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Complementation of a DNA repair defect in xeroderma pigmentosum cells by transfer of human chromosome 9

(xeroderma pigmentosum complementation group A/microcell fusion/UV irradiation/monochromosomal hybrids)

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ABSTRACT **Complementation of the repair defect in xeroderma pigmentosum cells of complementation group A was achieved by the transfer of human chromosome 9. A set of mouse–human hybrid cell lines, each containing a single Ecogpt-marked human chromosome, was used as a source of donor chromosomes. Chromosome transfer to XPTG-1 cells, a hypoxanthine/guanine phosphoribosyltransferase-deficient mutant of simian virus 40-transformed complementation group A cells, was achieved by microcell fusion and selection for Ecogpt. Chromosome-transfer clones of XPTG-1 cells, each containing a different human donor chromosome, were analyzed for complementation of sensitivity to UV irradiation. Among all the clones, increased levels of resistance to UV was observed only in clones containing chromosome 9. Since our recipient cell line XPTG-1 is hypoxanthine/guanine phosphoribosyltransferase deficient, cultivation of Ecogpt$^+$ clones in medium containing 6-thioguanine permits selection of cells for loss of the marker and, by inference, transferred chromosome 9. Clones isolated for growth in 6-thioguanine, which have lost the Ecogpt-marked chromosome, exhibited a UV-sensitive phenotype, confirming the presence of the repair gene(s) for complementation group A on chromosome 9.**

Xeroderma pigmentosum (XP) is an autosomal-recessive human disease and cells cultured from XP patients exhibit higher sensitivity to UV-radiation and cell transformation than normal cells (1). Nine complementation groups, designated A through I (1–3) and a variant (XP-V) (1) have been identified for the XP condition. Biochemical defects in nine of the XP groups (A–I) have been defined in the nucleotide excision repair pathway that removes pyrimidine dimers (3–5), whereas XP-V is defective in post-replication repair (1). Transient complementation of XP cells of several groups has been achieved by microinjection of total cell extracts (6–7) and mRNA (8) from normal cells but it has not been possible to define the gene(s) or gene products involved in excision repair synthesis. Another approach toward the study of the repair process has been the isolation of repair-defective mutants of Chinese hamster cells (9). However, none of these mutants has been shown to be related to the XP condition (9). In addition, a human gene isolated by complementation of the CHO mutants (10–12) is also not related to the repair defect in XP cells.

Although the UV-sensitive (UV^S) phenotype of XP cells provides an excellent model for gene isolation by DNA transfection, attempts in this (R.S.A., unpublished results) and other laboratories (22) to rescue the repair gene have been unsuccessful. This may be attributed to inefficient DNA transfer into XP cells, inadequate selection procedures, and/or a gene of large size. It is also possible that more than one gene is required to complement the repair defect. We, therefore, explored the possibility of transferring intact or large fragments of human chromosomes to XP cells using the method of microcell-mediated chromosome transfer (MMCT). A panel of mouse–human hybrid cell lines, each containing a single Ecogpt-marked human chromosome (13) has been used as a chromosome source to transfer marked human chromosomes individually to hypoxanthine/guanine phosphoribosyltransferase (HGPRT)-deficient XP-A cells. In this paper, we report that human chromosome 9 complements the repair defect in XP-A cells.

MATERIALS AND METHODS

Cell Lines and Growth Conditions. An HGPRT$^-$ XP-A cell line, XPTG-1, isolated after mutagenesis of GM4312A cells (Human Genetic Mutant Cell Repository, Camden, NJ) and selection in 6-thioguanine (TG), was used as recipient for MMCT. XPTG-1 cells were also marked with a selectable marker, pSV2neo (14), which facilitates double selection in MMCT experiments. A panel of mouse–human hybrid cell lines, each containing either individual or multiple human chromosomes, were used as microcell donors.

All cell lines were routinely cultured at 37°C in a 10% CO_2/90% air atmosphere in Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) fetal bovine serum. The medium was supplemented with appropriate selective agents such as mycophenolic acid (25 μg/ml) plus xanthine (70 μg/ml) (MX medium) and G418 (400 μg/ml) to isolate chromosome-transfer clones. DMEM containing TG (TG medium) was used to isolate clones for the loss of the Ecogpt-marked human chromosome (13).

MMCT. Microcells prepared from donor cell lines as described (13) were fused with the recipient cells in monolayers by using 50% (wt/vol) PEG 1500 (13). After fusion, cells were cultured in DMEM for 48 hr (expression time) and then transferred to MX medium containing G418. Addition of G418 to medium selects against any intact donor cells that might have been present among microcells. Independent chromosome-transfer colonies, isolated individually, were propagated in MX medium for further analysis.

Analysis for Complementation. For initial identification of complementation, 10^6 cells from each chromosome-transfer clone were seeded in the middle of 100-mm tissue culture dishes in triplicate. The cells were rinsed with isotonic phosphate-buffered saline (PBS), UV-irradiated (6 J/m^2) at a rate of 0.5 J·m^{-2}·sec^{-1}, and incubated in fresh medium. After 3 days, plates were fixed with absolute methanol, stained with crystal violet, and visually evaluated for cell survival by using an inverted microscope. The clones identified to be UV

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Abbreviations: XP, xeroderma pigmentosum; TG, 6-thioguanine; S, sensitive; R, resistant; MMCT, microcell-mediated chromosome transfer; HGPRT, hypoxanthine/guanine phosphoribosyltransferase; AK_1, adenylate kinase 1.

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resistant (UV^R) at 6 J/m^2 were analyzed for post-UV-irradiation cell survival at doses from 1.5 J/m^2 to 12.0 J/m^2 by modification of a method described by Cleaver and Thomas (15).

Briefly, 10^6 cells plated in the middle of 100-mm tissue culture dishes in triplicate were cultured for 24 hr in MX medium, rinsed with PBS, UV-irradiated at various doses, and then cultured in MX medium. After 24 hr of further incubation, [^{3}H]thymidine (0.5 μCi/ml; specific activity, 40 Ci/mmol; 1 Ci = 37 GBq) was added to the cultures. After 72 hr of growth in the presence of [^{3}H]thymidine, cells were harvested and lysed in 10 mM Tris·HCl/1 mM EDTA (pH 7) containing 0.1% SDS. Relative incorporation of [^{3}H]-thymidine into cellular DNA was used as an index for post-irradiation cell survival. Cell survival measured by [^{3}H]thymidine incorporation as described here, was comparable to the data obtained by traditional, but more laborious, post-UV-irradiation colony formation assay (unpublished results).

Quantification of unscheduled DNA synthesis was performed by labeling UV-irradiated (10 J/m^2) cells with [^{3}H]thymidine followed by autoradiography.

Cytogenetic and Biochemical Analysis. Cytogenetic and biochemical analyses were performed to identify the complementing chromosome present in hybrid cells. For cytogenetic identification, metaphase spreads prepared by standard method were stained for G-11 and G-banding to determine the number and identity of the human chromosome (13). Biochemical analysis was performed for the expression of the adenylate kinase 1 (AK_1) isozyme by starch gel electrophoresis of cell extracts (16). Southern blot analysis (17) was performed using chromosome 9-specific DNA probes, D9S11 and D9S12, to confirm chromosome identity in hybrid cells and to follow the lineage of complementing chromosome through various cell lines by using Ecogpt as probe.

RESULTS

Chromosome Transfer to XP-A Cells and Identification of Complementing Chromosome. The experimental strategy used for complementation analysis and chromosome identification is shown in Fig. 1. A list of donor and recipient cell lines, MMCT clones, and back-selected clones produced in these experiments along with respective phenotypes is given in Table 1. Complementation analysis of a DNA repair defect involved tagging human chromosomes with a dominant selectable marker by DNA transfection and sequential transfer of the marked chromosomes through mouse cells to XP cells. Plasmid pSV2gpt containing the Ecogpt gene (18) was transfected into human MGH-1 cells to produce clones designated as MGHGT, each containing a single marked chromosome (13). These clones were used as donors to generate a bank of mouse–human monochromosomal hybrids, each containing a single human chromosome. In addition to monochromosomal hybrids, another set of cell–cell hybrids between MGHGT clones and mouse RAG cells were also produced.

Marked human chromosomes present in mouse–human hybrids were individually transferred to XPTG-1 cells. The chromosome-transfer clones, each containing 1 of 12 human chromosomes, were assessed individually for increased resistance to UV-irradiation at 6 J/m^2 in comparison with XPTG-1 cells. Elevated levels of UV resistance occurred in three of the clones (XPM24-1, XPM24-2, and XPM24-3), where hybrid RA24-1 was used as a chromosome donor (Table 1).

RA24-1 is a cell–cell hybrid between an $HGPRT^-$ mouse cell line RAG and MGHGT61-1 and contained multiple human chromosomes. For chromosome identification, marked human chromosome present in RA24-1 was transferred to $HGPRT^-$ mouse A9 and Chinese hamster CHTG49

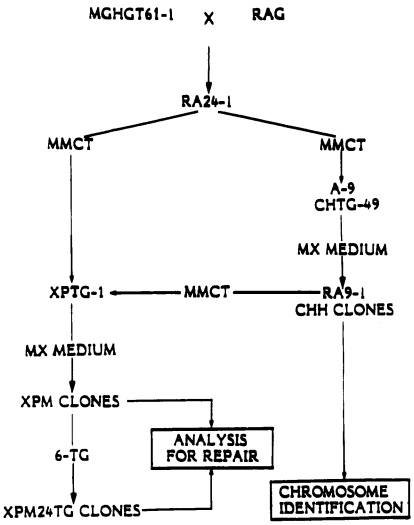

FIG. 1. Experimental scheme representing the transfer of an Ecogpt-marked human chromosome to XPTG-1 cells for complementation analysis and to A9 and CHTG49 cells for chromosome identification. MGHGT61-1 is a human cell line derived by transfection of pSV2gpt into MGH-1 cells. The marked chromosome present in RA24-1 was transferred to XPTG-1 cells by MMCT to generate XPM clones (left vertical pathway) and to A-9 and CHTG49 cells to produce mouse–human and Chinese hamster–human monochromosomal hybrids RA9-1 and CHH9, respectively (right vertical pathway). Hybrids RA9-1 and CHH9-1 were analyzed to determine the identity of the human chromosome. RA9-1 was used as an intermediary to transfer the identified single human chromosome 9 to XPTG1 cells to produce complemented clone XPM9-1. XPM stands for complemented clones that include XPM24-1, XPM24-2, XPM24-3 originating from RA24-1, and XPM9-1 originating from RA9-1. Cells from two of the clones (XPM24-2 and XPM9-1) were cultured in TG medium to generate XPM-TG clones that have lost the marked human chromosome (left vertical pathway). The XPM and XPM-TG clones were analyzed for UV^R/UV^S phenotype in comparison with XPTG-1 and MGH-1 cells.

cells to generate clones RA9-1 and RA9-2, in the mouse background, and clones CHH9-1, CHH9-2, and CHH9-3, in the hamster background (Fig. 1 and Table 1). Cytogenetic analysis of these clones revealed the presence of a single intact human chromosome 9 in RA9-1 (Fig. 2), RA9-2, and CHH9-1, but only a partial chromosome was present in CHH9-2 and CHH9-3. Cytogenetic identification of chromosome 9 in CHH9-1 was confirmed by flow karyotyping of isolated metaphase chromosomes. The DNA content of the human chromosome present in CHH9-1 was identical to

Table 1. Description of various cell lines used

Cell line(s)	Description
XPTG-1	UV^S, $HGPRT^-$ XP-A cell line
RA24-1	Mouse–human cell–cell hybrid
RA9-1 and RA9-2	Mouse–human monochromosomal hybrids produced by MMCT from RA24-1 to A9 cells
CHH9-1, CHH9-2, and CHH9-3	Chinese hamster–human monochromosomal hybrids, produced by MMCT from RA24-1 to hamster CHTG-49 cells
XPM24-1, XPM24-2, and XPM24-3	UV^R, complemented clones produced by MMCT from RA24-1 to XPTG-1
XPM9-1	UV^R, complemented clone produced by MMCT from RA9-1 to XPTG-1
XPM24-2TG1 and XPM9-TG1	UV^S, clones produced by selection for the loss of complementing marked chromosome

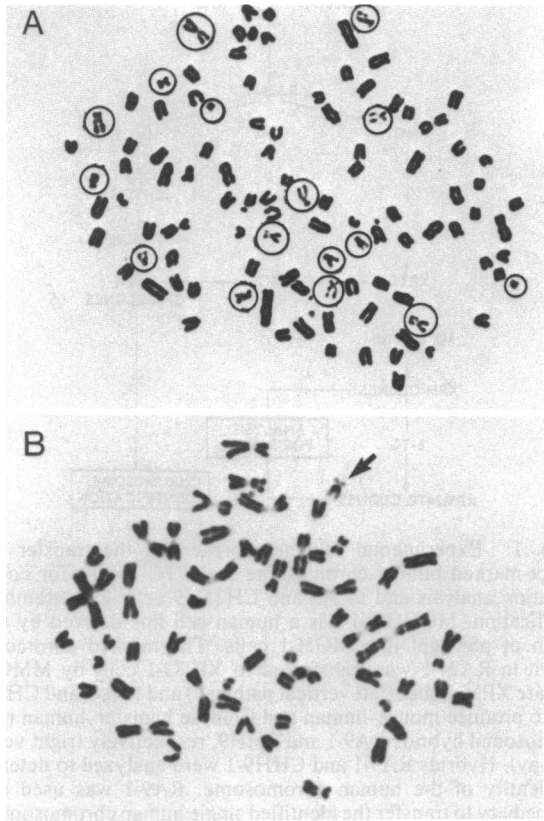

FIG. 2. Metaphase spreads stained by the G-11 method. (*A*) RA24-1 contains multiple human chromosomes (circles). (*B*) RA9-1 is a monochromosomal hybrid containing human chromosome 9 (arrow).

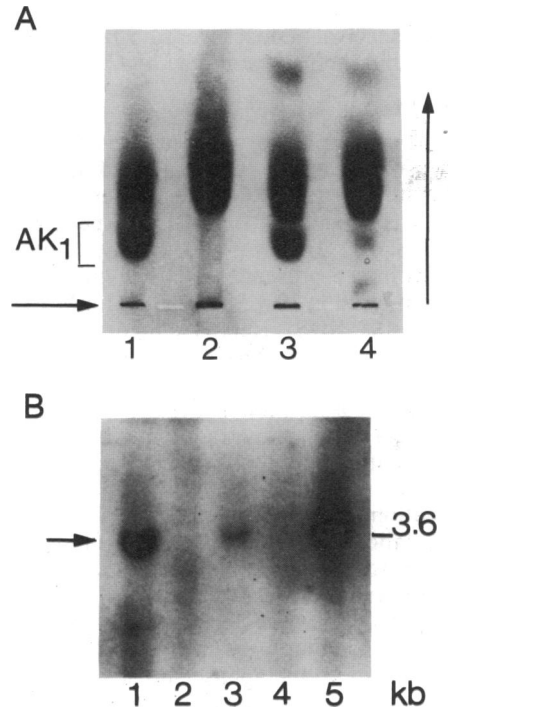

FIG. 3. (*A*) Zymogram of adenylate kinase isozymes. Lanes: 1, MGH-1 (human cells); 2, A9 (mouse cells); 3, RA9-1; 4, RA24-1. Slow-migrating human AK_1 near the origin is separated from mouse AK_1. (*B*) Southern blot analysis of genomic DNA digested with *Pst* I and probed with a chromosome 9-specific DNA segment D9S12. Lanes: 1, CHH9-1; 2, CHTG49; 3, RA9-1; 4, A9; 5, MGH-1. The hybridization bands are present only in lanes 1, 3, and 5 showing the presence of chromosome 9 in human and hybrid cells. Probe D9S12 did not hybridize with Chinese hamster (lane 2) or mouse (lane 4) DNA.

normal chromosome 9 from diploid lymphocytes (M. Van-Dilla, personal communication).

Identity of chromosome 9 in hybrid cell lines was also confirmed by biochemical analysis for the expression of an isozyme marker and two cloned DNA segments mapped to human chromosome 9. Our data revealed that human AK_1 isozyme, which maps to chromosome 9, was expressed in RA9-1 and RA24-1 (Fig. 3*A*). Two chromosome 9-specific probes, D9S11 and D9S12, also showed hybridization bands in the Southern blot of DNAs from RA9-1 and CHH9-1 (Fig. 3*B*).

Cells from clones RA9-1 and CHH9-1 cultured in DMEM for 7 days were selected in TG medium to generate cell populations that had lost human chromosome 9 (13). Cytogenetic analysis of these cells did not show the presence of intact or fragmented human chromosome. Slot blot analysis using human repetitive DNA (*Alu* sequence) as probe for the presence of human DNA was also negative (data not shown). Thus, our chromosome identification shows that a single marked human chromosome 9, present among other human chromosomes in RA24-1, was transferred to mouse A9 and hamster CHTG49 cells and was eliminated in a back-selected cell population.

A single human chromosome 9 present in hybrid RA9-1 was transferred again to XPTG-1 to confirm the presence of repair genes on this chromosome (Fig. 1). A single MMCT clone, XPM9-1 (Table 1), recovered in these experiments showed an elevated level of resistance to UV-irradiation.

Isolation of UV^S Clones from UV^R Clones by Back Selection. Since our recipient cell line XPTG-1 is $HGPRT^-$ and can grow in TG, this provides a system to select for cells that have lost the Ecogpt-marked human chromosome. Cells from UV^R clones XPM9-1 and XPM24-2 (Table 1) were grown in nonselective medium (DMEM) for 10 days to allow for random chromosome segregation and then cultured in TG medium. For each of the XPM9-1 and XPM24-2 parental clones, three independent colonies were isolated and designated as XPM9-TG1–3 and XPM24-2TG1–3. Analysis for UV^S phenotype of two of the clones, XPM9-TG1 and XPM24-2TG1, showed UV^S profiles similar to XPTG-1 cells. These data show that the loss of the marked human chromosome 9 from complemented MMCT clones correlated with the loss of UV^R phenotype.

Lineage of Marked Human Chromosome in Various Cell Lines. The integration of pSV2gpt in a human chromosome provides a method to follow the lineage of the marked chromosome through various cell lines. Southern blots of DNAs from MGHGT61-1, RA9-1, XPM9-1, XPM9-TG1, and XPTG-1, digested with *Sac* I and *Hin*dIII, were hybridized separately to ^{32}P-labeled probes prepared for pSV2gpt and the Ecogpt insert alone (Fig. 4). MGHGT61-1 DNA digested with *Hin*dIII showed three hybridization bands of 6.5, 5.3, and 3.9 kilobases (kb), respectively, with pSV2gpt as probe (data not shown), whereas only two bands (6.5 and 5.3 kb) were observed for Ecogpt (Fig. 4*B*). The absence of the 3.9-kb band with the Ecogpt probe can be explained on the basis of the site of integration within the plasmid and lack of homology of the probe to a region of the plasmid. When MGHGT61-1 DNA digested with *Sac* I was probed with pSV2gpt, a single hybridization band of 13.6 kb was observed (Fig. 4*A*). Since plasmid pSV2gpt has a single restriction site for *Hin*dIII and none for *Sac* I, comparison of the data from both Southern blots shows that at least two copies of pSV2gpt were integrated into a single site in MGHGT61-1 cells (13, 18). The DNAs from RA9-1 and XPM9-1 digested with *Sac*

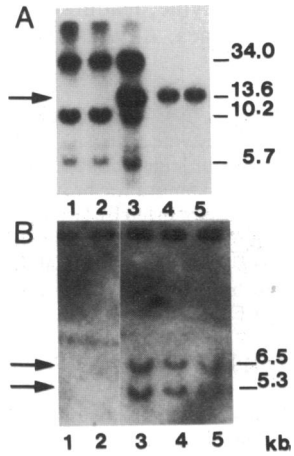

FIG. 4. Southern blot analysis of genomic DNA. Lanes: 1, XPTG-1; 2, XPM9-TG1; 3, XPM9-1; 4, RA9-1; 5, MGHGT61-1. (*A*) DNA digested with *Sac* I was probed with pSV2gpt. A common band of 13.6 kb (arrow), present in MGHGT61-1, RA9-1, and XPM9-1, is absent in parental recipient XPTG-1 cells and back-selected clone XPM9-TG1. Additional bands observed in XPTG-1, XPM9-TG1, and XPM9-1 (lanes 1, 2, and 3, respectively) are due to pSV2neo present in the recipient cells and do not correspond to the band for pSV2gpt. (*B*) DNA digested with *Hin*dIII was probed with an Ecogpt fragment excised from pSV2gpt. Two bands of 6.5 and 5.3 kb present in MGHGT61-1, RA9-1, and XPM9-1 (lanes 5, 4, and 3, respectively) are absent in XPTG-1 and XPM9-TG1 (lanes 1 and 2, respectively). These data show the presence of identical marked chromosome in parental donor cells, mouse–human hybrids, and a complemented clone that is not present in the parental recipient cells and back-selected clone.

I and *Hin*dIII and probed with pSV2gpt and Ecogpt, respectively, revealed the presence of bands identical to those observed for MGHGT61-1 (Fig. 4). These results show that a single marked chromosome present in the parental MGHGT61-1 cells was sequentially transferred through RA24-1 to XPTG-1 cells to generate respective MMCT clones. Bands corresponding to pSV2gpt are not present in DNA of XPTG-1 and XPM9-TG1 cells (Fig. 4). Absence of bands corresponding to pSV2gpt in the DNA of XPM9-TG1 shows the loss of the marked human chromosome 9 after back-selection (Fig. 4). Additional bands observed in lanes for XPTG-1, XPM9-TG1, and XPM9-1 do not correspond

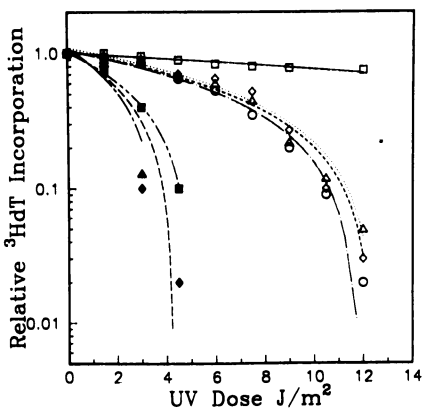

FIG. 5. Comparison of UV resistance of various cell lines determined by relative incorporation of [^{3}H]thymidine ([^{3}H]dT) during a 72-hr labeling period after UV irradiation. [^{3}H]Thymidine was added to the cultures 24 hr after irradiation. ■, UV^S XPTG-1 cells; □, normal human MGH-1 cells; ◇, complemented clones XPM24-1; △, XPM24-2; ○, XPM9-1, ▲ and ◆, back-selected clones XPM9TG-1 and XPM24-2TG1, respectively. Each data point represents an average of three experiments.

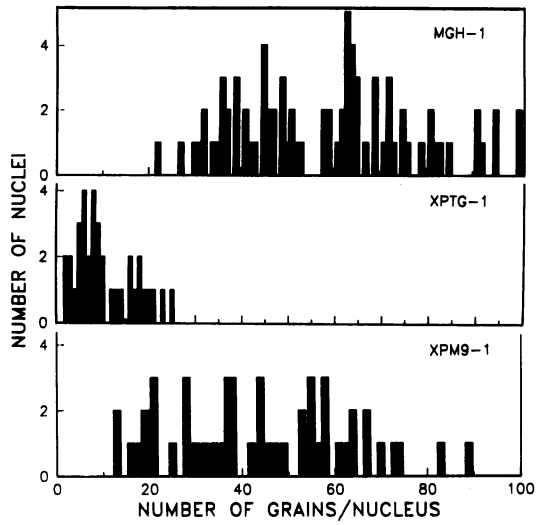

FIG. 6. Unscheduled DNA synthesis as measured by [^{3}H]thymidine incorporation and autoradiography. Cells UV-irradiated at 10 J/m^2 were labeled for 8 hr; histograms show distribution of grains in individual nuclei of normal human cell line MGH-1, UV^s cell line XPTG-1, and partially complemented clone XPM9-1.

with pSV2gpt (Fig. 4*A*) but rather are derived from the pSV2neo marker present in recipient cells, which shares homology with pSV2gpt.

Characterization of MMCT Clones for Complementation. Quantitative analysis for UV resistance was performed on three of the complemented clones (XPM24-1, XPM24-2, and XPM9-1) and two of the back-selected clones (XPM9-TG1 and XPM24-2TG1) and compared with XPTG-1 and normal human MGH-1 cells (Fig. 5). All three clones containing transferred chromosome 9 were significantly more UV^R than parental XP-A cells but less than MGH-1 cells (Fig. 5). Our data show that only a partial complementation of the UV^S phenotype is achieved by transfer of chromosome 9. The clones XPM24-2TG1 and XPM9-TG1 that have lost normal chromosome 9 were as sensitive to UV irradiation as the parental XPTG-1 cells (Fig. 5).

Another measure used for complementation of the repair defect was unscheduled DNA synthesis quantified by autoradiography after the incorporation of [^{3}H]thymidine into cells previously UV irradiated at 10 J/m^2. Considerable variation in the number of grains among different nuclei within the same cell line was observed. The average number of grains per nucleus was low (0–25 grains per nucleus) in XPTG-1 cells whereas the XPM9-1 cells (13–89 grains per nucleus) exhibited an unscheduled DNA synthesis profile close to MGH-1 cells (22–100 grains per nucleus) (Fig. 6). Heterogeneity for unscheduled DNA synthesis among nuclei in the same cell line has been observed in other studies as well (19).

Slot blot analysis of DNA from all complemented clones using total mouse DNA for probe was negative for the presence of any mouse DNA (data not shown). Thus complementation of the repair defect resulting from the transfer of a mouse chromosome or DNA is unlikely.

DISCUSSION

In this paper we present two lines of evidence that strongly indicate that human chromosome 9 carries gene(s) that complement the DNA repair defect in XP-A cells. (*i*) The only marked chromosome present in the parental normal human cells that was transferred to XPTG-1 cells through mouse–human hybrids RA24-1 and RA9-1 that complements the repair defect was identified to be human chromosome 9, and

(*ii*) the UV^R phenotype was eliminated in association with the loss of the marked chromosome in back-selected clones. Absence of any mouse DNA in complemented clones, as detected by slot blot analysis, shows that complementation could not be due to the transfer of mouse DNA or chromosomes.

In previous experiments using a similar approach, complementation of XP-A cells was achieved, but no definitive chromosome was identified (19). In another series of experiments, human chromosome 15 has been shown to complement the repair defect in XP-F cells (ref. 20 and unpublished results). However, chromosome 15 did not complement the repair defect when transferred into XP-A cells. These observations indicate that repair gene(s) for different complementation groups may be present on different chromosomes. This is also apparent from the fact that different human chromosomes complement the repair defects in CHO mutants belonging to different groups (21). Since chromosomes 9 and 15 have not, as yet, been tested against other complementation groups, the presence of additional repair gene(s) on these chromosomes cannot be ruled out.

Experiments to complement the repair defect by transfection of normal human DNA have been unsuccessful in this (R.S.A., unpublished results) as well as other laboratories (22). This suggested that the DNA repair gene in human cells may either be so large that an intact gene could not be recovered by DNA transfer methods or that more than one gene on the same or different chromosomes may be required for complementation. Thus, transfer of intact or large chromosomal fragments may be a more appropriate approach to identify and map the repair gene(s). Identification of a complementing chromosome is difficult if not impossible by direct transfer between two human cell lines. The mouse–human hybrids containing identified, dominantly marked human chromosomes provide excellent material for the complementation analysis of recessive mutations such as those of DNA repair in human cells, where species-specific transfer of a human chromosome may be required.

Use of HGPRT-deficient XPTG-1 cells in combination with Ecogpt-marked human chromosomes provides a method to select for the retention or loss of the marked chromosome. This made it possible to show the correlated loss of UV^R phenotype with the loss of the transferred chromosome 9, as inferred by the loss of the integrated Ecogpt marker. Although a marker could, in principle, be eliminated without loss of the chromosome, we have not observed it in mouse–human hybrids tested so far by using more than 10^6 cells in each experiment. Furthermore, concomitant loss of UV^R phenotype and Ecogpt sequences was observed in clones isolated in two independent experiments.

As in previous studies (19, 20), only partial reversal of UV^S phenotype in XP-A cells was observed in the chromosome-transfer clones. This has been interpreted to result from gene-dosage effects (20). Alternately subunit mixing of the enzymes from normal and mutated gene(s) could partially "poison" the active enzyme, leading to a lower activity in complemented UV^R clones. All these hypotheses remain to be tested.

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