Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5512–5516, July 1989
Genetics

Molecular cloning of a mouse DNA repair gene that complements the defect of group-A xeroderma pigmentosum

(DNA excision repair/DNA transfection)

KIYOJI TANAKA*, ICHIRO SATOKATA, ZENZABURO OGITA, TSUYOSHI UCHIDA, AND YOSHIO OKADA

Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan

Communicated by Frank H. Ruddle, April 24, 1989

ABSTRACT **For isolation of the gene responsible for xeroderma pigmentosum (XP) complementation group A, plasmid pSV2gpt and genomic DNA from a mouse embryo were cotransfected into XP2OSSV cells, a group-A XP cell line. Two primary UV-resistant XP transfectants were isolated from about 1.6 × 10^5 pSV2gpt-transformed XP colonies. pSV2gpt and genomic DNA from the primary transfectants were again cotransfected into XP2OSSV cells and a secondary UV-resistant XP transfectant was obtained by screening about 4.8 × 10^5 pSV2gpt-transformed XP colonies. The secondary transfectant retained fewer mouse repetitive sequences. A mouse gene that complements the defect of XP2OSSV cells was cloned into an EMBL3 vector from the genome of a secondary transfectant. Transfections of the cloned DNA also conferred UV resistance on another group-A XP cell line but not on XP cell lines of group C, D, F, or G. Northern blot analysis of poly(A)$^+$ RNA with a subfragment of cloned mouse DNA repair gene as the probe revealed that an ≈1.0-kilobase mRNA was transcribed in the donor mouse embryo and secondary transfectant, and ≈1.0- and ≈1.3-kilobase mRNAs were transcribed in normal human cells, but none of these mRNAs was detected in three strains of group-A XP cells. These results suggest that the cloned DNA repair gene is specific for group-A XP and may be the mouse homologue of the group-A XP human gene.**

Xeroderma pigmentosum (XP) is an autosomal recessive human disease, clinically characterized by the early onset of severe photosensitivity of exposed skin to sunlight, a very high incidence of skin cancers, and frequent neurological abnormalities. Cells from XP patients are hypersensitive to killing by ultraviolet light (UV), because they have a defect in the repair of UV-induced DNA damage (1). Genetic complementation analysis by cell fusion has led to the identification of at least 10 genetic complementation groups, designated groups A through I and a variant (2–4). Group-A through -I XP cells have defects in the early step of DNA excision repair (5–9). The XP variant was first thought to have normal excision repair and impaired post-replication repair (10), but recent studies have suggested that it is also defective in excision repair (9, 11, 12). However, the genetic basis of the physiological defect of XP has not yet been characterized. No XP genes or gene products have yet been identified. Transfection of genomic DNA from normal cells into XP cells and then the isolation of UV-resistant XP transfectants should facilitate molecular cloning of the XP genes. However, introduction of the genomic DNA from normal cells by ordinary DNA transfection methods has thus far been unsuccessful in introducing genes that code for UV resistance into XP cells. That is, the frequency of appearance of UV-resistant colonies was the same regardless of previous treatment of the cells, indicating that the frequency of reversion to a UV-resistance phenotype was substantially higher than the frequency of transfection of the DNA repair gene in the conditions used (13, 14). For obtaining true UV-resistant XP transfectants by the DNA transfection methods, it seems necessary to reduce the possibility of selecting DNA repair-proficient XP revertants. We adopted the procedure of cotransfection of genomic DNA from heterologous (mouse) normal cells with pSV2gpt and initially selected pSV2gpt-transformed XP (group A) cells that identified small subpopulations of XP cells that were competent to take up mouse genomic DNA. After UV-irradiation of the pSV2gpt-transformed XP colonies, we then selected UV-resistant XP colonies. In this way we isolated two primary UV-resistant XP transfectants and one secondary UV-resistant XP transfectant and found that these transfectants retained common mouse sequences. We also molecularly cloned a mouse gene that complements the defect of group-A XP cells.

MATERIALS AND METHODS

Cell Lines and Plasmids. Simian virus 40-transformed human fibroblast cell lines were provided by the following individuals: Masayoshi Namba [Okayama University (WI38VA13 cells; normal human)]; Hiraku Takebe and Mituo Ikenaga [Kyoto University (XP2OSSV and XP2YOSV cells; group-A and group-F XP, respectively)]; Dirk Bootsma and Jan H. J. Hoeijmakers [Erasmus University, Rotterdam (XP12ROSV cells; group-A XP)]; Alan Sarasin [Institut de Recherche Scientifique sur le Cancer/Centre National de la Recherche Scientifique (XP4PASV cells; group-C XP)]; Kenneth H. Kraemer [National Institutes of Health (XP6BESV cells; group-D XP)]; and B. Klein [State University Leiden, The Netherlands (XP3BRSV cells; group-G XP)]. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 or 10% (vol/vol) fetal calf serum (General Scientific Laboratories, Los Angeles). Plasmids pSV2gpt and pSV2neo were obtained from Paul Berg (Stanford University). Plasmid p7014, which contains four kinds of mouse repetitive sequences (MIF, Bam5, R, and B1) was obtained from Hideo Yamagishi (Kyoto University).

UV Survival. For colony formation, cells were inoculated into 100-mm Petri dishes at densities of 4 × 10^3 to 10^4 cells per dish. The following day, the cells were UV-irradiated at (0.4 J·m^{-2}·sec^{-1}). The cells were then cultured for 10–14 days in normal growth medium, fixed with 80% (vol/vol) methanol, and stained with Giemsa, and colonies were counted.

UV-Induced Unscheduled DNA Synthesis. The rate of UV-induced unscheduled DNA synthesis was measured as described (5).

DNA and RNA Extractions. DNA from a whole mouse embryo (ICR strain) of about 14 days was extracted as described by Maniatis *et al.* (15). Total RNA was purified as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: XP, xeroderma pigmentosum.

*To whom reprint requests should be addressed.

described (16) and poly(A)$^+$ RNA was selected through oligo(dT)-cellulose chromatography (17).

DNA Transfection. Samples of 10^6 XP cells were seeded into 100-mm Petri dishes (Corning 25020) in DMEM supplemented with 10% fetal calf serum. The following day the cells were transfected with 5 μg of pSV2gpt and 20 μg of high molecular weight DNA from the ICR mouse embryo per Petri dish by the calcium phosphate precipitation methods (18). After 2–3 weeks of incubation in selection medium (19), the pSV2gpt-transformed XP colonies formed were UV-irradiated (4 J/m^2) (Toshiba germicidal lamp; 1.2 J·m^{-2}·sec^{-1}) two or three times at intervals of 4 days. Healthy colonies that survived were selected, transferred to Petri dishes, and UV-irradiated again to confirm that they were UV-resistant.

Filter Hybridization. Restriction enzyme-digested DNA and poly(A)$^+$ RNA were partitioned on agarose gel, transferred to a nitrocellulose sheet (Schleicher & Schuell) or a nylon membrane (Hybond-N, Amersham), and hybridized to random-primer-extended probes (Amersham) as recommended by suppliers.

Cosmid or Phage Library. Cosmid or phage DNA libraries of the secondary UV-resistant XP transfectant were constructed as described (15) using cosmid pHC79 or EMBL3 phage vectors. These libraries were screened by colony or plaque hybridization on a nylon membrane (Colony/PlaqueScreen, NEF-978A, NEN), as recommended by the supplier.

RESULTS

Transfection of the Mouse DNA Repair Gene into Group-A XP Cells by the Calcium Phosphate Precipitation Method. For DNA transfection, a simian virus 40-transformed group-A XP cell line, named XP2OSSV, was used as the recipient. High molecular weight genomic DNA isolated from a 14-day mouse embryo (ICR strain) was used as a donor. Mouse genomic DNA was cotransfected with plasmid pSV2gpt. The pSV2gpt-transformed XP colonies were first selected in medium containing mycophenolic acid. After incubation in this medium for 2–3 weeks, the XP colonies formed were UV-irradiated (4 J/m^2) two or three times at intervals of 4 days. The frequency of appearance of mycophenolic acid-resistant transfectants was 1–3 $\times$ 10^{-4}/μg of pSV2gpt. Two stable UV-resistant XP colonies were obtained from among 1.6 $\times$ 10^5 pSV2gpt-transformed XP colonies, and named XR13 and XR49. These two UV-resistant XP transfectants were isolated independently in different transfection experiments, but their UV-survival curves were identical and they were slightly more UV-sensitive than WI38VA13 cells, a simian virus 40-transformed normal human cell line (Fig. 1). Consistent with the UV survivals, their rates of UV-induced unscheduled DNA synthesis were also identical and almost equal to that of donor mouse embryonic cells, which was about 70% of that of normal human cells (Fig. 2). These findings suggested that the acquisition of UV resistance by XP cells was due to the acquisition of DNA excision repair capability, and the intermediate restoration of DNA repair ability in the UV-resistant XP transfectants might be due to the heterologous nature (mouse) of the transferred DNA. Southern hybridization analysis of the genomic DNA from the transfectants and parental XP cells using an insert from plasmid p7014 (20), which contains four kinds of mouse repetitive sequences (MIF, Bam5, R, and B1), as probes showed that many mouse sequences were retained in the UV-resistant XP transfectants but that none of these were present in the parental XP2OSSV cells (Fig. 3).

Next, secondary transfection experiments were done. For this, pSV2gpt and genomic DNA from the primary transfectants were again cotransfected into XP2OSSV cells. The pSV2gpt-transformed XP colonies were first selected in medium containing mycophenolic acid and then UV-irradiated (4 J/m^2) two or three times at intervals of 4 days. After cotransfection of genomic DNA from XR13, one of the

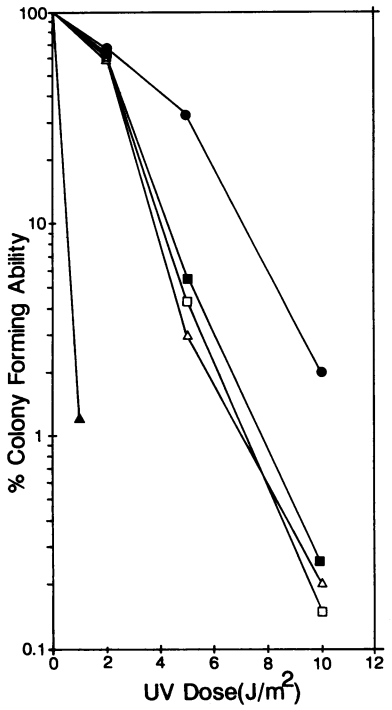

FIG. 1. UV-survival curves as measured by colony-forming ability in normal human cells (WI38VA13 cells, ●), UV-resistant primary (XR13 cells, □; XR49 cells, △) and secondary (XRII130 cells, ■) XP transfectants, and group-A XP cells (XP2OSSV cells, ▲). Points are averages for duplicate dishes.

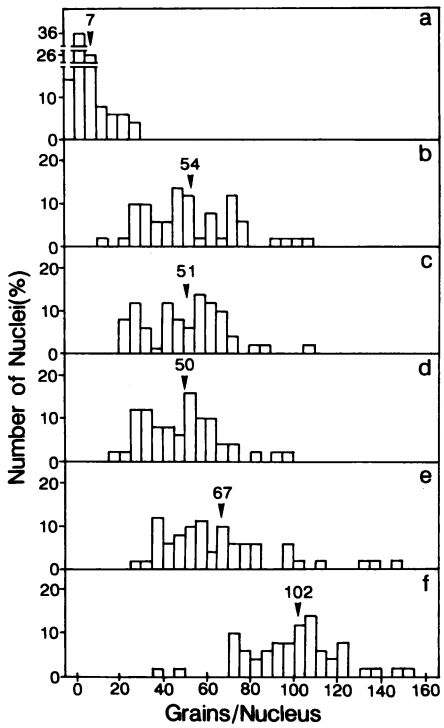

FIG. 2. UV-induced unscheduled DNA synthesis of XP2OSSV cells (*a*), XR13 cells (*b*), XR49 cells (*c*), XRII130 cells (*d*), ICR donor mouse embryonic cells (*e*), and WI38VA13 cells (*f*). The ordinate shows the frequency of labeled nuclei and the grain number is on the abscissa. Arrowheads indicate mean grain numbers per nucleus calculated from numbers in 50 nuclei.

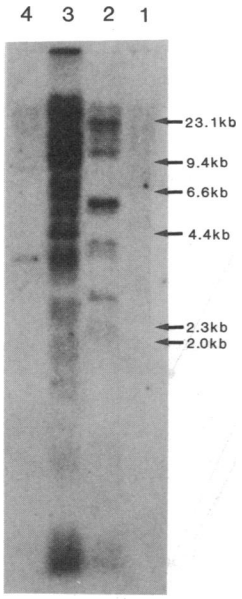

FIG. 3. Southern hybridization of *Bam*HI-digested genomic DNA (10 μg each) with the insert of plasmid p7014, which contains four kinds of mouse repetitive sequences (MIF, Bam5, R, and B1). Lanes: 1, XP2OSSV DNA; 2, XR13 DNA; 3, XR49 DNA; 4, XRII130 DNA. Molecular markers are the phage λ DNA cleaved with *Hin*dIII.

primary transfectants, plus pSV2gpt, about 1.9×10^5 pSV2gpt-transformed XP colonies were screened for UV resistance, but no secondary UV-resistant XP transfectant was obtained. Using genomic DNA from XR49, the other UV-resistant primary XP transfectant, however, we obtained one UV-resistant secondary XP transfectant, named XRII130, by screening 2.9×10^5 pSV2gpt-transformed XP colonies. The UV-survival curves and rate of UV-induced unscheduled DNA synthesis of the UV-resistant secondary XP transfectant were identical to those of the primary transfectants (Figs. 1 and 2). Southern hybridization of genomic DNA from the UV-resistant secondary XP transfectant with mouse repetitive sequences as probes revealed that this secondary transfectant retained a few mouse sequences (Fig. 3).

Molecular Cloning of a Mouse Gene That Complements the Defect of Group-A XP Cells. Only a few fragments hybridized with mouse repetitive sequences were present in the UV-resistant secondary XP transfectant, so we decided to clone the mouse sequences retained in the secondary XP transfectant into cosmid or phage vectors and try to find the gene that complements the defect of group-A XP cells within these sequences.

First, two *Bam*HI fragments of about 3 kilobases (kb) retained in the secondary transfectant (the smaller fragment is not seen clearly in Fig. 3) were cloned into phage L47 vectors. The cloned *Bam*HI fragments were then subcloned into the pUC19 vector and the mouse repetitive sequences were removed from these subcloned fragments. The unique mouse sequences obtained were then used as probes (probes a and b in Fig. 5) in further cloning studies. A cosmid DNA library from the secondary transfectant was constructed, and positive cosmid clones with mouse sequences were isolated by colony hybridization with probes a and b. Using the E_6–E_7 fragment of pHC79/443, one of the positive cosmid clones, as a probe, Southern hybridization of the *Eco*RI-digested genomic DNA from parental XP2OSSV cells, UV-resistant primary and secondary XP transfectants, and an ICR mouse embryo revealed that the E_6–E_7 fragment was retained in all these UV-resistant XP transfectants and the ICR mouse embryo but not in XP2OSSV cells (Fig. 4). The restriction enzyme maps of the UV-resistant XP transfectants were constructed by using subfragments from pHC79/443 as probes. The E_1–E_3 mouse sequences were not found (the E_3 site is present) in XR13 cells, but the E_3–E_7 mouse sequences were found in all three UV-resistant XP transfectants (Fig. 5). Although the UV-resistant XP transfectants were isolated independently in different transfection experiments, they retained the same mouse sequences, suggesting that the mouse gene that complements the defect of group-A XP cells is very closely linked to the E_3–E_7 fragments. Since the mouse sequences on the right side of the pHC79/443 in Fig. 5 are conserved in all these UV-resistant transfectants, "gene walking" toward the right by using a cosmid vector was carried out. A cosmid library from the UV-resistant secondary XP transfectant was screened with the B_5–E_6 fragment of pHC79/443 as a probe (probe c). However, all six positive cosmid clones had integrated rearranged XRII130 genomes, and we, therefore, gave up cosmid walking with probe c. We then constructed an EMBL3 phage library from the secondary transfectant and isolated positive phage clones by plaque hybridization with probe c again. One of the positive clones, EMBL3/24B, retained mouse sequences containing the E_6–B_8 fragment of the secondary transfectant (Fig. 5). Using the B_8–*Sal* I fragment of EMBL3/24B as a probe (probe d), we obtained an overlapping phage, EMBL3/60A. All the inserted sequences of EMBL3/60A were still mouse sequences (Fig. 5). We also screened the cosmid DNA library from the secondary transfectant again with probe d. Clone pHC79/700, which includes the whole insert of EMBL3/60A, was obtained, suggesting that there were no gene rearrangements in pHC79/700 (Fig. 5). The mouse sequences ended in the E_{11}–E_{12} fragment of pHC79/700. On the other hand, in XR13 cells, the E_{10}–E_{11} fragment was missing but the E_9–E_{10} fragment was present, indicating that the mouse sequences retained in XR13 cells were shorter at either end than those in XRII130 cells. XR49 cells retained exactly the same mouse sequences as those retained in XRII130 cells. Therefore, the mouse gene that complements the defect of group-A XP cells seems to be located in the E_3–E_{10} mouse sequences (Fig. 5).

DNA from each cosmid or phage clone (10 μg) was cotransfected with pSV2neo (2 μg) into 10^6 XP2OSSV cells. About 2000 pSV2neo-transformed XP colonies from each were UV-irradiated (4 J/m^2) two or three times at intervals of 4 days. None of them showed enhanced UV resistance. However, cotransfections of EMBL3/24B, EMBL3/60A, plus pSV2neo conferred UV resistance on XP2OSSV cells, and 19 UV-resistant XP colonies were obtained from among 3345 pSV2neo-transformed XP colonies (Table 1). On the other hand, no UV-resistant XP colonies were obtained by cotransfection using pHC79/443, EMBL3/24B, plus pSV2-neo or by transfection of pSV2neo alone, although 3245 and 2000 pSV2neo-transformed XP colonies were obtained, respectively, in these transfection experiments. The cotransfections of EMBL3/24B, pHC79/700, plus pSV2neo also developed many UV-resistant colonies in XP2OSSV cells (Table 1). The UV-survival curves of the UV-resistant XP transfectants that were cotransfected with EMBL3/24B,

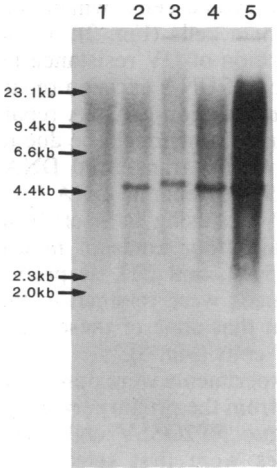

FIG. 4. Southern hybridization of *Eco*RI-digested genomic DNA (10 μg each) from parental XP2OSSV cells (lane 1), XR13 cells (lane 2), XR49 cells (lane 3), XRII130 cells (lane 4), and donor ICR mouse embryo cells (lane 5) with the E_6–E_7 fragment of pHC79/443 as a probe.

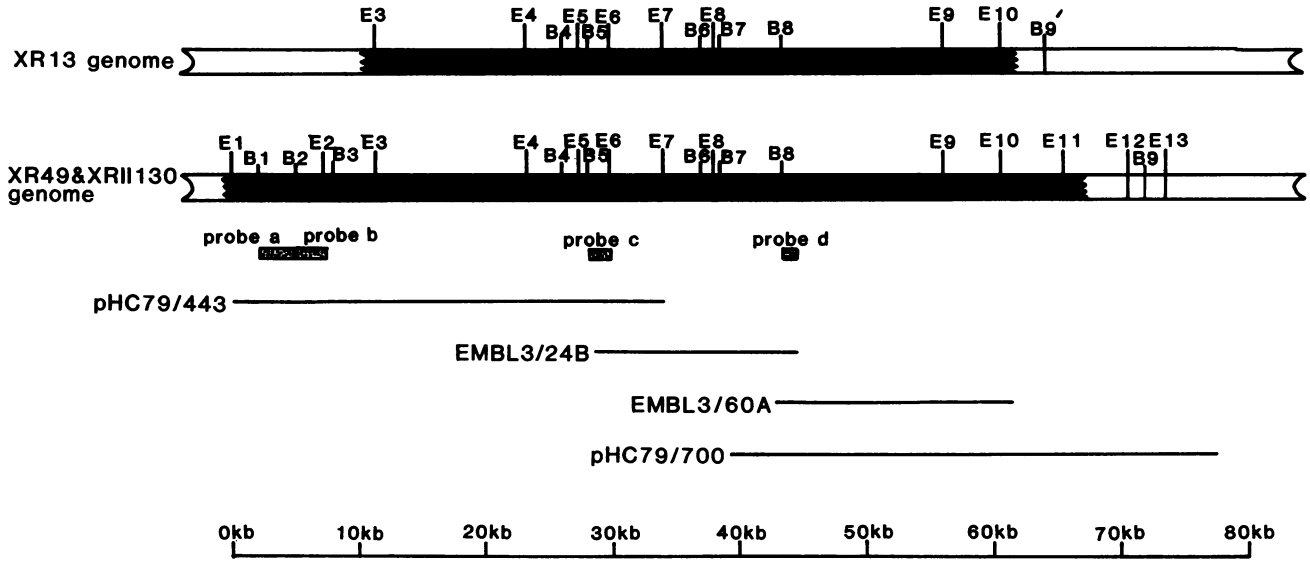

FIG. 5. Restriction enzyme maps of genomic DNA from UV-resistant XP transfectants and the cloned fragments. Open boxes, human (XP) sequences; solid boxes, mouse sequences. E, *Eco*RI site; B, *Bam*HI site. Horizontal bars represent the cosmid or phage inserts isolated. Stippled boxes represent the probes used for cosmid or phage library screenings.

EMBL3/60A (pHC79/700), plus pSV2neo were almost identical to that of the secondary transfectant (data not shown). These results clearly indicate that (*i*) homologous recombination between EMBL3/24B and EMBL3/60A (pHC79/700) occurred in XP cells and (*ii*) a mouse gene that complements the defect of group-A XP cells is located in the region encompassed by inserts of EMBL3/24B and EMBL3/60A and that this gene is less than 30 kb long.

Transfections of the Cloned Mouse DNA Repair Gene into Another Group-A XP Cell Line and into XP Cell Lines of Other Complementation Groups. To determine whether the cloned DNA repair gene could complement the defects of another group-A XP cell line or lines of other groups of XP cells, pSV2neo (2 μg) plus DNA of EMBL3/24B (5 μg) and EMBL3/60A (5 μg) were cotransfected into 10^6 cells from the following cell lines: XP2OSSV (group A), XP12ROSV (group A), XP4PASV (group C), XP6BESV (group D), XP2YOSV (group F), or XP3BRSV (group G) cells. As a negative control, pSV2neo (10 μg) alone was also transfected into these cells. The pSV2neo-transformed XP colonies were first selected and then UV-irradiated (4 J/m^2 for group-A, -D, and -G XP cells or 6 J/m^2 for group-C and -F XP cells). As shown in Table 2, 33 and 65 UV-resistant XP transfectants were obtained among 12,480 and 12,710 pSV2neo-transformed colonies of XP2OSSV and XP12ROSV cells, respectively, but none were obtained in the other groups of XP cells. No UV-resistant XP cells were obtained from any XP cell line by transfection of pSV2neo alone. Thus, the recovery by our cloned DNA repair gene is specific for group-A XP cells.

Detection of mRNA. To determine the size of mRNA transcribed from our cloned DNA repair gene, the inserts of EMBL3/24B and EMBL3/60A were fragmented (0.3–2.0 kb) and used as probes for Northern blot analysis of poly(A)$^+$ RNA from three lines of group-A XP cells, a secondary transfectant, normal human cells, and a donor mouse embryo. By using the 0.7-kb subfragment of the B_7–B_8 fragment as a probe, an ≈1-kb mRNA was detected in mouse embryo and secondary transfectant. The amount of transcript is much higher in the mouse embryo than in the secondary transfectant. Moreover, ≈1.3- and ≈1.0-kb mRNAs were detected in normal human cells, but none of these mRNA were detected in three group-A XP cell lines (Fig. 6).

DISCUSSION

For isolation of the gene that complements the defect of group-A XP, plasmid pSV2gpt and mouse genomic DNA were cotransfected into XP2OSSV group-A XP cells. Two primary and one secondary UV-resistant XP transfectants were obtained by screening 6.4 (1.6 + 4.8) × 10^5 pSV2gpt-transformed XP colonies. The secondary transfectant retained fewer mouse repetitive sequences (Fig. 3). The pos-

Table 1. Cotransfection of EMBL3/24B and EMBL3/60A confers UV resistance on XP2OSSV cells

Donor DNA	UV-resistant colonies, no. per dish	Total UV-resistant colonies, no. per 10 dishes	Total Geneticin-resistant colonies, no. per 10 dishes
Exp. 1			
pSV2neo (2 μg) + pHC79/443 (5 μg) + EMBL3/24B (5 μg)	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	3245
pSV2neo (2 μg) + EMBL3/24B (5 μg) + EMBL3/60A (5 μg)	1, 2, 0, 5, 0, 2, 1, 1, 2, 5	19	3345
pSV2neo (5 μg)	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	2000
Exp. 2			
pSV2neo (3 μg) + EMBL3/24B (5 μg) + pHC79/700 (5 μg)	3, 9, 2, 5, 4, 3, 8, 8, 6, 9	57	2470
pSV2neo (5 μg)	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	3700

Table 2. Cotransfection of EMBL3/24B and EMBL3/60A DNA confers UV resistance on group-A XP cell lines but not on group-C, -D, -F, or -G XP cell lines

Complementation group	Cell line	Transfection	No. UV^r colonies/no. $Geneticin^r$ colonies
A	XP2OSSV	+	33/12,480
	XP2OSSV	−	0/12,440
A	XP12ROSV	+	65/12,710
	XP12ROSV	−	0/13,720
C	XP4PASV	+	0/7000
	XP4PASV	−	0/3640
D	XP6BESV	+	0/10,300
	XP6BESV	−	0/6760
F	XP2YOSV	+	0/2895
	XP2YOSV	−	0/2840
G	XP3BRSV	+	0/24,000
	XP3BRSV	−	0/7380

+, Cotransfection of PSV2neo, EMBL3/24B, and EMBL3/60A; −, transfection of pSV2neo alone; UV^r, UV resistant; $Geneticin^r$, Geneticin resistant.

sibility that the secondary transfectant contained mouse DNA by chance is extremely unlikely. The limitations and difficulties experienced in genomic DNA transfection experiments using human cells have been reported (21, 22). These explain the extremely low transfection frequency of DNA repair gene observed in our work and the negative results obtained in other laboratories (13, 14). We cloned the mouse sequences retained in the secondary transfectant into cosmid and EMBL3 phage vectors and found that all three independent transfectants retained common mouse sequences (Figs. 4 and 5) and cotransfections of cloned EMBL3/24B and EMBL3/60A (pHC79/700) phage DNA conferred UV resistance at much higher frequency compared to the frequency after genomic DNA transfections (Table 1). These results indicate that we have cloned the mouse DNA repair gene that complements the defect of group-A XP cells.

Our cloned gene complemented the defect in DNA excision repair of two lines of group-A XP cells, but not group-C, -D, -F, or -G XP cell lines. Therefore, our gene does not seem to be a gene for nonsense or missense suppressors or for an enzyme involved in compensatory repair bypass, such as *Micrococcus luteus* UV endonuclease or T4 endonuclease V, which restore UV-induced unscheduled DNA synthesis of all complementation groups of XP cells *in vivo* (5, 23). Northern blot analysis using a subfragment of our cloned mouse DNA repair gene as a probe revealed that an ≈1.0-kb mRNA was transcribed in the donor mouse embryo and secondary transfectant, and ≈1.0- and ≈1.3-kb mRNAs were transcribed in normal human cells, but none of these mRNA were detected in three strains of group-A XP cells (Fig. 6). These results

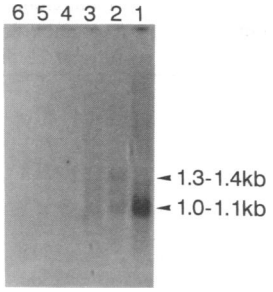

FIG. 6. Northern blot analysis of poly(A)$^+$ RNA (20 μg each) from donor mouse embryo cells (lane 1), WI38VA13 cells (lane 2), XRII130 cells (lane 3), and three group-A XP cell lines, XP12ROSV (lane 4), XP12BESV (lane 5), and XP2OSSV (lane 6) using a 0.7-kb subfragment of the B_7–B_8 fragment of EMBL3/24B as a probe.

strongly suggest that we have cloned the mouse homologue of the group-A XP gene and that mRNA of group-A XP gene are missing in group-A XP cells tested.

By using the method of microinjection of poly(A)$^+$ RNA from normal human cells into group-A XP cells, Hoeijmakers *et al.* (24) reported that the size of mRNA that restored the UV-induced unscheduled DNA synthesis in group-A XP cells was 1.2–1.4 kb, which agrees well with the size of our gene's transcript.

Based on the nomenclature proposed at the Seventh Human Gene Mapping Conference (25), we term our cloned gene mouse homologue of *XPAC* (XP group A complementing) gene.

We are very grateful to Drs. Yoshihide Hayashizaki, Akira Horii, and Ken-ichi Matsubara (Osaka University) and Drs. Yoshimichi Nakatsu, Kenji Shimizu, and Mutsuo Sekiguchi (Kyushu University) for helpful suggestions on construction of cosmid and phage libraries. This work was supported by a grant from the Foundation for Promotion of Cancer Research and Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

1. Cleaver, J. E. (1968) *Nature (London)* **218,** 652–656.
2. de Weerd-Kastelein, E. A., Keijzer, W. & Bootsma, D. (1972) *Nature (London) New Biol.* **238,** 80–83.
3. Fischer, E., Keijzer, W., Thielman, H. W., Popanda, O., Bohnert, E., Edler, E. G., Jung, E. G. & Bootsma, D. (1985) *Mutat. Res.* **145,** 217–225.
4. Kraemer, K. H., Lee, M. M. & Scotto, J. (1987) *Arch. Dermatol.* **123,** 241–250.
5. Tanaka, K., Sekiguchi, M. & Okada, Y. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4071–4075.
6. Fornace, A. J., Kohn, K. W. & Kann, H. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 39–43.
7. Zelle, B. & Lohman, P. H. M. (1979) *Mutat. Res.* **62,** 363–368.
8. Clarkson, J. M., Mitchell, D. L. & Adair, G. M. (1983) *Mutat. Res.* **112,** 289–299.
9. Thielmann, H. W., Edler, L., Popanda, O. & Friemel, S. (1985) *J. Cancer Res. Clin. Oncol.* **109,** 227–240.
10. Lehmann, A. R., Kirk-Bell, S., Arlett, C., Paterson, M. C., Lohman, P. H. M., de Weerd-Kastelein, E. A. & Bootsma, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 219–223.
11. Kondo, S., Satoh, Y. & Kuroki, T. (1987) *Mutat. Res.* **183,** 95–101.
12. Wood, R. D., Robbins, P. & Lindahl, T. (1988) *Cell* **53,** 97–106.
13. Royer-Pokora, B. & Haseltine, W. A. (1984) *Nature (London)* **311,** 390–392.
14. Schultz, R. A., Barbis, D. P. & Friedberg, E. C. (1985) *Somat. Cell Mol. Genet.* **11,** 617–624.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Chirgwin, J. M., Przybyla, A. F., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
17. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
18. Corsaro, C. M. & Pearson, M. L. (1981) *Somat. Cell Mol. Genet.* **7,** 603–616.
19. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2072–2076.
20. Fujimoto, S., Tsuda, T., Toda, M. & Yamagishi, H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2072–2076.
21. Hoeijmakers, J. H. J., Odijk, H. & Westerveld, A. (1987) *Exp. Cell Res.* **169,** 111–119.
22. Mayne, L. V., Jones, T., Dean, S. W., Harcourt, S. A., Lowe, J. E., Priestley, A., Steingreimsdottir, H., Sykes, H., Green, M. H. L. & Lehmann, A. R. (1988) *Gene* **66,** 65–76.
23. de Jonge, A. J. R., Vermeulen, W., Keijzer, W., Hoeijmakers, J. H. J. & Bootsma, D. (1985) *Mutat. Res.* **150,** 99–105.
24. Hoeijmakers, J. H. J., van Duin, M., Koken, M. H. M., Westerveld, A., Eker, A. P. M., Jaspers, K., Weeda, G., van der Eb, A. J. & Bootsma, D. (1988) *J. Cell. Biochem.* Suppl. **12A,** 244.
25. Shows, T. B., McAlpine, P. J. & Miller, R. L. (1984) *Cytogenet. Cell Genet.* **37,** 340–390.