A SURVEY OF DNA REPAIR INCISION
ACTIVITIES AFTER ULTRAVIOLET
IRRADIATION OF A RANGE OF HUMAN,
HAMSTER, AND HAMSTER–HUMAN
HYBRID CELL LINES

ANDREW COLLINS*, CAROL JONES
AND CHARLES WALDREN

Eleanor Roosevelt Institute for Cancer Research Inc.,
University of Colorado Health Sciences Center, Denver, Colorado 80262, U.S.A.

SUMMARY

We describe a rapid and simple assay for DNA damage and repair in mammalian cells,
based on the partial unwinding of nicked DNA in alkali, and involving transfer of this DNA
to nitrocellulose and the digestion of single-stranded DNA with S1 nuclease, all steps taking
place on tissue-culture chamber slides. Direct breakage of DNA by ionizing radiation has
been examined, and we have developed a standard procedure for measuring enzymic breakage
of DNA as an index of excision-repair capacity following ultraviolet irradiation.

We report a wide range of repair capacities among various hamster and human cell lines,
with considerable overlap between the two species. Hybrids between hamster and human
cells tend to display repair activity characteristic of the hamster parent.

INTRODUCTION

There is evidence that cell lines established from rodents, including the hamster,
have a lower capacity for excision repair of ultraviolet (u.v.) irradiation damage
than human cell lines (Burg, Collins & Johnson, 1977; Collins, 1977; Klimek, 1966;
Painter & Cleaver, 1969; Trosko & Kasschau, 1967). To investigate whether this
difference in repair capacity is the result of regulatory factors, or of qualitative
differences in the enzyme(s) involved, we have studied excision repair in a range
of human cell lines, in variants of the Chinese hamster ovary (CHO K1) cell line,
and in hybrid cell lines established by fusion of Chinese hamster and human
cells.

The rate-limiting step in excision repair appears to be at (or before) the enzymic
incision of DNA at damage sites; only if the later stage of polymerization, or re-
synthesis, is blocked by DNA synthesis inhibitors do the normally transient DNA
breaks due to incision accumulate to a readily detectable level (Johnson & Collins,
1978). Excision of dimers (the step following incision) apparently continues for
several hours in the presence of the inhibitors (Snyder, Carrier & Regan, 1981). The
* Present address for correspondence: Cancer Research Campaign Mammalian Cell DNA
Repair Group, University of Cambridge, Department of Zoology, Downing Street, Cambridge
CB2 3EJ, England.
frequency of breaks introduced during brief incubation of u.v.-irradiated cells with hydroxyurea (HU) and 1-β-D-arabinofuranosylcytosine (araC) is commonly used as an index of the cells' capacity for excision repair (Collins, Downes & Johnson, 1980; Snyder et al. 1981; Squires, Johnson & Collins, 1982).

The first part of this paper is concerned with the development of a rapid assay for DNA breaks, suitable for screening several cell lines simultaneously. The method is a modification of the 'alkaline strand-separation' technique originally described by Ahnström & Edvardsson (1974), and involves lysis of cells in alkali. Intact cellular DNA unwinds only slowly in alkaline solution and neutralization causes almost complete renaturation, but breaks in DNA act as unwinding points and allow irreversible separation of DNA strands. The proportion of DNA rendered single-stranded may be determined by hydroxyapatite chromatography (Ahnström & Edvardsson, 1974) or by digestion with single-strand-specific nuclease S1 (in the modification by Sheridan & Huang, 1977). The method has been of value in estimating DNA breaks induced by ionizing radiation (Ahnström, 1974; Sheridan & Huang, 1977), enzymic DNA breakage following u.v. irradiation (Collins, 1977; Erixon & Ahnström, 1979), and DNA damage and repair following treatment with chemical mutagens (Collins, Ord & Johnson, 1981), but it is time-consuming and unsuitable for large-scale screening. The method described below employs Lab-Tek® chamber slides on which identical samples of cells are grown, prelabelled with [3H]thymidine, irradiated, incubated for repair, and lysed in alkali; the DNA is transferred to nitrocellulose filters and digested with S1 nuclease. The assay method is rapid, convenient and economical, and while it has been devised for studying u.v.-induced DNA repair, it is equally applicable to investigations of damage and repair induced by other agents.

Having optimized the conditions for measurement of excision repair, we conducted a survey of 17 stable hamster–human hybrid cell lines, each of which contained the standard set of CHO chromosomes plus up to 12 specific human chromosomes. All chromosomes except numbers 13 and 23 were represented. We did not find any increased capacity for incision of DNA after u.v. irradiation in hybrids (compared with the hamster parent), which might have indicated donation of repair activity by human chromosome(s). Thus we could not map the human genes responsible for repair. Instead, we were led to a consideration of the variation in repair activity that occurs among cell lines of a single species, of the nature of the difference (if any) between human and hamster repair activities, and of the likely connection between the repair process and other aspects of cellular metabolism. Also we have clarified some of the factors to be taken into account in further studies of repair in hybrids.

MATERIALS AND METHODS

Hamster (CHO) cell lines

Group I: Ade−F and series 822 hybrids. The latter were produced from Ade−F fused with human foetal brain fibroblasts (using u.v.-inactivated Sendai virus). Hybrids were isolated in purine-free medium and assayed for human isozymes as described (Jones, Kao & Taylor, 1980).
Table 1. Characteristics of hamster and hamster–human hybrid cell lines

Hamster cell lines: K1 (pro−), 77256 (pro−, gly−), A563 (pro−, gly−), Ade−F (pro−, pur−), GAT− (pro−, gly−, pur−, dT−), 55-1 (pro−, pur−), 665-18a (pro−, gly−, pur−, asn−)*, 734-31b4 (pro−, gly−, pur−, asn−, HGPRT−)

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Hamster parent</th>
<th>Human parent</th>
<th>Human chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23</td>
</tr>
<tr>
<td>822-17</td>
<td></td>
<td></td>
<td>− − + − − − − − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-19b</td>
<td></td>
<td></td>
<td>+ + − + + − − − − + (+) + + − − − − + + + + + +</td>
</tr>
<tr>
<td>822-33†</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-45c</td>
<td>Ade−F</td>
<td>Foetal brain fibroblasts</td>
<td>+ + − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-49b</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-goat†</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-58</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>822-59b</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>706-B6-11</td>
<td>665-18a</td>
<td></td>
<td>+ − − nd + (+) + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>749-20</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>805-3</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>805-7</td>
<td>734-31b4</td>
<td>Lymphocytes</td>
<td>+ − − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>805-28</td>
<td></td>
<td></td>
<td>− − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>805-32</td>
<td></td>
<td></td>
<td>− − − + + + + − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>640-51</td>
<td>GAT−</td>
<td></td>
<td>− − − − − − − − − − − − − − − − − − − − − − − − − −</td>
</tr>
<tr>
<td>725-18</td>
<td>55-1</td>
<td></td>
<td>− − − − − − − − − − − − − − − − − − − − − − − − − −</td>
</tr>
</tbody>
</table>

Human chromosomes in hybrids were identified by assay for human isozymes (see Jones et al. 1980, 1981; Patterson et al. 1981).

+ , Weak isozyme activity; nd, the assay was not done.
* 665-18a is listed as the parent of 734-31b and of hybrids; it was not tested for repair activity.
† These lines were selected as hybrids but lack intact human chromosomes.
A. Collins, C. Jones and C. Waldren

Group II: 734–31b and hybrids 706, 749 and 805 formed with human lymphocytes (Jones, Patterson & Kao, 1981).

Others: K1, A683, 77236, 55-1, GAT; and hybrids 640 and 725 formed with human lymphocytes (Jones et al. 1980; Patterson, Graw & Jones, 1981).

The nutritional requirements of these cell lines, and derivation and chromosome content of hybrids, are given in Table 1.

Cells were cultured, and experiments performed, in growth medium F12 (Ham, 1965) with foetal calf serum at 8% for Group I, 5% for Group II. The other cells were grown on different occasions with 8% or 5% foetal calf serum, or with 3% foetal calf and 3% calf serum; the different serum conditions appeared to have no effect on repair activities. The medium for Group II (with the exception of 706) contained adenine at $3 \times 10^{-5}$ M.

Human cell lines

HT1080 (a near diploid line derived from a fibrosarcoma) and a mutant of HT1080 deficient in hypoxanthine-guanine phosphoribosyltransferase (HT1080 HGPRT-) were given by Dr C. Croce (Wistar Institute) to Dr D. Patterson at the Eleanor Roosevelt Institute. These, and HeLa cells, were cultured in F12 medium with 5% foetal calf serum or with 3% foetal calf and 3% calf serum (with no apparent effect on repair activity). Simian virus 40 (SV40)-transformed WI38 (normal human fibroblasts) and SV40-transformed fibroblasts from a patient with xeroderma pigmentosum (XP) (complementation group A) were obtained from Dr S. Zimmer (University of Kentucky); normal human fibroblasts, Flow 2000, from Flow Laboratories; and fibroblasts from Down’s syndrome, 137, 230, 2504 and 2767, from Dr C. Scoggin at the Eleanor Roosevelt Institute: all these were grown in medium with 6% foetal calf and 6% human cord serum, or with 10% foetal calf serum.

All cell lines (hamster and human) were cultured in plastic dishes. The medium used satisfied the nutritional requirements of the various auxotrophs; i.e. experiments were not intended to examine repair under ‘starvation’ conditions.

Prelabelling of cells for incision assay

To ensure that cells at the time of assay were in equivalent logarithmic growth states, they were harvested by trypsinization from subconfluent cultures, and equal numbers (1.5–2 x 10^6 hamster cells, or 2–2.5 x 10^6 human cells) were inoculated in 0.3 ml of growth medium into each of the 8 chambers of tissue-culture chamber slides (Lab-Tek® Division, Miles Laboratories, Inc.). They were incubated for 1 day (or 2 days in the case of slower growing human lines) with [methyll-³H]thymidine (New England Nuclear, 6.7 Ci/mmol) at 0.2 µCi/ml, and then for 3 h in non-radioactive medium before irradiation.

Irradiation

Normally, 30 min before irradiation with u.v., hydroxyurea ($10^{-4}$ M) and araC ($10^{-6}$ M) (both from Sigma Chemical Co.) were added to the cells in all chambers. Cells were irradiated after removing the medium and the plastic chambers from the slide. A frame was constructed to fit exactly over the 8 cell culture squares on the slide, with two squares each covered by aluminium foil, two squares each covered by different thicknesses of Saranwrap® brand plastic film (Dow Chemical), and the last two squares open. Thus when exposed to u.v. light (from a germicidal lamp emitting at a rate of 2 J m⁻² s⁻¹, attenuated to 0.2 J m⁻² s⁻¹ for lower u.v. doses), duplicate samples of cells received one of three graded u.v. doses or no irradiation. Two drops of medium (with HU and araC) were replaced over each square, and the slide was incubated for 20 min (or for other times as indicated). Sometimes a single u.v. dose was given to all squares by omitting the Saranwrap® screens. X-irradiation was performed on cells in phosphate-buffered saline at 0°C, at a dose rate of 90 rad/min (230 kV, 15 mA). For γ-irradiation, a Gammacell 60 ⁶⁰Co source was used, at a dose rate of 1.8 krad/min.
Alkaline lysis, transfer of DNA to nitrocellulose, and digestion with S1 nuclease

Slides were rinsed with 0.9% sodium chloride solution, excess solution removed with a paper tissue, and 50 µl of alkaline sucrose solution (5%, w/v, sucrose, 0.3 M-NaOH, 0.5 M-NaCl) gently pipetted onto each square. After 15 min at 4 °C, the alkaline cell lysate was neutralized and brought to pH 4.5 with 15 µl of 2 M-acetic acid, and a 10 mm diameter disk, cut with a cork-borer from BA85 nitrocellulose membrane filter (Schleicher and Schuell, Inc.), placed over each square. Above this was put a disk of the same size cut from GF/B glass-fibre paper (Whatman, Inc.) to absorb the lysis solution. The plastic chamber unit previously removed from the slide was inverted and replaced over the disks on the slide, and a 25 g weight placed on it, to apply even pressure during transfer of DNA from glass slide to nitrocellulose. After 1 h at room temperature, the weighted chamber unit was removed, the glass fibre disks removed and dried, and to each nitrocellulose disk was added, in situ, 50 µl of deoxyribonuclease S1 (Calbiochem-Behring Corp.) diluted to 0.24 I.U./ml in sodium acetate buffer (0.03 M, pH 4.5) with zinc sulphate at 3 x 10^-6 M. Each disk was thoroughly wetted in the solution, and the slides were incubated for 45 min at 45 °C (conditions found in control experiments to result in complete solubilization of single-stranded DNA bound to nitrocellulose). The glass-fibre disks were then replaced to absorb the supernatant from the digestion, removed and dried. The nitrocellulose disks were covered with 5% trichloroacetic acid, and after 20 min at 4 °C they were washed in 5% trichloroacetic acid, distilled water, and dried. ³H in DNA was measured by scintillation counting of disks in toluene with Permafluor I (Packard Instrument Company, Inc.). Scintillation counting was repeated with the appropriate dried glass-fibre disk present with each nitrocellulose disk; this allowed estimation of the total recovery of c.p.m.

RESULTS

Development of the standard procedure

Distribution of cells in chambers. The procedure depends on the presence of equal numbers of cells, evenly distributed, in each chamber on the slide. Adding cell suspension in 50 µl to 0.25 ml of medium already in the chamber gave a more even distribution of cells (as judged by microscopic examination after the cells had attached) than did simple pipetting of the cells in the final volume into the chamber.

Transfer of DNA to nitrocellulose. To examine the rate and extent of transfer of DNA to nitrocellulose in this system, hamster cells were prelabelled with [³H]-thymidine, incubated with HU and araC, irradiated with u.v. (10 J m^-2) and lysed in alkaline sucrose as described in Materials and Methods. After neutralization of the alkaline lysates, nitrocellulose and glass-fibre disks were placed on each square and left under pressure (see Materials and Methods) for varying times; the radioactivity present on the nitrocellulose disk, on the glass-fibre disk, and remaining on the glass, were measured. After 10 min, 74% of total recovered radioactivity was on the nitrocellulose, and 1 h later this percentage had risen to 85%. Of the radioactivity not adsorbed to nitrocellulose, two-thirds was in the glass-fibre disk and one-third on the glass. In similar experiments with unirradiated hamster cells about 90% of total radioactivity was adsorbed to nitrocellulose by 1 h.

It was found that when the number of cells inoculated per chamber was reduced to 10⁶, a smaller proportion of DNA bound to nitrocellulose, although the fraction of this DNA subsequently digested by S1 nuclease was the same as at higher cell concentrations.
Period of alkaline lysis. Breaks in the DNA molecule permit unwinding of the two strands in alkali. As well as depending on the number of DNA breaks, unwinding is a function of the period of alkaline lysis. Fig. 1 shows the effect of varying this parameter. Hamster cells on two chamber slides, prelabelled with [3H]thymidine, were incubated with HU and araC for 30 min. Cells on one slide were irradiated with u.v. (10 J m⁻²) and incubation continued for 20 min to introduce enzymic breaks at DNA damage sites. The other slide was an unirradiated control. Alkaline sucrose added to the squares of cells was neutralized at different times, and the DNA was transferred to nitrocellulose and digested with S₁ nuclease. The radioactivity recovered on the nitrocellulose filters (representing undigested DNA) is shown in Fig. 1 as a percentage of that obtained from unirradiated cells lysed for the minimum time. There is no change in digestibility of unirradiated DNA over 15 min of lysis, but some indication of unwinding thereafter. DNA from irradiated cells shows a steady increase in digestibility with increase in lysis time, indicating a time-dependent separation of DNA strands in alkali.

The glass-fibre disks used to absorb the lysis solution were dried and used again to absorb the supernatant from the S₁ nuclease digestion. After drying, the radioactivity present in these disks, as well as that present on the nitrocellulose filters, was...
DNA repair in human and hamster cells

measured, so that the c.p.m. retained on nitrocellulose could be expressed as a percentage of total recovered c.p.m. (neglecting radioactivity retained on the glass slide). In the unirradiated sample, after 15 min lysis, the c.p.m. on nitrocellulose represented 84% of recovered c.p.m., compared with 39% in the corresponding irradiated sample. Not all radioactivity is recovered by this procedure; the total recovered c.p.m. per sample diminished as the c.p.m. retained on nitrocellulose became less, i.e. with increased lysis time and especially following irradiation. However, routine counting of the total c.p.m. as well as the c.p.m. on nitrocellulose, and calculation of the extent of binding of DNA from unirradiated samples, serve as useful checks on the performance of the assay.

For the standard assay procedure, the period of 15 min was selected for alkaline lysis. Results are generally expressed as the percentage loss of nitrocellulose-bound c.p.m. in the irradiated sample compared with the unirradiated sample; thus a higher percentage loss of c.p.m. indicates a greater extent of unwinding during lysis and digestion of unwound DNA by S1 nuclease, reflecting a greater frequency of DNA breaks.

Unwinding and digestion of DNA as a function of frequency of breaks. Hamster cells cultured on chamber slides and labelled with [3H]thymidine were X- or y-irradiated and immediately lysed in alkaline sucrose, the DNA transferred to nitrocellulose and digested with S1 nuclease. Fig. 2 shows that breaks introduced in DNA by ionizing radiation serve as unwinding points and increase the digestibility of DNA in a dose-dependent way. The frequency of DNA breaks induced by X-rays or y-rays is between 1.7 and 3.1 breaks/krad per 109 daltons (Erixon & Ahnström, 1979; Kohn, Erickson, Ewig & Friedman, 1976; Lydersen & Pettijohn, 1977; Ormerod & Stevens, 1971), and the abscissa in Fig. 2 is accordingly expressed in both krad and DNA breaks per 109 daltons, using the equivalence of 2.5 breaks/krad per 109 daltons. Fig. 2 serves as a calibration curve in estimating the frequency of DNA breaks from the percentage loss of nitrocellulose-bound c.p.m.

Incubation of cells after X-irradiation results in a reduction in the number of DNA breaks and hence in the extent of unwinding of DNA in alkali and digestion by S1 nuclease. For example, cells irradiated with 900 or 1350 rad were incubated for 1 h at 37 °C; the amount of DNA retained on nitrocellulose after alkaline lysis and S1 digestion was indistinguishable from that retained from unirradiated cells, i.e. virtually all detectable DNA breaks had been rejoined during the incubation.

Detecting enzymic incision at u.v. damage sites

Irradiation of hamster or human cells with low doses of u.v., followed by incubation with HU and araC, leads to the accumulation of repair-related DNA breaks to levels readily detected by this assay (Fig. 3). The number of breaks in DNA (indicated by the percentage loss of nitrocellulose-bound DNA) shows an increase with time of incubation, up to 20 min or longer, in these cells and in various other cells tested (results not shown). Twenty minutes was therefore chosen as the standard period of incubation for subsequent screening assays.

The frequency of breaks detected is increased (at least in the case of HeLa cells)
Fig. 2. Detection of DNA breakage induced by ionizing radiation. Prelabelled hamster cells (CHO-K1; ×, △) or human cells (HeLa; ○) were X-irradiated (×) or γ-irradiated (○, △) and immediately lysed in alkali for 15 min. After S1 nuclease digestion the acid-insoluble c.p.m. remaining on nitrocellulose were measured; results are expressed as loss of c.p.m. relative to the unirradiated sample. Bars indicate standard error of mean; data from γ-irradiated hamster and human cells were combined at each dose (○). The scale of the abscissa shows estimated DNA break frequency as well as radiation dose.

if cells are preincubated with HU and araC (Fig. 3). This ensures that repair DNA synthesis is maximally inhibited, so that repair sites remain open. Experiments were carried out over a range of u.v. doses to determine the optimum period of preincubation (Fig. 4). The cells studied were two related human lines and a hamster line. In each case incubation for either 30 min or 60 min with HU and araC before u.v. irradiation (and for 20 min after irradiation) produced essentially the same frequency of DNA breaks at a given u.v. dose. Preincubation of hamster cells for up to 4 h does not increase the frequency of detectable DNA breaks (data not shown). Fig. 4 also shows the lower level of DNA breakage seen, in all cases, without preincubation.
On the basis of these and other results (Collins & Johnson, 1981) we conclude that 30 min preincubation with HU and araC is optimal for the accumulation of repair-related DNA breaks after u.v. irradiation.

Fig. 4 is in the form of a u.v. dose-response curve. The percentage digestion of DNA at a given dose reflects the frequency of DNA breaks and therefore the activity of repair endonuclease during post-irradiation incubation with inhibitors. Differences among cell lines can be readily detected by this assay. Thus, from Fig. 4, after u.v. irradiation with 1.2 J m⁻² the hamster line 77256 gives rise to about 30% single-stranded DNA, corresponding to 0.8 break per 10⁸ daltons (see Fig. 2), while HT1080 cells at the same dose are capable of higher levels of breakage (over 3 breaks per 10⁸ daltons). At 4.8 J m⁻², the difference is more pronounced. It is also evident that the HGPRT⁻ derivative of HT1080 accumulates fewer breaks. We therefore embarked on the comparative study of numerous hamster and human and hamster–human hybrid cell lines, regarding DNA break frequency as an index of excision-repair capacity.

We first examined DNA break accumulation after u.v. in a group of hybrids
A. Collins, C. Jones and C. Waldren

derived from the hamster line Ade-F (Fig. 5). (In Figs. 5–8 the ordinate is given in terms of DNA breaks per 10⁹ daltons as well as the percentage loss of nitrocellulose-bound DNA, using for calibration the data of Fig. 2.) Although these hybrids contain a wide selection of human chromosomes, in no case is there an elevation of DNA break frequency towards levels characteristic of the human cells previously tested.

![Figure 4](image)

**Fig. 4.** Enhanced accumulation of DNA breaks by incubation with HU and araC before u.v. irradiation. Prelabelled hamster cells, CHO-77256 (A), or human cells, HT1080 (a) and HT1080 HGPRT- (c), were incubated with HU and araC for 0 (○), 30 (△) or 60 (■) min before irradiation with a range of u.v. doses. The cells were then incubated for 20 min with HU and araC, lysed in alkali, digested with S1 nuclease, and the acid-insoluble c.p.m. remaining on nitrocellulose were measured.

Another set of hybrids is depicted in Fig. 6; the human chromosomes of these were contributed by lymphocytes. Mutant 734-31b₂ (the hamster parent of hybrids 749 and 805) is derived from 665-18a, the parent of the hybrids 706; 706-B6-1 appears to have markedly lower repair capacity than 706-B8-1. The other hybrids (and their parental cell 734-31b₂) all show relatively high repair activities. Once again, we cannot detect any donated human repair capacity.

In another experiment, the hybrid cell line 640-51 was compared for repair activity with its parent GAT⁻; no difference was seen (data not shown).

We concluded from these results: (1) that certain hamster lines (such as 734-31b₂) may have such a high level of repair activity that (in this assay at least) elevation of that activity in hybrids, even if it occurs, will be impossible to detect; (2) that, in hybrids from a hamster line of moderate activity (Ade⁻F), the introduction of various human chromosomes has no significant effect on repair activity.
Before attempting to interpret these results further, we investigated the variation in repair activity between different (non-hybrid) hamster lines. CHO-K1 and Ade^−F (and also GAT^− - not shown) give similar DNA break frequencies (Fig. 7A). Another cell line derived from CHO-K1, A563, appears to be slightly less active in repair, and 55-1 is markedly more active (Fig. 7B). 55-1 is a purine auxotroph; a hybrid between 55-1 and human lymphocytes, 725-18, which is able to synthesize purines, has very similar repair capacity to 55-1.

There is a wide range of repair activities among human cell lines, too (Fig. 8). Several (HeLa, HT1080 and Flow 2000) produce such a high level of susceptibility to S1 digestion that they reach the limit of resolution of the assay; above 80% digestion we can say only that the DNA break frequency exceeds 8 per 10^9 daltons. At the other extreme, the SV40-transformed fibroblasts from a patient with xeroderma pigmentosum (XP(A)), a disease thought to be associated with very low u.v. endonuclease activity; Erixon & Ahnström, 1979; Fornace, Kohn & Kann, 1976) show the expected low activity in our assay when compared with an SV-40-transformed line of WI38 normal human cells. The HGPRT− mutant of HT1080 is, as mentioned above, less active in repair. Fibroblasts from patients with Down's syndrome produce levels of DNA breakage generally slightly lower than the levels from normal cells.
DISCUSSION

The breaks introduced enzymically into DNA in u.v.-irradiated mammalian cells may be measured by several methods. Alkaline sucrose-gradient sedimentation is appropriate for detecting high levels of DNA breakage (Dunn & Regan, 1979), and sensitivity is increased by employing very brief cell lysis periods before sedimentation to ensure that DNA sediments in a high molecular weight form (Cleaver, 1974; Collins, Schor & Johnson, 1977). The DNA alkaline elution technique (Fornace et al. 1976) provides accurate estimation of low levels of DNA breakage. However, the alkaline strand-separation technique (Ahnström & Edvardsson, 1974) combines the advantages of sensitivity and simplicity, and using it we have made measurements of break frequencies over the range 0.2 to 20 breaks per $10^8$ daltons (Squires et al. 1982). The procedure described in this paper minimizes the manipulations involved in cell culture, irradiation, repair-incubation, alkaline lysis, and determination of single-stranded/double-stranded DNA content, since all these steps take place in situ.
Fig. 7. u.v.-dose-dependent accumulation of DNA breaks in various CHO cell lines. A, K1 (□), Ade- F (△), B, A563 (○), 55-1 (▽), 725-18 (▽) (hybrid). Experimental details as for Fig. 5. Bars indicate standard error of mean.

in the compartments of tissue-culture chamber slides. Using slides inoculated the previous day it is possible to obtain, within a few hours, information on the excision-repair capacities of several cell types over a range of u.v. doses; typically 48 data points are collected in such an experiment.

An essential feature of the technique is the transfer of DNA, after alkaline lysis and neutralization, to nitrocellulose filters. The binding of DNA to nitrocellulose apparently requires that the DNA be at least partially single-stranded (Probst,
Fig. 8. u.v.-dose-dependent accumulation of DNA breaks in various human cell lines. A, HeLa (○), SV40-W138 (△), SV40-XP(A) (□). B, HT1080 (○), HT1080 HGPRT- (△). C, Flow 2000 (x), Down's syndrome cells 137 (○), 239 (△), 2504 (▽), 2767 (□). Bars indicate standard error of mean. Experimental details as for Fig. 5.
DNA repair in human and hamster cells

We have previously used nitrocellulose binding to detect regions of single-strandedness that appear in DNA during excision repair in the presence of araC (without alkaline lysis), and concluded (Johnson & Collins, 1978) that a small percentage of single-stranded DNA in an otherwise duplex molecule is sufficient to cause attachment to nitrocellulose. We now find that virtually all the DNA from unirradiated but alkali-lysed cells binds to the nitrocellulose disks. When intact DNA molecules in alkali are neutralized, the rapid renaturation to double-stranded form may involve the creation of intermittent mis-matched regions. While these are insignificant as far as susceptibility to S1 nuclease is concerned, they apparently enable the DNA to bind strongly to nitrocellulose.

Both the alkaline lysis-hydroxyapatite chromatography technique and the alkaline lysis-S1 nuclease digestion procedure discussed here depend on the unwinding of DNA in alkali as a function of the frequency of breaks. For a given break frequency, the percentage of single-stranded DNA as measured by hydroxyapatite chromatography is lower than the percentage as measured by S1 nuclease digestion (compare Fig. 2 with fig. 2 of Collins & Johnson, 1979). This may reflect the omission of EDTA from the lysis solution used here, or a real difference in the resolution achieved by the two approaches. Using either method, doses of X-rays above about 3 krad are detected with poor accuracy, since the single-strandedness of DNA approaches 100%. To resolve break frequencies above 8 per 10⁸ daltons a shorter period of alkaline lysis or a solution of lower pH or lower salt concentration should be used to produce less unwinding of DNA.

Using Fig. 2 to convert values of percentage digestion of DNA into DNA break frequencies, we can estimate rates of enzymic incision at damage sites. For example, from Fig. 3, HeLa cells irradiated with 1 J m⁻² (which induces 13 cyclobutane pyrimidine dimers per 10⁸ daltons of DNA; Squires et al. 1982) make nicks, presumably at dimer sites, at a rate of 1-2 per 10⁸ daltons in the first 10 min of incubation, and 3-8 per 10⁸ daltons over a 20 min period. The hamster cells shown in the same figure make nicks at a lower rate (0.45 per 10⁸ daltons in 20 min) and the rate appears to fall off after 30 min. It may be that, under normal conditions (i.e. in the absence of HU and araC), enzymic incision in these cells occurs at maximum rate immediately after irradiation and then slows. But repair is likely to be a tightly controlled enzymic activity, and the accumulation of an intermediate (i.e. DNA that is nicked and excised but not resynthesized or ligated) in the presence of inhibitors may have a feedback effect, tending to block further incision.

The enhanced accumulation of DNA breaks when cells are incubated with inhibitors before as well as after u.v. irradiation (Figs. 3, 4) emphasizes the dependence of repair on the supply of DNA precursors. HU inhibits ribonucleotide reductase, which produces deoxyribonucleotides (Reichard, 1972), while araC (in the form of araCTP) competes with dCTP for the DNA polymerase site (Graham & Whitmore, 1970). Preincubation with HU depletes the deoxyribonucleotide pool, potentiating the competitive inhibition by araC and more completely disrupting repair DNA synthesis (Collins et al. 1980).

Figs. 4-8 are in the form of u.v. dose-response curves, and indicate a range of
repair activities among hamster cell lines that overlaps the range of repair activities in various human cell lines. Thus the supposition that hamster cells in general are substantially less efficient than human cells in excision repair of u.v. damage is challenged, and with it the underlying reason for using hamster–human hybrids in an attempt to identify the human chromosomes coding for repair. Our results suggest that the genetic information specifying the enzymes involved in the incision stage of repair may be identical in hamster and human cells, and that differences between cell lines may reflect regulatory effects, acting at the level of enzyme synthesis or activity. We have already pointed out the importance of the DNA precursor pool in allowing cells to complete the synthetic stage of repair (Collins & Johnson, 1979). It is likely that the overall rate of repair (set by the rate-limiting step of incision) is regulated in conjunction with purine and pyrimidine metabolism (i.e. the pathways leading to production of nucleic acid precursors). However, as yet there are no direct measurements of DNA precursor pools and their fluctuations in repairing cells, and the mode of regulation of repair remains a matter for speculation. The HGPRT− HT1080 line shows lower repair than normal; 55-1, a purine-requiring hamster line, shows elevated repair capacity compared with CHO-K1, but Ade−F, defective at another stage in purine metabolism, does not differ from K1 in its repair capacity; and a hybrid from 55-1, which does not require purines, retains the elevated level of repair. Further study of repair in these and other cell lines should give information on the integration of repair within the general metabolism of the cell.

The fact that none of the hamster–human hybrids tested (even those derived from a hamster parent showing low incision capacity) was able to introduce significantly more repair-related breaks into DNA than the parent cell may indicate simply that human chromosome 13 or 23 or a combination of human chromosomes not found in these hybrids, is required to code for human repair activity; or that the human chromosome(s), though present, was imperfect in the repair locus. However, in view of our conclusions on the regulation of repair, it seems very likely that human repair genes, even if introduced into hybrid cells, would be subject to whatever regulatory conditions prevail in the recipient cell; we should therefore not be surprised at a failure to detect elevated repair activity. For a successful identification of the human chromosome(s) bearing genes for repair, hybrids should be made using a hamster parent with demonstrably deficient incision capacity, ideally a mutant analogous to the human XP(A). Alternatively, cells of another species may turn out to have genetically distinct repair activity on which a donated human repair activity can be superimposed.

The results obtained with human cells are in general agreement with the survey carried out by Squires et al. (1982), using the alkaline lysis–hydroxyapatite technique. In both studies, SV40-transformed lines show low capacity for incision after u.v. irradiation. In the latter study, normal human cells (including Flow 2000) reach a higher level of DNA breakage than tumour cells (e.g. HeLa or HT1080); such a difference is beyond the resolving power of the rapid assay with the alkaline lysis solution described here.
The measurements of cellular-repair capacity by the accumulation of DNA breaks after u.v. irradiation and incubation with HU and araC, has certain advantages over conventional methods that depend on repair-related [3H]thymidine incorporation. First, it avoids the problem of distinguishing repair DNA synthesis from the generally far greater replicative DNA synthesis; and second, it is not dependent on the specific activity of [3H]thymidine in the cellular pool (which is much influenced by the physiological state of the cell). The variation in effectiveness of araC that might arise from differences in the dCTP pool is countered by preincubation of cells with HU as well as araC. The rapid assay method described here lends itself to the screening of numerous cell lines, for example putative DNA repair mutants; it might also be put to diagnostic use in detecting repair deficiencies in human fibroblasts.

This investigation is Contribution 348 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 Center, Denver, Colorado. We acknowledge the skilled assistance of Mr R. Mochizuki, and valuable discussions with Drs J. Davidson, R. Johnson and D. Patterson. Supported by USPHS grants from the NIEHS (ES-01555), the NICHD (HD-02080) and the NCI (CA-20810); the Ellis L. Phillips Foundation, Jerico, New York; and the Elsa U. Pardee Foundation (Midland, Michigan). A.C. was the holder of an American Cancer Society (Eleanor Roosevelt) International Cancer Fellowship awarded by the International Union Against Cancer, while on leave from the Cancer Research Campaign Mammalian Cell DNA Repair Group.

REFERENCES


(Received 15 January 1982)