Survival of UV-irradiated mammalian cells correlates with efficient DNA repair in an essential gene
(dihydrofolate reductase)

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Communicated by Robert T. Schimke, January 24, 1986

ABSTRACT The survival of UV-irradiated mammalian cells is not necessarily correlated with their overall capacity to carry out DNA repair. Human cells typically remove 80% of the pyrimidine dimers produced by a UV dose of 5 J/m² within 24 hr. In contrast, a Chinese hamster ovary (CHO) cell line survives UV irradiation equally well while removing only 15% of the dimers. Using a newly developed technique to measure dimer frequencies in single-copy specific sequences, we find that the CHO cells remove 70% of the dimers from the essential dihydrofolate reductase (DHFR) gene but only 20% from sequences located 30 kilobases or more upstream from the 5' end of the gene in a 24-hr period. Repair-deficient human cells from xeroderma pigmentosum complementation group C (XPC) are similar to the CHO cells in overall repair levels, but they are extremely sensitive to killing by UV irradiation. In the XPC cells, we find little or no repair in the DHFR gene; in contrast, in normal human fibroblasts and epidermal keratinocytes, >80% of the dimers induced in the gene by 20 J/m² are removed in 24 hr. Since the CHO and normal human cells exhibit similar UV resistance, much higher than that of XPC cells, our findings suggest a correlation between efficient repair of essential genes and resistance to DNA-damaging agents such as UV light.

In attempts to analyze the complex relationships between persisting DNA damage and biological endpoints such as survival and mutation, it has generally been assumed that DNA repair is uniform throughout the genome. Indeed, most methods for assaying DNA repair are insensitive to intragenomic heterogeneity in the distribution of repair sites (1). We have developed an assay for measuring DNA repair of UV-induced pyrimidine dimers in specific nucleotide sequences in the genome and have used it to demonstrate proficient repair of the 50-fold amplified dihydrofolate reductase (DHFR) gene in otherwise excision repair-deficient Chinese hamster ovary (CHO) cells (2). We have now improved the sensitivity of the technique so that pyrimidine dimers can be detected at the level of single-copy genes. This should permit the assessment of damage and repair in a wide variety of genes under different conditions and in various cell types. We present here the first application of this approach in a comparative analysis of repair in the essential DHFR gene in wild-type CHO (K1) cells, in a normal human cell line, in a primary culture of human epidermal keratinocytes, and in cells from a patient with xeroderma pigmentosum.

Cells from complementation group C of the human autosomal recessive cancer-prone disease xeroderma pigmentosum (XPC) are extremely sensitive to UV irradiation and partially defective in excision repair (3). Intragenomic heterogeneity of repair in confluent cultures of XPC cell strains has been reported by Mansbridge and Hanawalt (4), who found that some domains (accounting for a maximum of 20% of the genome) are more proficiently repaired than others. Furthermore, the repairable domains appear to be associated with the nuclear matrix (5). These observations are consistent with our finding of repair heterogeneity in CHO cells, but it was not clear which regions were preferentially repaired in XPC. It was therefore of interest to determine whether the DHFR gene was included in the proficiently repaired genomic domains in these cells.

Although the CHO and XPC cells each repair a similar small fraction (~20%) of the induced dimers in a 24-hr period, the UV resistance (survival) of the CHO cells is much greater than that of the XPC cells. Since the overall repair efficiency in these cases clearly does not correlate with UV resistance, we have considered the possibility that UV resistance might exhibit a better correlation with the proficient repair of essential genes.

In our repair assay, the frequency of pyrimidine dimers is measured as endonuclease-sensitive sites by the use of the enzyme T4 endonuclease V, which specifically cleaves DNA strands at pyrimidine dimer sites (6). By Southern hybridization with specific probes for the gene of interest, the fraction of molecules without dimers (zero class) can be quantitated. By bromodeoxyuridine labeling and CsCl equilibrium density-gradient centrifugation, we separate the parental DNA from any sequences replicated after the irradiation. This is necessary because daughter DNA strands would be expected to be dimer-free and would, therefore, cause an overestimation of the zero class.

We have found that in a case in which resistance to UV light does not correlate with the overall repair of the genome, it does correlate with proficient repair of the essential DHFR gene.

MATERIALS AND METHODS

Cell Culture. CHO-K1 cells (obtained from R. T. Schimke) were grown in F-12 medium ( Gibco) without glycine, hypoxanthine, and thymidine, supplemented with 10% dialyzed fetal bovine serum plus penicillin and streptomycin. The human epidermal keratinocytes were obtained from a meloplasty, plated as described by Liu et al. (7), and grown for 10 days in Dulbecco’s minimal essential medium supplemented with 10% fetal calf serum before irradiation. Normal human fibroblasts (GM38) were originally obtained from the Institute for Medical Research (Camden, NJ), and the XPC (XP4RO) cells were from the American Type Culture Collection. Both were grown in minimal essential medium containing 10% fetal calf serum and antibiotics. The GM38 cells were prelabeled for 3 days with [3H]thymidine at 0.1 μCi/ml (1 Ci = 37 GBq) in the presence of 3 μM unlabeled thymidine. The keratinocytes were prelabeled for 10 days with [14C]thymidine (0.5 μCi/ml). To ensure active transcrip-

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Abbreviations: DHFR, dihydrofolate reductase; kb, kilobase(s).
tion of the DHFR gene, actively growing cells were used. The irradiation source was a calibrated unfiltered germicidal lamp with an incident dose rate of 0.33 J/m² per sec of UV light at 254 nm and providing \( \approx 7 \times 10^{-3} \) pyrimidine dimers per m² per kilobase (kb).

**Repair Assay.** After irradiation, the cells were either lysed immediately or incubated for 24 hr in the presence of 10 \( \mu \)M bromodeoxyuridine and 1 \( \mu \)M fluorodeoxyuridine. They were lysed in 10 mM Tris-HCl/1 mM EDTA/0.5% NaDodSO₄, and incubated for 24 hr at 37°C in proteinase K (0.1 mg/ml) (Sigma). The DNA was extracted with an equal volume of phenol, phenol/chloroform (1:1), and chloroform (the chloroform contained 1/25th isoamyl alcohol). The samples were then precipitated with ethanol, resuspended and treated with RNase A (100 \( \mu \)g/ml) for 1-3 hr at 37°C, precipitated again in ethanol, resuspended in 10 mM Tris-HCl/1 mM EDTA, and the absorbance (260 nm) was read. The genomic DNA was treated with endonuclease Kpn I or HindIII (6 units per \( \mu \)g of DNA for 12 hr at 37°C) and the restrictions were checked on minigels. Samples were centrifuged to equilibrium in CsCl gradients (8) in the presence of 10 mM EDTA (final concentration). The fractions containing parental density DNA (identified by scintillation spectrometry or by slot blot hybridization (2) of the fractionated gradient) were pooled, dialyzed, and concentrated. Two 10-\( \mu \)g fractions of each DNA sample were taken, and then all samples were adjusted to equal volumes in a final concentration of 10 mM Tris-HCl/10 mM EDTA/0.1 M NaCl/T4 endonuclease buffer (10% ethylene glycol/6.85 mM KH₂PO₄/3.15 mM K₂HPO₄/10 mM 2-mercaptoethanol/2 mM EDTA/100 mM KCl). T4 endonuclease V at an equivalent volume of 0.1 \( \mu \)l (incision activity is \( \approx 1 \times 10^{12} \) sites per \( \mu \)g/min at 37°C) per \( \mu \)g of DNA or endonuclease buffer was added, and the samples were incubated at 37°C for 15 min. Concentrated alkaline loading buffer (10 \( \times = 500 \) mM NaOH/10 mM EDTA/25% Ficoll/0.25% bromoresol purple) was added and the samples were electrophoresed on 0.4% agarose gels for \( \approx 20 \) hr at \( \approx 35 \) V in a buffer consisting of 30 mM NaOH/1 mM EDTA. After standard gel washes as described (2), the DNA was transferred to Zetabind nylon support membrane (Microfiltration Products Division). Prehybridizations, hybridizations, and washes were according to the manufacturer’s suggestions with minor modifications: 20 \( \times \) SSPE (3.6 M NaCl/0.2 M Na₂HPO₄/22 mM EDTA, buffered to pH 7.4 with NaOH) was substituted for SSC (0.15 M NaCl/0.015 M Na citrate); 20 mM NaPO₄ and dextran sulfate were omitted. In the CHO cell experiments, a hybridization with 50–100 \( \mu \)g of C₀₁₀₀ DNA per ml (2) prepared from the same cells was included between the prehybridization and the hybridization with a radioactive probe to suppress hybridization to repetitive sequences present in the genomic probes. Hybridization was done in a 2-ml solution containing 50% formamide, 0.5 \( \times \) Denhardt’s solution (1 \( \times \) Denhardt’s solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 1.2% NaDodSO₄, 5 \( \times \) SSPE, and denatured salmon sperm DNA at 200 \( \mu \)g/ml for 2 days at 42°C. Nick-translation \( ^32 \)P-labeled probe (\( \approx 10^8 \) cpm/\( \mu \)g) in the amount of 2 \( \times 10^8 \) cpm was included in the hybridization solution. After prewashes of the support membrane, the final wash was at 0.1 \( \times \) SSPE/0.1% NaDodSO₄. Support membranes were exposed to Kodak XAR-5 X-ray film for 1–10 days, when necessary, under an intensifying screen. The film was scanned with a Helena Laboratories Quick Scan R + D Densitometer.

**Colony-Forming Ability.** Colony-forming ability was assayed for CHO-K1 cells by allowing 200 cells per 10-cm dish to attach for 5 hr. Cells were washed in phosphate-buffered saline, irradiated, and reincubated for 6 days, after which colonies were stained with methylene blue and counted. Plating efficiency for the unirradiated cells was \( \approx 80\% \). The normal human GM38 cells, plated at 600 cells per dish and incubated for 9 days, exhibited a plating efficiency of 58%. UV survival data for the XP4RO cells after 254 nm were obtained from ref. 9.

**RESULTS AND DISCUSSION**

DNA was extracted from cells, treated with an appropriate restriction enzyme, and the parental DNA was isolated as described above. Two equal fractions of each sample, of which only one was treated with T4 endonuclease V, were electrophoresed in parallel in alkaline agarose gels to resolve single-stranded fragments. After transfer of the gel to the support membrane and hybridization with appropriate \( ^32 \)P-labeled probes, the membrane was exposed to x-ray film, and the extent of hybridization was quantitated by densitometry. The intensity of the full-length fragment under study in the T4 endonuclease V-treated sample represents the zero class—i.e., the fraction of fragments free of endonuclease-sensitive sites. Random introduction and removal of endonuclease-sensitive sites in a homogeneous population of fragments is

![Fig. 1](https://example.com/f1.png)
assumed. When the intensity of the band in the T4 endonuclease V-treated sample is compared to that of the untreated sample, the Poisson analysis can then be applied to calculate the dimer frequency per fragment from the zero class. The sensitivity of this determination at biologically relevant doses of irradiation can be enhanced by choosing relatively large fragments of DNA for analysis, as we have done.

The repair analysis of the DHFR gene of CHO cells and in upstream sequences is illustrated in Fig. 1, where the genomic map is also shown. The intragenic 14-kb Kpn I fragment in Fig. 1A comprises roughly the 5' half of the transcribing region of the gene. Dimer removal was determined by comparing the initial dimer frequency (0-hr sample) to that after 24 hr. Qualitatively, the repair can easily be seen by the reappearance of the 14-kb band in Fig. 1A (lane d versus lane b). Densitometry of the developed film showed that the fraction of dimer-free molecules at 0 hr [intensity of the band in lane b (+ T4 endonuclease V) relative to that of the band in lane a] was 0.06. Using a Poisson table, this zero class corresponds to the formation of 2.8 dimers initially in the 14-kb fragment, or to 0.98 dimers per J/m² per 100 kb. At 24 hr (lanes c and d), a similar calculation yields a zero class of 0.43, corresponding to a dimer frequency of 0.84 in the 14-kb fragment. This represents a removal of 70% of the dimers within that period. The initial dimer frequency was somewhat higher than that found under similar conditions in the amplified DHFR gene of the derivative CHO cell line (2). Using the value of 1.8 dimers per 14-kb fragment from that study, the estimated repair efficiency would be 55%. In Fig. 1B, frequencies of endonuclease-sensitive sites in a 22-kb fragment located at least 30 kb to the 5' end of the gene are analyzed. There is no detectable repair in this upstream segment in 24 hr. We also measured endonuclease-sensitive sites in the overall genome using permeabilized CHO cells from this same (K1) cell line and quantitation of DNA repair by size determination on alkaline sucrose gradients (11). In 24 hr, ≈15% of the dimers were removed from the genome overall (data not shown). This is a demonstration of selective DNA repair of an essential gene in a normal mammalian cell line, and it prompts our suggestion that microheterogeneity of DNA repair may be a general phenomenon.

We have also examined repair in the DHFR gene in two normal human cell lines and in cells from a patient with xeroderma pigmentosum. This autosomal recessive disease is characterized by partial deficiency in excision repair following UV damage. In this particular XPC cell strain (XP4RO), only 10–20% of the dimers induced by a dose of 10 J/m² were repaired as determined by unscheduled DNA synthesis or by repair replication (12).

A comparison of repair in the DHFR gene in normal human

![Figure 2](image_url)

Fig. 2. Formation and removal of pyrimidine dimers in the DHFR gene in human cells. The genomic digest was with HindIII to provide a 23-kb fragment within the DHFR gene that hybridizes with the depicted 1.8-kb genomic probe obtained from G. Attardi (California Inst. of Technology, Pasadena, CA). (A) Visualization of dimer removal in GM38, here at passage 22. Lanes a-d and e-h are after UV irradiation at 10 and 20 J/m², respectively. Lanes a, b, e, and f are initial time points (0 hr), and lanes c, d, g, and h are after 24 hr. Arrows indicate a HindIII-digested λ marker. (B) Lanes a-d are from XP cell strain XP4RO irradiated with 10 J/m². Lanes e-h are from primary cultures of human epidermal keratinocytes irradiated at 20 J/m². Lanes a, b, e, and f are initial time points, whereas lanes c, d, g, and h are after 24 hr. Paired lanes are without or with (+) T4 endonuclease V treatment. Lane i is a HindIII-digested λ marker. (C) Map indicating the location of the fragment under study and the probe used (in part from ref. 13). Arrows show HindIII restriction sites.
fibroblasts, primary cultures of epidermal keratinocytes, and XP4RO fibroblasts is shown in Fig. 2. Repair was measured after 10 and 20 J/m² in GM38 cells (Fig. 2A) and after 20 J/m² in the keratinocytes (Fig. 2B, lanes e–h). In both normal human cell types, >80% of the dimers induced in DHFR gene sequences were repaired in 24 hr. This finding was expected since these cells are repair proficient, and therefore all genomic regions should be repaired. In contrast, little or no repair was seen in the DHFR gene of XP4RO cells at 24 hr after UV irradiation at 10 J/m² (Fig. 2B, lanes a–d). Repair was also assayed in XP4RO cultures at a late passage number and in another XPC cell strain, XP1BE (data not shown). In each of the different experiments, the repair of the DHFR gene in XPC cells was low or negligible (typically, 0–20%; in one experiment, 30%), indicating that the efficiently repairable domains in XPC cells evidently do not include the essential DHFR gene.

It remains to be determined which genomic regions in these cells are proficiently repaired and what kind of defect in XPC results in the exclusion of repair in essential domains. The XPC cells may not have the ability to confine their limited repair capacity to essential regions. The lack of appropriately selective repair may explain the large differences in UV sensitivity between the CHO cells and XPC cells shown in Fig. 3. Whereas CHO cells survive as well as normal human cells, the survival of the XPC cells is only 1% of normal at 3 J/m². The “quality” rather than the “quantity” of DNA repair appears to be the important parameter for cellular viability. At least in CHO cells, but most likely also in other types of mammalian cells, certain regions are being preferentially repaired. We are proceeding to study the localization and fine structure of such “repair domains” for DHFR and for other genes as well. It will be important in future studies to learn more about the features of mammalian chromatin structure and gene expression that determine the accessibility of particular genomic regions to repair.

The results from the repair and survival experiments are summarized for the different cell types in Table 1. While the CHO and XPC cells are similarly repair deficient for pyrimidine dimers in the overall genome, the CHO cells repair the DHFR gene, whereas the XPC cells do not. In conclusion, our results suggest that cellular survival may be better correlated with the ability to repair essential regions of the genome than with overall DNA repair levels.

We thank J. Feder and C. A. Smith for helpful discussions, E. Wauthier for preparation of T4 endonuclease V, G. Attardi for human DHFR probes, J. Hamlin and L. Chasin for CHO probes, and Ann Ganesan for critical reading of the manuscript. This work was supported by Grants GM 09901-24 from the National Institute of General Medical Sciences and NP 1611 from the American Cancer Society. V.A.B. acknowledges support from the University of Copenhagen.

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**Table 1. Comparison between survival and DNA repair in the overall genome and in an essential gene**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>% UV survival after 3 J/m²</th>
<th>% repair in 24 hr after UV irradiation</th>
<th>Overall genome</th>
<th>DHFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human</td>
<td>85</td>
<td>85</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>85</td>
<td>15</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>XPC</td>
<td>1</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 3.** Comparative survival of UV-irradiated cells. •, CHO-K1 cells; •, normal human fibroblasts GM38; •, XPC-XP4RO fibroblasts (from ref. 9). Cells were plated and irradiated as described in Materials and Methods.