XERODERMA PIGMENTOSUM D–HeLa HYBRIDS WITH LOW AND HIGH ULTRAVIOLET SENSITIVITY ASSOCIATED WITH NORMAL AND DIMINISHED DNA REPAIR ABILITY, RESPECTIVELY

R. T. JOHNSON, SHOSHANA SQUIRES, G. C. ELLIOTT
Cancer Research Campaign Mammalian Cell DNA Repair Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EF, U.K.

G. L. E. KOCH
Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, U.K.

AND A. J. RAINBOW
Departments of Radiology and Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada

SUMMARY
Fusion between HeLa and fibroblasts from complementation group D xeroderma pigmentosum (XPd) followed by challenge with small doses of ultraviolet light (u.v.) results in the production of hybrid cells expressing either HeLa (HD1) or XPd-like (HD2) sensitivity to u.v. and related repair capacity. Assays used include unscheduled DNA synthesis (UDS), DNA break accumulation in the presence of inhibitors of DNA repair synthesis and host cell reactivation of irradiated adenovirus. Complementation assay in heterokaryons reveals limited ability of HD2 to restore UDS in XPd nuclei. We believe this complementation is more apparent than real since proliferating hybrids of HD2 and XPd parentage are without exception u.v.-sensitive and express limited excision repair. On the other hand hybrids between HD2 and XPc, XPe or XPF fibroblasts show true complementation resulting in a return to normal u.v. sensitivity and elevated repair ability.

INTRODUCTION
Defects in DNA repair pathways are important in relation to mutagenesis, normal development and malignant conversion (Maher, Ouellette, Curren & McCormick, 1976; Arlett & Lehmann, 1978; Maher et al. 1982), and yet of the several clearly defined human conditions whose cells display abnormal sensitivity to killing by DNA-damaging agents little is known of the molecular basis underlying the sensitivity and, excepting xeroderma pigmentosum (XP), there is no consensus about the expression of defective DNA repair in ataxia telangiectasia (AT), Cockayne's Syndrome (CS) and Fanconi's anaemia (FA) (Lehmann, 1982a, recently reviewed this area). Each of these four genetic syndromes represent genetically complex situations; for each condition complementation studies with heterokaryons (or, for FA, with hybrids) have indicated the involvement of two or more genes (Giannelli, Pawsey & Avery, 1982; Murnane & Painter, 1982; Lehmann, 1982b; Zakrzewski & Sperling, 1980).

Key words: excision repair, xeroderma pigmentation, somatic cell hybrids.
Perhaps the recessive nature of known repair defects has also dissuaded attempts to increase the breadth of human repair-defective phenotypes by mutation and selection of established cells. Most of the few human cell cultures that are known to be sensitive to DNA-damaging agents have been derived from HeLa (e.g. see Baker, Van Voorhis & Spencer, 1979). The earliest strains, selected on the basis of slow growth after exposure to methylmethanesulphonate, proved to be sensitive both to alkylating agents (Wilkinson & Nias, 1971) and to ultraviolet light (Wilkinson, Kiefer & Nias, 1970) though an associated DNA repair defect was not established at that time. Later, HeLa cells selected for their sensitivity to u.v. light by the bromodeoxyuridine-visible light procedure proved to have greatly diminished ability to remove dimers (Isomura, Nikaido, Horikawa & Sugahara, 1973). Another u.v.-sensitive HeLa variant described by Chalmers et al. (1976) not only showed reduced dimer removal but also accumulated single-strand DNA breaks. Unfortunately the origin of this interesting cell was not described.

Few repair-defective human mutants have been described that did not arise from HeLa cells. Several of these were derived from embryonic fibroblasts doubly transformed by Rous sarcoma virus and simian virus 40 (SV 40) (Suzuki & Fuse, 1981), and at least one, RSr, has given rise by mutation and selection to resistant clones with elevated repair ability (Suzuki & Kuwata, 1979). Since viral transformation is associated with modified repair capacity (Day et al. 1980; Squires, Johnson & Collins, 1982) and can broaden the spectrum of cell sensitivity (Heddle & Arlett, 1980; Simon, Hazard, Maher & McCormick, 1981) these cell lines may prove difficult to decipher.

There have been few studies of repair involving permanent cell hybrids except to demonstrate the relief of u.v. sensitivity in XP by fusion with rodent cells (Goldstein & Lin, 1972; Lin & Ruddle, 1981), or mitomycin sensitivity in FA by fusion with normal or complementing cells (Zakrzewski & Sperling, 1980). The aim of the work described in this paper was to assemble intraspecific hybrids between X-irradiated HeLa cells and fibroblasts from XP complementation group D and subsequently to promote karyotype instability by multiple small doses of u.v. light. In this way we hoped to obtain immortal cell lines via the appropriate HeLa genes, but which had lost the HeLa chromosome carrying the wild-type D allele and thus expressed the XPD phenotype. Of the first two hybrids analysed one expressed normal u.v. sensitivity and excision repair, and the other considerable u.v. sensitivity and XPD-like repair.

MATERIALS AND METHODS

Cells and media

HeLa cells adapted for suspension culture were obtained originally from Dr T. T. Puck. We thank Drs F. Giannelli (XP102LO), C. Arlett (XP4LO, XP1BR, XP4BR, XP23OS, XP2RO), D. Bootsma (XP11BE), M. Taylor (XP2BI), A. Sarasin (XPH, XP-SC-8) (Moshell et al. 1983), and A. Lehmann (XP2LE) for their kindness in providing xeroderma pigmentosum cell cultures. Human embryonic lung fibroblasts (HEL) were purchased from Gibco Ltd. All cells were grown in Eagle's minimal essential medium (Gibco Biocult) supplemented with glutamine, sodium pyruvate, non-essential amino acids, penicillin and streptomycin, and foetal bovine serum (5% for HeLa; 15% for XP; 10% for hybrids).
Hybrid production and selection

A total of $10^6$ suspension HeLa cells previously X-irradiated with 500 rad to reduce initial proliferation rate were fused in suspension by means of u.v.-inactivated Sendai virus with $10^6$ XP_{D} passage 19 fibroblasts as described (Rao & Johnson, 1972a) and plated into two 50 mm Petri dishes (Nunc Gibeo Bicocult). Thereafter the dishes were irradiated in phosphate-buffered saline each day with low doses of u.v. in order to destabilize the hybrid karyotype and to select for cells capable of surviving small amounts of damage as follows: $1 \text{ J m}^{-2}$ per day for 3 days, $1.5 \text{ J m}^{-2}$ per day for 4 days and $2 \text{ J m}^{-2}$ per day for 7 days. During this period the limited proliferation appeared to be mainly of HeLa-like cells. By day 15, six clones consisting of large, flattened cells were picked from the two dishes and grown up in a Linbrite multiwell dish (Linbro Ltd) in the presence of a 40 J m$^{-2}$ u.v.-killed feeder suspension of the parental XP_{D} fibroblasts. For 6 days each culture was u.v. irradiated with $2 \text{ J m}^{-2}$ per day, and the two surviving clones were split into several dishes. Each split was further irradiated each day with $2 \text{ J m}^{-2}$ for 9 more days and sub-clones were picked and grown up twice more. The extensive subcloning was carried out to select putative hybrid cultures from populations that still gave rise occasionally to HeLa-like cells. The resulting subclones (HD1, HD2) grew well and had a morphology unlike either HeLa or parental fibroblast. A second series of hybrids between HeLa cells resistant to 10 $\mu$g ml$^{-1}$ 6-thioguanine and XP_{D} (XP102LO) passage 23 fibroblasts was produced, selecting for the hybrids by their ability to grow in HAT medium (Littlefield, 1964). No u.v. was used during the initial stages of hybrid growth. In this paper one hybrid from this series, HC2, is illustrated in part.

Further hybrids, between a clone of HD2 cells resistant to 10 $\mu$g ml$^{-1}$ 6-thioguanine (HD2TG) and fibroblasts from different xeroderma pigmentosum complementation groups or from a normal embryo, were produced by Sendai virus-mediated fusion, selecting for the hybrids in HAT medium.

Chromosome preparations

These were prepared as described (Schor, Johnson & Waldren, 1975).

Host cell reactivation of u.v.-irradiated adenovirus

Control and u.v.-irradiated suspensions of adenovirus type 2 (Ad2) were assayed for their ability to form structural viral antigens (Vag) in fibroblasts from normal and xeroderma pigmentosum individuals, in HeLa cells and in the HeLa-XP_{D} hybrids, HD1 and HD2 using methods described by Rainbow (1980).

Unscheduled DNA synthesis

All cells were seeded in 30 mm Petri dishes one day before irradiation. For u.v. irradiation medium was replaced by saline and graded doses of u.v. (predominantly at 254 nm) were given with a germicidal lamp. Dosimetry was measured with an IL500 radiometer (International Light) calibrated as described (Downes, Collins & Johnson, 1979). [methyl-$^3$H]thymidine (48 Ci mmol$^{-1}$) (Radiochemical Centre, Amersham) was added to all dishes at 10 $\mu$Ci ml$^{-1}$ and the cells incubated for 1 h. All dishes were thoroughly washed with saline and the cells fixed in Carnoy's fixative. After standard extraction with trichloroacetic acid the cells were prepared for autoradiography using Ilford G5 nuclear emulsion (Ilford) as described (Collins & Johnson, 1979).

Single-cell survival and growth curves

A suspension of cells from a logarithmic culture was irradiated at a concentration of $10^6$ cells ml$^{-1}$ in Dulbecco's phosphate-buffered saline (PBS) at room temperature and with agitation. The suspension was exposed to increasing amounts of u.v. light, at dose rates of 0/01 to 1 J m$^{-2}$s$^{-1}$. Irradiated and mock-irradiated control cells were plated out in triplicate after each dose in Petri dishes in growth medium and incubated at 37°C in a CO$_2$ incubator for 7 to 11 days when surviving colonies were fixed and stained. Only colonies with 50 or more cells were scored and the percentage survival was calculated. The plating efficiencies for HeLa and the hybrids were in the range of 25–50%. To achieve reasonable plating efficiency for human fibroblasts $10^4$ to $2 \times 10^4$ u.v.-killed cells
of the same strain were also added to each dish. The supralethal u.v. dose was selected empirically as that which reduced survival by more than 100-fold relative to the control. The u.v.-killed cells replaced the need to use feeder-layer plates and raised control plating efficiencies from 1-2 % to 4-8 % for XP0 102LO fibroblasts. Single-cell survival curves were constructed using data from at least three separate experiments for each cell type by plotting the log of the surviving fraction against the u.v. dose. Values for the survival curve parameters of $D_0$ (the dose required to reduce the surviving fraction by the factor $e^{-1}$ in the linear part of the semi-log curve), $n$ (the extrapolation number), and $D_s$ (the initial shoulder width: $D_s = D_0 \ln n$) were calculated with standard errors from the computed lines of best fit.

Measurement of enzymic DNA breaks accumulated during post-u.v. incubation

The procedure described by Squires et al. (1982) was followed to assess the frequency of single-strand breaks accumulated after u.v. in [methyl-$^3$H]thymidine-labelled parental DNA in the presence of $10^{-4}$m-$\beta$-arabinofuranosylcytosine (araC). Briefly, at various times after irradiation cells were lysed in situ on ice with 1 ml of alkaline sucrose solution (5 % (w/v) sucrose, 0-01 M-Na$_2$EDTA, 0-15 M-NaCl, 0-1 M-NaOH; or 5 % (w/v) sucrose, 0-01 M-Na$_2$EDTA, 0-3 M-NaCl, 0-15 M-NaOH), for high or low breakage rates, respectively. The ratio of native to denatured DNA (a function of the number of single-strand unwinding points) was assessed by means of hydroxyapatite chromatography, essentially as described by Erixon & Ahnström (1979), and the number of breaks representing this ratio calculated by reference to the elution behaviour of similarly chromatographed X-irradiated DNA bearing known numbers of DNA breaks as described (Squires et al. 1982).

Complementation analysis in heterokaryons

To assess the identity of the u.v.-sensitive phenotype expressed by HD2, heterokaryons were prepared between the hybrid and XP fibroblasts using u.v.-inactivated Sendai virus (Rao & Johnson, 1972a). Despite the great difference in size between the nuclei in these cells, the precaution was taken of uniformly prelabelling each hybrid partner by growing them for three generations in the presence of [methyl-$^3$H]thymidine (0-05 μCi ml$^{-1}$; 20 Ci mmol$^{-1}$; Radiochemical Centre, Amersham) so as to identify unequivocally one type of nucleus. Labelled medium was removed 12 h before fusion. All fusion mixtures were added to 30 mm Petri dishes in growth medium and, except for the XP$_A$ heterokaryons, each set was u.v.-irradiated in PBS with 50 Jm$^{-2}$ at 5 or 24 h after fusion. For the XP$_A$ fusion the cells were irradiated in situ 22 h after fusion with 10 Jm$^{-2}$. Irradiated populations were incubated in medium containing [methyl-$^3$H]thymidine (2:5 μCi ml$^{-1}$; 48 Ci mmol$^{-1}$) for 40 min at 37 °C for XP$_A$; (5 μCi ml$^{-1}$; 48 Ci mmol$^{-1}$) for 1 h for the others, after which time the cells were washed, fixed in situ with warm (37°C) methanol, repeatedly extracted with cold (4 °C) 5 % trichloroacetic acid and processed for autoradiography with Ilford G5 emulsion (Ilford Ltd) as described (Collins & Johnson, 1979). Silver grains were counted above the small (i.e. XP) nuclei mainly in diheterokaryons, but also in a smaller number of triheterokaryons (1HD2 :2XP and 2HD2 :1XP). Between 50 and 100 small nuclei were scored in heterokaryons for each time point; 100-200 unfused xeroderma pigmentosum cells from the same cultures served as controls for unscheduled synthesis. To avoid scoring XP nuclei in S phase small, heavily labelled nuclei in heterokaryons were ignored. In addition, to discriminate between unscheduled DNA synthesis (UDS) and replicative synthesis, UDS complementation was assessed in several parallel experiments in the presence of the inhibitors of DNA synthesis, araC ($10^{-4}$m) and HU ($10^{-2}$m). The fusion mixtures were also pretreated with the inhibitors for 1 h before irradiation to increase their efficiency, and to inhibit DNA synthesis in those nuclei in S phase. Despite the fact that both replicative and repair synthesis are inhibited by araC plus HU, the former is by far the most sensitive. UDS usually appears unchanged compared to controls or in some circumstances may even appear enhanced (Mullinger, Collins & Johnson, 1983).

Recovery of DNA synthesis after irradiation

A total of $10^8$ cells prelabelled with [¹⁴C]thymidine (0-1 μCi ml$^{-1}$; 50 mCi mmol$^{-1}$; Radiochemical Centre, Amersham) for 2 days were added to 50 mm Petri dishes and grown for 1 day before u.v. irradiation. At various times thereafter the medium was removed from duplicate plates and fresh medium was added for 50 min containing 10 μCi ml$^{-1}$ of [methyl-$^3$H]thymidine
(48 Ci mmol⁻¹) for measurement of DNA synthesis. After each pulse the cells were washed in saline and the ³H and ¹⁴C acid-insoluble radioactivities determined using the procedure described (Collins & Johnson, 1979). In some later replication recovery experiments, approximately 5 × 10⁴ cells were seeded in each of a number of 30 mm dishes and grown in non-radioactive medium for 2 days prior to u.v. irradiation. At several times after irradiation, duplicate cultures were pulsed for 30 min with 2 μCi ml⁻¹ [methyl-³H]thymidine. After washing the cells several times with cold PBS, acid-soluble and insoluble label was extracted, counted and analysed as described previously (Elliott & Johnson, 1985).

Comparative fingerprinting of concanavalin A (ConA) acceptor glycoproteins

Two-dimensional gel analyses of whole cell lysates (HeLa and XP₀ parents, and hybrids HD1 and 2) were carried out as described by O'Farrell (1975) and developed for glycoproteins by post-electrophoretic staining with ¹²⁵I-labelled concanavalin A (Koch & Smith, 1982). Comparisons between glycoprotein patterns were carried out between fingerprints that had been prepared in strict parallel to exclude the variation that can occur from one set of analyses to another. Fingerprints were superimposed on a light-box and common spots identified on the basis of their relative positions. Horizontal arrays of spots separated by single charge differences were treated as single glycoproteins.

RESULTS

Verification of the hybrid nature of the cells

Morphologically the putative XP₀-HeLa hybrids resemble neither parent, either at the single cell level or in colony form (not shown). In one strain, HD1, the chromosome content is approximately the sum of HeLa (64) plus XP₀ (46) with a range from 90 to 127 and a mode at 110; in HD2, the average chromosome number is somewhat reduced, with a range from 71 to 96 and mode at 88. In each cell line the chromosome number has been quite stable for almost 3 years: No systematic analysis of the karyotype has been attempted.

The identification of hybrids between HeLa and XP₀ cells was based on the observation that cell lines derived from different developmental lineages express different ConA acceptor glycoproteins, which can be identified by two-dimensional gel analysis. Fig. 1 shows the two-dimensional gel patterns obtained from HeLa and XP₀ cells. Only the lower molecular weight region (<55 × 10⁶ Mₒ) of each map is provided because previous studies have shown that most of the characteristic glycoproteins are in this size range. Some of the glycoproteins specific for HeLa and XP₀ cells are marked on the respective maps.

Fig. 1 shows the glycoprotein fingerprints of HD1 and HD2. Formal identification of a spot as originating from either parent line requires a comparison of three separate maps. Normally this is done by direct superimposition of the autoradiographs on a light box. To simplify the comparison of prints, details (marked on the HD2 map) have been treated separately. These details show that there are both HeLa-specific and XP₀-specific glycoproteins in each map.

Several other specific glycoproteins also appear to be expressed by the hybrids but they are relatively weak and can only be examined adequately on the originals. Some of the glycoproteins are present in both HeLa and XP₀ cells and provide useful internal markers for the comparisons.
Fig. 1. Two-dimensional fingerprints of the ConA acceptor glycoproteins of HeLa, XPD, and hybrids. Spots identified as HeLa-specific are marked with small arrowheads and XPD-specific spots with large arrowheads. The details are taken from the corresponding positions on the HeLa, XPD, and HD2 maps.

These data permit us to conclude that strains HD1 and HD2 are true hybrids. Some of the data, i.e. the HD2 map, has been published elsewhere (Koch & Smith, 1982) but is included here to establish the identity of the cells.

Sensitivity to ultraviolet light

As expected XPD fibroblasts and HeLa cells differed greatly in their sensitivity to u.v. light (Fig. 2) confirming results of other workers (Maher et al. 1976; Lee & Puck, 1960; Robbins, 1978). Both the $D_q$ and $D_s$ values are much smaller in XPD than in HeLa, though the extrapolation number, $n$, is similar. The survival curves of the hybrids fall into two clear groups. One (HD1) is indistinguishable from that of HeLa, while the survival curve of HD2 much more closely resembles that of XPD, although
Human hybrid with XPD-like DNA repair

Fig. 2. Survival of u.v.-irradiated parent and hybrid cells. Curves were fitted by least-squares analysis. (△) XP_D (102LO); (□), HeLa, (■), HD1, (■), HD2. Values for $D_q$ and $D_o$ in J m$^{-2}$ with their standard errors are as follows. $D_q$: XP_D 0·57 ± 0·09; HeLa, 3·51 ± 0·25; HD1, 2·7 ± 0·56; HD2, 0·6 ± 0·82. $D_o$: XP_D 1·34 ± 0·06; HeLa, 6·4 ± 0·07; HD1, 6·2 ± 0·2; HD2, 2·3 ± 0·21.

differing from XP_D in its slightly greater $D_o$ value. The u.v. sensitivity of hybrid HC2, obtained from a later fusion, closely resembles that of HD1 (data not shown).

Unscheduled DNA synthesis

Fig. 3 shows the dose-response of u.v.-induced unscheduled DNA synthesis in the three hybrids and the parents. HD2 resembles the XP_D parent and is only capable of limited repair synthesis, but HD1 shows much more. UDS in HeLa is lower than in HD1 but this may be more apparent than real for several reasons. First, the HeLa strain used in this work rarely flattens to a great extent, making accurate grain counting difficult; and second, a greater total amount of repair synthesis would be expected in the larger hybrid cell, perhaps reflecting the sum of HeLa plus XP_D activities.

Recovery of replicative DNA synthesis after u.v. in the hybrids

Several studies have shown that u.v. irradiation results in a dose-related depression of hydroxyurea-sensitive DNA replication in normal and XP cells, which is followed by recovery over the next few hours in the former but not by cells belonging to XP_A or XP_D complementation groups (Rudé & Friedberg, 1977; Mayne & Lehmann, 1982; also see Fig. 7D).
R. T. Johnson and others

Fig. 3. u.v.-induced unscheduled DNA synthesis in HeLa XP<sub>D</sub> 102LO and hybrid cells. Cells were irradiated with graded doses and incubated with [3H]thymidine for 1h. (■) HD1; (○) HeLa; (□) HD2; (▲) XP<sub>D</sub> (102LO). For clarity standard error bars of the mean are not given, but at 10 Jm<sup>-2</sup> they are: HeLa, ± 0.95; HD1, ± 1.03; HD2, ± 1.14; XP<sub>D</sub>, ± 0.23.

The results obtained with the u.v.-sensitive and insensitive hybrids are shown in Fig. 4 (and in part, for XP<sub>D</sub> fibroblasts XP1BR in Fig 7d) and reveal that DNA synthesis is depressed after u.v. in all cells including the HeLa parent in a dose-related manner, but that it fails to recover in the sensitive hybrid at doses above 2 Jm<sup>-2</sup> during the first day after irradiation. These results are in agreement with the growth characteristics of the different strains after irradiation; the sensitive hybrids showing considerable proliferative lag after 4 Jm<sup>-2</sup> (data not shown).

**u.v.-dependent DNA break accumulation**

The u.v. dependence of DNA break accumulation in the XP<sub>D</sub>-HeLa hybrids and in their parent cells is shown in Fig. 5. Cells were incubated with HU and araC for 30 min before and after irradiation to accumulate single-strand DNA breaks produced as a result of u.v. endonuclease activity. The hybrids fall into two distinct groups, one corresponding to HeLa and the other to XP<sub>D</sub>. Over the first 30 min after u.v. the hybrids HD1 and HC2 are indistinguishable from HeLa cells with respect to the number of DNA breaks accumulated in the presence of inhibitors while the pattern of break accumulation in HD2 closely resembles that of XP<sub>D</sub>. 
Human hybrid with XPD-like DNA repair

Fig. 4. Inhibition of thymidine incorporation after u.v. irradiation in proliferating hybrid and HeLa cells. [14C]thymidine-labelled cultures were pulse-labelled for 1 h with [3H]thymidine at various times after u.v. (a, 2 Jm⁻²; b, 4 Jm⁻²; c, 8 Jm⁻²). The points correspond to amounts of acid-insoluble radioactivity in irradiated versus mock-irradiated cells, expressed as a ratio of [3H]: [14C]. Points represent the average of two experiments. (□) HeLa; (○) HD1; (△) HD2.

Host cell reactivation (HCR) of u.v.-irradiated adenovirus

This sensitive assay has been used to detect the defective DNA repair capacities of several u.v.-sensitive cells and permits the discrimination of different XP complementation groups and of XP heterozygotes from normal cells (Rainbow, 1980; Rainbow & Howes, 1982). We have, therefore, examined the ability of hybrids HD1 and HD2 to repair u.v.-irradiated adenovirus, quantitating this function in terms of the amount of viral antigen expressed compared to that of unirradiated controls. For comparison HeLa cells, normal human fibroblasts and XPA fibroblasts were also assayed. In Fig. 6 it can be seen that HeLa and each hybrid showed a reduced host
cell reactivation of u.v.-irradiated adenovirus compared to normal human fibroblast strains. It is also clear that the survival curves for u.v.-irradiated Ad2 in hybrid HD1 consisted of more than one component. The first, most sensitive, component accounted for the survival of the majority of the virions. Using this first component of the survival curves, the host cell reactivation in the various cells was compared with that in normal human fibroblasts. Percentage HCR values (Rainbow, 1980) were about 20% for the HeLa cells, 16% for the HD1 and about 6% for the HD2. The difference between the HeLa and HD1 was small. The viral antigen (Vag) survival was in fact significantly higher in the HD1 hybrid compared to HeLa for the largest u.v. dose given to the virus, due to the marked two-component nature of its survival curve.

Percentage HCR values for the XP fibroblasts and the SV40 XP strain from complementation group A were both about 6%, consistent with previously published results for XP homozygous strains from this complementation group. The percentage HCR value for XP group D strains has also been reported to be 5–7% using the Ad2 Vag assay (Rainbow, 1980; Rainbow & Howes, 1982). Though there is some scatter of HCR values in hybrid HD2 its repair capacity appears to be similar or very slightly better than that of XP cells as judged by this assay.

Fig. 5. Accumulation of DNA repair breaks as a function of u.v. dose, in parental and hybrid cells. Prelabelled cultures of XPD (■) and HeLa (□), and hybrids HD1 (▲), HD2 (△) and HC2 (●) were replated at 2 × 10^6 cells per 35 mm dish in MEM with 10% foetal bovine serum for 2 or 24 h, incubated with HU and araC 30 min before irradiation with a graded series of u.v. doses, and then incubated for 30 min with HU and araC before alkaline lysis and hydroxyapatite chromatography to determine the frequency of repair-related breaks in DNA. The numbers of strand breaks accumulated were taken from the calculated time courses of at least three experiments (see Squires et al. 1982). At a dose of 4 J m⁻² the errors estimated from the deviations of the measured values of the breaks at different time points from the calculated values were: (■) ± 0.55; (□) ± 0.88; (▲) ± 0.42; (△) ± 0.05; (●) ± 0.33.
Fig. 6. u.v. survival curves for viral antigen formation in HeLa cells, normal human and XPA fibroblasts (A), hybrid HD1 (B), hybrid HD2 (C). The frequency of Vαg positive cells was determined in duplicate at three serial dilutions for each treatment of the virus. Each point represents the results from a separate experiment and the HeLa data were fitted to a straight line using least-squares analysis. This line is shown for reference in each survival curve. A. (●) HeLa; (△) CRL 1221 normal human fibroblasts; (▽) GM 4429B SV40-transformed group A fibroblasts; (□), CRL 1223 XP group A fibroblasts. B. (○) HD1. C (▲) HD2.

**Complementation analysis between HD2 and XP fibroblasts in heterokaryons and proliferating hybrids**

Certain combinations of cells from XP individuals brought together in heterokaryons result in significant restoration of repair synthesis when challenged by u.v. This system provides the strongest evidence that several loci are involved in generating the XP phenotype (see, e.g., Giannelli et al. 1982). To determine whether the expression of reduced unscheduled DNA synthesis in HD2 could be complemented by known repair-defective cells, heterokaryons were produced between XP fibroblasts from each of the eight known excision-deficient complementation groups,
Table 1. Unscheduled DNA synthesis in hybrid HD2–xeroderma pigmentosum heterokaryons

<table>
<thead>
<tr>
<th>XP cell</th>
<th>Complementation group</th>
<th>XP fibroblasts at 5 h</th>
<th>XP fibroblasts at 24 h</th>
<th>XP nuclei in heterokaryons at 5 h</th>
<th>XP nuclei in heterokaryons at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP4L0</td>
<td>A</td>
<td>0-14(0-21)</td>
<td>N.D.</td>
<td>14-9(10)</td>
<td>N.D.</td>
</tr>
<tr>
<td>XP11BE</td>
<td>B</td>
<td>0-8(0-9)</td>
<td>1-7(1-4)</td>
<td>11-1(8-9)</td>
<td>31-8(17-2)</td>
</tr>
<tr>
<td>XP4BR</td>
<td>C</td>
<td>4-7(2-4)</td>
<td>2-2(1-4)</td>
<td>37(17-4)</td>
<td>17-2(10-7)</td>
</tr>
<tr>
<td>XP1BR</td>
<td>D</td>
<td>7-2(2-5)</td>
<td>7-6(3-2)</td>
<td>19-5(9-2)</td>
<td>11-8(5-6)</td>
</tr>
<tr>
<td>XP23OS</td>
<td>E</td>
<td>34-2(11-2)</td>
<td>25-3(8-0)</td>
<td>58-2(11-2)</td>
<td>46-3(16)</td>
</tr>
<tr>
<td>XP2BI</td>
<td>F</td>
<td>5-6(2-1)</td>
<td>N.D.</td>
<td>28-1(10-3)</td>
<td>N.D.</td>
</tr>
<tr>
<td>XPH(XP-Sc-8)</td>
<td>G</td>
<td>1-8(1-1)</td>
<td>1-5(1-0)</td>
<td>30-8(22-1)</td>
<td>9-4(8-4)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>6-6(3-1)</td>
<td>4-1(1-9)</td>
<td>28-4(16-5)</td>
<td>21-4(10-3)</td>
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</tbody>
</table>

All u.v. irradiations were 50 Jm⁻² at 5 h, except for the XPA heterokaryons, 10 Jm⁻² at 3 h.
The values in parenthesis are the standard deviations. ND, not determined.

A to H. The results of these crosses are shown in Table 1 and confirm that products made by HD2 are able to complement the defects in each of the XP groups including, unexpectedly, XPD. Complementation is rapidly achieved and by 5 h nuclei from each XPD group show improved unscheduled synthesis. For XPC up to 15 times the amount of repair synthesis is achieved compared to unfused cells and for XPC UDS is increased by about eightfold. In comparison, complementation of XPD is less pronounced at about threefold. By 24 h though there is still substantial complementation, many of the cells show less UDS than earlier after fusion. This is particularly clear for XPD and XPC. In parallel experiments using the inhibitors araC and HU in combination to arrest replicative but not repair synthesis, as described in Materials and Methods, complementation for UDS was observed between hybrid HD2 and XP fibroblasts from the following complementation groups: D, C, E, F and G (data not

Fig. 7. Characterization of u.v. sensitivity and repair capacity of hybrids between HD2 and XPD or XPC fibroblasts. The former series is represented by $\pi_1$ (△), and the latter by $\delta_2$ (□).

a. Clonal survival of $\pi_1$ and $\delta_2$. Most points represent the average of two experiments; the lines are drawn by eye.
b. Recovery of replicative DNA synthesis after a single dose (4 Jm⁻²) of u.v.; the behaviour of XP1BR (△) and hybrid O-I (□), a product of HD2 and normal human fibroblast, are shown for comparison. The error bars represent standard deviations of the mean from duplicate samples.
c. Incision activity in $\pi_1$, $\delta_2$ and HD2 (■), shown for comparison. After graded doses of u.v., araC and HU were added for 1 h before the cells were lysed in alkali. Each point represents the mean of two experiments.
d. Unscheduled DNA synthesis measured by autoradiography in $\pi_1$, $\delta_2$ and HD2 (■). After u.v., cells were incubated for 1 h with 2-5 μCi/ml⁻¹ [methylo²H]thymidine. The range of values represent standard errors of the mean.
shown). Because of the unexpected degree of restoration of UDS in heterokaryons consisting of hybrid HD2 and XPB fibroblasts we have examined this further by constructing several more series of hybrids this time between HD2 and XPB or other XP fibroblasts. Proliferating hybrids permit more extensive assays to be carried out to assess their repair capacity than are possible with heterokaryons. Clones were picked from fusions with normal embryonic lung fibroblasts, with XPB (parental
Table 2. Phenotypic characteristics of HD2–XP or normal fibroblast hybrids

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Fibroblast partner</th>
<th>% survival after* 10 J m⁻² u.v.</th>
<th>UDS† (ratio, hybrid:HD2 10 J m⁻²)</th>
<th>Incision‡ bks/10⁶ daltons (5 J m⁻², 60 min)</th>
<th>Recovery of§ DNA synthesis (4 J m⁻², 6 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁</td>
<td>XPC (XP106LO)</td>
<td>52</td>
<td>6</td>
<td>7.5</td>
<td>+</td>
</tr>
<tr>
<td>δ₁</td>
<td>XPC (XP4BR)</td>
<td>33</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ₂</td>
<td>XPC (XP2RO)</td>
<td>67</td>
<td>2</td>
<td>3.6</td>
<td>+</td>
</tr>
<tr>
<td>π₁</td>
<td>XPD (XP1BR)</td>
<td>0.8</td>
<td>2</td>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
<td>π₂</td>
<td>XPD (XP201BR)</td>
<td>3.5</td>
<td>2</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>π₄</td>
<td>XPD (XP201BR)</td>
<td>3</td>
<td>2</td>
<td>3.9</td>
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</tr>
<tr>
<td>D₁</td>
<td>XPD (XP102LO)</td>
<td>0.3</td>
<td></td>
<td>4.2</td>
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</tr>
<tr>
<td>β₂</td>
<td>XPE (XP2RO)</td>
<td>43</td>
<td>5</td>
<td>8.0</td>
<td>+</td>
</tr>
<tr>
<td>β₃</td>
<td>XPE (XP2RO)</td>
<td>64</td>
<td>5</td>
<td></td>
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<tr>
<td>γ₁</td>
<td>XPF (XP230S)</td>
<td>36</td>
<td>4</td>
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<tr>
<td>K₄</td>
<td>XP2LE</td>
<td></td>
<td></td>
<td>54</td>
<td>4</td>
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<tr>
<td>O-1</td>
<td>Normal embryonic lung</td>
<td>60</td>
<td>4</td>
<td>8.2</td>
<td>+</td>
</tr>
<tr>
<td>O-2</td>
<td>Normal embryonic lung</td>
<td>41</td>
<td>6</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>O-3</td>
<td>Normal embryonic lung</td>
<td>41</td>
<td>6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>HD2</td>
<td>0.5</td>
<td>1</td>
<td></td>
<td>3.2</td>
<td>-</td>
</tr>
</tbody>
</table>

* Relative to unirradiated control.
† Data obtained from autoradiographs; irradiated cells incubated with [³H]thymidine.
‡ The number of single-strand breaks was determined by the standard alkaline unwinding-hydroxyapatite chromatography procedure after inhibiting repair for 1 h with araC plus HU.
§ Compared to unirradiated controls.
|| Not yet assigned to a complementation group.
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102LO and XP1BR), and with XP C (XP106LO, XP4BR), XP E (XP2RO) and XP F (XP23OS). Each HD2-fibroblast hybrid contains, as expected, many chromosomes, usually with an average of about 120. In response to u.v. irradiation, however, they fall into two clear groups. All hybrids involving XP C, XP E or XP F, or normal fibroblasts are u.v.-insensitive (Fig. 7A), rapidly recover DNA synthetic ability (Fig. 7B) and have high levels of UDS (Fig. 7C) and incision activity (Fig. 7D). On the other hand, hybrids involving XP D are u.v.-sensitive (Fig. 7A), are slow or unable to recover normal rates of DNA synthesis after low levels of irradiation (Fig. 7B), have lower levels of UDS (Fig. 7C) and incision (Fig. 7D). The figures show data mainly from HD2-XP C and HD2-XP D hybrids only. A summary of the phenotypic traits of the several series of hybrid so far examined as given in Table 2. Taken together these results provide reasonable evidence that the original HeLa-XP D hybrid, HD2, expresses the repair capability of the XP D parent and therefore that the parent HeLa D allele is silent or absent.

Turning to the problem of elevated UDS in HD2-XP D heterokaryons (Table 1) it is clear that there is a similar degree of UDS enhancement in proliferating hybrids constructed between the same parents (Table 2). In these cells therefore the slight though positive UDS enhancement is hardly in accord with their other phenotypic traits. For example, the slight increase in incision ability and survival in the π series compared to HD2 is far less than might be predicted from the two- or threefold increase in unscheduled DNA synthesis.

Discussion

In this paper we have described the production of repair-defective permanent human cultures using a method common in somatic cell genetics. The aim was to assemble hybrid cells initially bearing, though presumably not expressing, recessive repair-related alleles and to use these as the starting point for generating u.v.-sensitive progeny that retain a limited repair ability. Though hamster or human intraspecific cell hybrids tend not to lose many chromosomes (Kao, Johnson & Puck, 1969; Rao & Johnson, 1972b; Bengtsson et al. 1975; Stanbridge, Flandermeyer, Daniels & Nelson-Rees, 1981), we believed it likely that limited chromosome loss could occur at a significant rate from such cells by analogy with results obtained on rates of mutation and expression of recessive markers in Chinese hamster pseudotetraploid cells and intraspecific hybrids (Chasin, 1973; Chasin & Urlaub, 1975). Limited chromosome loss associated with the segregation of several identifiable characters has also been established in human cell hybrids between lymphocytes and the permanent line D98/AH2 (Bengtsson et al. 1975), and the expression of malignancy in HeLa-fibroblast hybrids is also associated with the loss of specific chromosomes (Stanbridge et al. 1981; Stanbridge & Wilkinson, 1978). In many respects these latter hybrids closely resemble the HeLa-XP D cells described in this paper.

In the hybrids produced between HeLa and XP D and initially selected for their ability to tolerate small amounts of u.v. two phenotypes have been generated: HD1, expressing HeLa-like u.v. sensitivity and repair ability, and HD2, a u.v.-sensitive cell
with limited excision repair similar to that of the XP<sub>D</sub> parent. The former hybrid has on average 10 chromosomes more than the latter suggesting that chromosome loss and expression of the sensitive phenotype are related. In another system, this time in interspecies hybrids, the loss of a specific human chromosome has been shown to correlate with the re-expression of limited repair (de Wit, Odijk, Bootsma & Westernveld, 1984). Phenotypically, HD2 cells resemble XP<sub>D</sub> in a considerable number of repair or repair-related responses to u.v. irradiation. In terms of sensitivity, incision rate, unscheduled DNA synthesis, replication recovery and host cell virus reactivation, the two cells are very similar. Nonetheless, somatic cell fusion experiments reveal that HD2 is able to complement to a limited degree, the XP<sub>D</sub> defect in u.v.-irradiated heterokaryons. The simplest interpretation of this result is that the repair defects expressed in XP<sub>D</sub> cells and the u.v.-sensitive hybrid are associated with different genetic loci. However, the results obtained with proliferating cells produced from HD2 backcrossed with XP<sub>D</sub> fibroblasts strongly suggest that this is not the case. True complementation for repair function occurred when the XP partner was drawn from groups C, E or F, but not from D (see Table 2). The elevated UDS in both heterokaryons and hybrids with HD2-XP<sub>D</sub> background remains a mystery. It is far less than in other hybrids or heterokaryons and implies that this trait, at least in XP<sub>D</sub> cells, cannot be taken as evidence of significant repair activity. This view is sustained by the common observation that XP<sub>D</sub> cells show relatively high levels of repair replication in response to u.v. yet very poor survival (Paterson, 1982). Despite the fact that incision events can be accumulated by repair synthesis inhibitors (Squires et al. 1982; this paper) XP<sub>D</sub> cells are unable to remove pyrimidine dimers as judged by the u.v. endonuclease assay (Paterson, Lohman & Sluyter, 1973; Zelle & Lohman, 1979). Another strange feature of XP<sub>D</sub>, emphasizing the complexity of its repair defect (Lehmann, 1982b), is that a significant fraction of dimers cannot be photoreactivated in these cells (Paterson, 1982).

Turning to the HD2-XP hybrids it is possible that these represent HD2 revertants to normal u.v. sensitivity and thioguanine resistance rather than secondary hybrids. We believe this is unlikely for several reasons. First, we have no evidence of spontaneous or induced reversion of either trait in HD2TGR. Second, fusion between 'wild-type' HD2 and HD2, resistant to both 6-thioguanine and ouabain, does not result in the production of u.v.-resistant cells (data not shown). Third, the profile of concanavalin-binding, fibroblast-specific glycoproteins in the HD2-XP hybrids is quantitatively much more pronounced than in the HD2 TG-resistant parent. HD2TGR has much reduced expression of XP<sub>D</sub> glycoproteins compared to the original HD2 hybrid (G.L.E. Koch, unpublished data).

Previously established human cell lines defective in repair have been produced by mutagenesis and selection of tumour cells (Isomura et al. 1973), or by their infection with transforming viruses (Suzuki & Fuse, 1981). Very few such strains are known. Alternatively, permanent lines have been obtained by SV40 or Epstein-Barr transformation of diploid cells from repair-defective individuals (Heddle & Arlett, 1980; Altamirano-Dimas, Sklar & Strauss, 1979). More recently, transformed xeroderma pigmentosum fibroblast cultures have been reported following u.v. irradiation
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(Maher et al. 1982). This system may ultimately provide useful permanent XP cell lines, though, to date, most of the anchorage-dependent cell cultures so obtained have not developed into permanent lines (see, e.g., Zimmerman & Little, 1983).

Our approach using somatic cell hybrids to achieve immortalization of the XP genome and chromosome segregation from these hybrids to allow expression of recessive XP allele offers another way of obtaining repair-defective human cells. The stability of hybrid HD2 and the ease with which it can be grown should help to make it a useful tool in the analysis of the XP0 defect.

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