker region of the nucleosome is supported by the following observations:

1) Using the shortest labeling period of 15 minutes we always find approximately 30% of the DNA repair label to be resistant to micrococal nuclease digestion. If repair was occurring only in the linker portion of the nucleosome the repair label at short labeling times should be completely nuclease sensitive. 2) The estimated size of the UV repair patch is generally larger than the linker portion of the nucleosome (23). 3) Williams and Friedberg showed that there was no detectable difference in dimer excision between the linker or core particle portions of the nucleosome (13). 4) During DNA replication, label incorporated after short pulses is nuclease sensitive, but with time acquires the same nuclease sensitivity as the bulk chromatin (24-26).

The above results with UV light are in contrast to obser-
vations of repair following exposure to alkylating agents. With methyl methanesulfonate or methyl nitrosourea a preferential distribution of DNA repair synthesis was found in the linker portion of the nucleosome even with a 2 hour labeling period (9,10). This suggests that the influence of chromatin structure on the repair of alkylation and UV damage is different.

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REFERENCES
