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# EXCISION REPAIR IN XERODERMA PIGMENTOSUM GROUP C CELLS IS REGULATED DIFFERENTLY IN TRANSFORMED CELLS AND PRIMARY FIBROBLASTS

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Excision repair in xeroderma pigmentosum group C cells occurs at about 20-30% of normal levels. In confluent fibroblasts a unique characteristic of this low repair is that it is clustered, representing very efficient repair in a small region of the genome. In SV40-transformed fibroblasts and Epstein-Barr virus-transformed lymphocytes of complementation group C, however, excision repair is randomly distributed. This may be a consequence of the high rate of proliferation of both of these cell types, because random repair is also observed in rapidly proliferating group C fibroblasts. The distribution of sites that can be mended in group C cells, therefore, varies according to the transformed and proliferative state of the cells, demonstrating that transformed cells do not always exhibit repair characteristics identical to those of primary fibroblasts. 

Xeroderma pigmentosum (XP) is a human disorder caused by defects in a family of genes, represented by complementation groups A through I, that control repair of ultraviolet (UV) damage to DNA (1-3). In XP complementation group C, repair of UV damage to DNA is reduced to about 20-30% of normal. Several previous studies have indicated that this residual repair is highly clustered in plateau phase group C cells (4-9). To facilitate investigation of the multiple genetic defects in this disease, a series of SV40-transformed fibroblasts and Epstein-Barr virus-transformed lymphocytes have been developed for various complementation groups (10-15). It is important to know how faithfully these transformed lines reflect the repair of UV damage that occurs in untransformed cells of the same group, because other kinds of repair are known to be altered by transformation (16). In previous experiments, I showed that XP group C cells in exponential growth (6) or exposed to the polymerase alpha inhibitor aphidicolin (7) no longer repair UV damage in clusters. In light of these results, the rapid proliferation and absence of a nonproliferative plateau phase in transformed cells might preclude detection of clustered repair, even at high cell densities.

Abbreviations: XP, xeroderma pigmentosum; UV, ultraviolet; dThd, thymidine; BrdUrd, bromodeoxyuridine; FdUrd, fluorodeoxyuridine.

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In this study, I have determined the intragenome distribution of repair in XP group C cell lines transformed by two different methods and find that neither displays the clustered repair characteristic of primary group C fibroblasts.

## Materials and Methods

Normal (FS1), XP group C (XP17ME), and SV40-transformed group C (XP4PA) (15) fibroblasts were grown in Eagle's minimal essential medium with 10% fetal calf serum. An Epstein-Barr virus-transformed lymphoblastoid cell line of complementation group C (GM2249A) was grown in RPMI medium plus 10% fetal calf serum. To obtain uniformly labeled DNA, cultures were incubated for at least 3 days with 0.01 to 0.05 $\mu$Ci/ml [$^{14}$C]thymidine ([$^{14}$C]dThd) (spec. act., 53 mCi/mmol). The medium was changed regularly until normal and XP fibroblast cultures were confluent, and they were then maintained in 1% serum for 2 to 7 days to ensure that they were nonproliferating. XP4PA was grown similarly to confluence, but because of its transformed phenotype, proliferation continued and mitotic figures were always evident. GM2249A was grown to saturation density (2 x $10^6$ cells/ml) and maintained with daily complete changes of medium. At this density, the cell number remained constant for at least 4 days, although cells remained capable of synthesizing DNA (17).

To obtain DNA labeled in repair sites, [$^{14}$C]dThd-labeled cultures were first grown in bromodeoxyuridine (BrdUrd) (10 $\mu$M) and fluorodeoxyuridine (FdUrd) (2 $\mu$M) for 60 min to start synthesis of density-labeled DNA and then irradiated with UV light (6.5, 13, or 26 J/m$^2$; 1.3 J/m$^2$/sec; 254 nm) and incubated with [$^3$H]dThd (10 $\mu$Ci/ml; 80 Ci/mmol), BrdUrd (10 $\mu$M), FdUrd (2 $\mu$M), and hydroxyurea (2 mM) for 6 h. Hydroxyurea was included to suppress $^3$H incorporation by semiconservative replication and thereby improve resolution of repair. At the end of the labeling period, cultures were harvested, washed in phosphate-buffered saline, centrifuged, and drained, and DNA was isolated as previously described (18). The repaired DNA of normal density was separated from hybrid-density DNA labeled by semiconservative DNA synthesis in isopycnic gradients at neutral pH (18). The average single-strand molecular weight (weight average) from isopycnic gradients was 1.50 ± 0.14 (SD) x $10^7$ daltons (6).

Repaired DNA from normal-density regions of neutral isopycnic gradients was dialyzed against a buffer (20 mM Tris, 2 mM EDTA, 40 mM NaCl, pH 8.0) suitable for UV endonuclease treatment. DNA (200 $\mu$l) was incubated with 20 $\mu$l of purified UV endonuclease extract from *Micrococcus luteus* at 37°C for 2 h. At the end of digestion, DNA was centrifuged in alkaline sucrose gradients at 25,000 rev/min in an SW28 rotor for 5 h. DNA radioactivity was then determined and molecular weights were calculated for $^3$H- and $^{14}$C-labeled DNA (18). For DNA isolated from normal and XP cells immediately after a UV dose of 13 J/m$^2$, UV endonuclease treatment produced weight-average molecular weights of 4.68 ± 0.22 x $10^6$ and 4.35 ± 0.16 x $10^6$, respectively (6). These values represent the production of 62.0 ± 7.1 pyrimidine dimers per 2 x $10^8$ daltons by a dose of 13 J/m$^2$ in human fibroblasts. The radioactivity in each fraction of the gradient was displayed as a percentage of radioactivity in the whole gradient to facilitate comparisons between $^3$H and $^{14}$C distributions.

## Results

Repaired DNA, isolated from neutral isopycnic gradients, was digested with UV endonuclease to cleave it at all unrepaired cyclobutane pyrimidine dimers, making it possible to determine the size distribution of unrepaired and repaired DNA. This method, first used by Mansbridge and Hanawalt (4), depends on the principle that if cells are allowed to repair after irradiation, $^3$H label will be inserted into repair patches and the distribution of $^3$H will depend on the distribution of the repaired sites in relation to flanking unrepaired dimers in

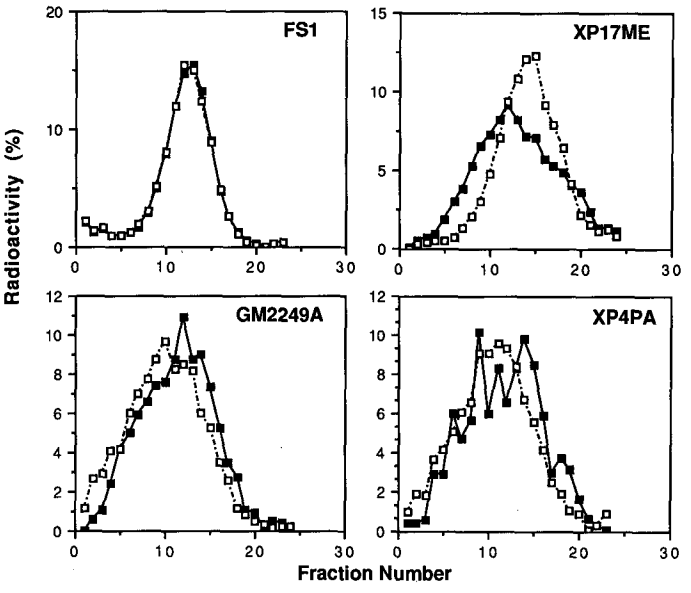


Figure 1. Alkaline sucrose gradient profiles of DNA purified from normal-density DNA in isopycnic gradients and digested with *M. luteus* UV endonuclease. XP group C cells were labeled uniformly with [$^{14}$C]dThd (0.05 $\mu$Ci/ml, 53 mCi/mmol), irradiated with UV light (13 J/m$^2$), and labeled with [$^3$H]dThd (10 $\mu$Ci/ml, 80 Ci/mmol), BrdUrd (10 $\mu$M), FdUrd (2 $\mu$M), and hydroxyurea (2 mM) for 6 h. Top left: FS1, normal primary fibroblasts. Top right: XP17ME, XP group C primary fibroblasts irradiated as confluent monolayers. Bottom left: GM2249A, Epstein-Barr virus-transformed lymphoblastoid cells. Bottom right: XP4PA, SV40-transformed fibroblasts. □, $^{14}$C; ■, $^3$H. Centrifugation was from right to left at 25,000 rev/min for 5 h.

the genome. The clustering of repair patches will yield $^3$H-labeled molecules significantly larger than the majority of the DNA, which will be cleaved into lengths corresponding to the spacing of unrepaired DNA lesions. If, on the other hand, the repair patches and the unrepaired lesions are distributed similarly throughout the DNA, the $^3$H in the repair patches and the $^{14}$C label will give similar size distributions in alkaline sucrose gradients. Because repair of (6-4) photoproducts proceeds much more rapidly than that of cyclobutane dimers (19, 20), the distribution of $^3$H-labeled patches will be biased toward the distribution of repaired (6-4) photoproducts with respect to unrepaired cyclobutane dimers after short labeling times of a few hours.

Repaired DNA from normal cells showed no difference between the distributions of $^3$H-labeled repaired DNA and $^{14}$C-labeled DNA. Repaired DNA from a primary XP group C fibroblast line (XP17ME) showed the profile characteristic of group C (Fig. 1) (4-6). Repaired DNA was distributed with a size distribution larger than that of the $^{14}$C-labeled bulk DNA, indicating that the repair patches were clustered along the DNA fragments. Neither the SV40-transformed group C fibroblast line (XP4PA) nor the lymphoblastoid group C cell line (GM2249A) exhibited such profiles. For these cell types, the $^3$H-labeled repaired DNA was similar to the $^{14}$C-labeled bulk DNA, indicating that repair was not distributed in a clustered manner (Fig. 1). Analysis of a series of experiments on the basis of the $^3$H and $^{14}$C

Table 1. Ratios of $^{3}H$ (repaired DNA) and $^{14}C$ (bulk DNA) weight-average molecular weights in normal and XP group C cells labeled with [$^{3}H$]dThd after irradiation with 6.5 to 26 J/m$^{2}$ UV light*

| Cell line and condition | Dose (J/m$^{2}$) | Time (h) | $^{3}H/^{14}C$ mol. wt. |
|---|---|---|---|
| Normal (FS) | | | 0.97 - 1.03† |
| XP group C (confluent fibroblasts) | | | 1.34 - 1.93† |
| XP group C (exponentially growing) | | | 0.80 - 1.40‡ |
| XP4PA (SV40-transformed) | 13 | 6 | 0.85 |
| | 26 | 6 | 1.04 |
| GM2249A (Epstein-Barr | 6.5 | 6 | 0.79 |
| virus-transformed) | 6.5 | 24 | 0.74 |
| | 26 | 6 | 0.69 |
| | 26 | 24 | 0.84 |

*In previous studies these ratios were found to be independent of the UV doses between 6.5 and 13 J/m$^{2}$ for labeling times between 6 and 42 h. For normal and XP group C fibroblasts, therefore, values for these doses and labeling times were pooled from the previous studies to provide a context with which to compare the results obtained in this study for XP4PA and GM2249A.

†Range of values reported by Karentz and Cleaver (5) and Cleaver (6,7). The value for the XP17ME profile in Fig. 1 was 1.44.

‡Range of values reported by Cleaver (6).

molecular weight ratios confirmed that neither XP4PA nor GM2249A exhibited clustered repair, unlike that seen in numerous investigations of XP group C fibroblasts (Table 1). The $^{3}H/^{14}C$ molecular weight ratios for transformed cells were on average below 1.0, as expected of molecules containing less than one $^{3}H$-labeled patch, on average, whereas the $^{14}C$ label was uniformly distributed (6, 7).

## Discussion

The clustering of repair in confluent XP group C fibroblasts has become a diagnostic characteristic of these cells. Every fibroblast culture representative of this group that has been investigated has shown such nonuniform distribution of repair. These include the original cell lines studied by Mansbridge and Hanawalt (4); four studied by Mullenders *et al.* (8,9); one by Player and Kantor (21); and a total of six lines studied in our own laboratory (5-7). A sufficient number of cell lines have now been described by independent investigators to regard clustering as a consistent feature of repair in confluent untransformed fibroblasts from XP group C. The present study, however, shows that transformed group C cells, even at high density, do not show clustered repair.

The reason for the absence of clustered repair in transformed group C cells depends on the interpretation of the clustered repair itself. Clustered repair may be due either to repair

of specific transcriptionally active genes (22, 23) or to a restricted diffusion of a low number of repair enzyme complexes (6, 7). An increase in the gene-specific mode of repair was not observed in XP group C cells in one investigation (24). Preferential repair of certain genes can, however, occur even in normal cells without causing clustered repair (Fig. 1) (23). The two kinds of nonuniform distributions of repair, therefore, occur at different levels of resolution and do not represent the same process.

Repair in transformed XP group C cells will most closely correspond to that observed in XP group C fibroblasts stimulated to enter the DNA synthesis phase (Table 1) (6); in neither of these cases is clustered repair observed. Clustered repair may be inconsistent with the rapidly proliferating state. I previously suggested that clustered repair may be the result of a regulatory role for DNA polymerases that exercise a dual role in repair and replication (6, 7). Inhibition of DNA polymerase by aphidicolin has previously been shown to eliminate clustered repair in confluent primary fibroblasts, which further identifies the polymerase step of excision repair as a site of regulation of repair in XP group C (7).

These results therefore indicate that transformation of XP group C cells, which alters the regulatory controls that lead to a nonproliferative state at high cell density, leads to a condition in which clustered repair can no longer be observed. Such differences may be important in understanding the precise defect in XP group C cells and must be borne in mind when using transformed cells because they are consequently not equivalent to primary fibroblasts in all aspects of excision repair.

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