Mutation Research, 217 (1989) 83–92
DNA Repair
Elsevier

MTR 06318

The cloned human DNA excision repair gene *ERCC-1* fails to correct xeroderma pigmentosum complementation groups A through I

M. van Duin, G. Vredeveldt, L.V. Mayne [1,*], H. Odijk, W. Vermeulen, B. Klein [2], G. Weeda [2], J.H.J. Hoeijmakers, D. Bootsma and A. Westerveld

Department of Cell Biology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam (The Netherlands),
[1] MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR (U.K.) and
[2] Department of Medical Biochemistry, University of Leiden, 2333 AL Leiden (The Netherlands)

(Received 15 July 1988)
(Revision received 29 August 1988)
(Accepted 30 August 1988)

Keywords: DNA excision repair; *ERCC-1* gene; Xeroderma pigmentosum; Transfection; UV-sensitive CHO mutants

Summary

The human DNA excision repair gene *ERCC-1* complements the ultraviolet light (UV) and mitomycin C (MMC) sensitivity of CHO mutants of complementation group 1. We have investigated whether *ERCC-1* is the mutated gene in cell lines from xeroderma pigmentosum (XP) complementation groups A through I by analyzing the endogenous gene in XP cells and by introduction of the gene followed by repair assays. Our studies show that *ERCC-1* is not deleted or grossly rearranged in representative cell lines of 9 XP groups. Furthermore, Northern blot analysis revealed correct transcription of *ERCC-1* in all groups. The cloned human *ERCC-1* gene was introduced into immortalized XP cells by DNA transfection (groups A, C, D, E and F). The presence of the integrated transfected sequences was verified on Southern blots and by selection for 2 dominant marker genes that flank the *ERCC-1* gene on the transfected cos43-34 DNA. *ERCC-1* failed to confer a normal UV survival and UV-induced unscheduled DNA synthesis (UDS) to transfected populations. In the case of the remaining XP complementation groups (B, G, H and I), nuclear microinjection was used to introduce an *ERCC-1* cDNA construct driven by an SV40 promoter into primary fibroblasts. Coinjection of the SV40 large T gene and analysis of its expression served as a control for the injection. The *ERCC-1* cDNA failed to induce increased levels of UDS in the microinjected fibroblasts. We infer from these experiments that *ERCC-1* is not the mutated gene in the 9 XP complementation groups examined. From a similar type of experiments we conclude that *ERCC-1* is not the defective gene in UV-sensitive Cockayne's syndrome cells.

Cell fusion experiments have so far identified 7 complementation groups of UV-sensitive Chinese hamster mutants (Thompson et al., 1981, 1987; Zdzienicka et al., 1988). Cell lines of groups 1 and 2 have been exploited successfully in DNA-media-

Correspondence: Dr. M. van Duin, Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam (The Netherlands).

* Present address: Sussex Centre for Medical Research.

ted gene transfer experiments aimed at the isolation of complementing human DNA repair genes. This has resulted in the cloning of human *ERCC-1* and *ERCC-2* genes (Westerveld et al., 1984; Van Duin et al., 1986; Weber et al., 1988). *ERCC-1* and *ERCC-2* specifically correct the mutant phenotype of CHO group 1 * and group 2 * mutants respectively, indicating that both genes are the human homologues of the mutated loci in the corresponding CHO complementation groups (Van Duin et al., 1988a; Weber et al., 1988). In addition to an enhanced sensitivity to UV, CHO group 1 mutants display an extreme sensitivity to MMC and other bifunctional alkylating agents (Hoy et al., 1985). A defective incision step of the excision of DNA damage is thought to underlie the phenotype of CHO group 1 mutants (Thompson et al., 1982). The cloned *ERCC-1* gene corrects the impaired excision repair of both UV- and MMC-induced DNA adducts (Westerveld et al., 1984). Furthermore, cross-sensitivities of group 1 mutants to other chemical agents are corrected by the cloned gene (Zdzienicka et al., 1987).

The phenotype of CHO group 1 mutants is reminiscent of the rare human autosomal recessive disorder xeroderma pigmentosum (XP). Patients suffering from this trait are hypersensitive to sunlight exposure and have an elevated risk for skin tumor development (Kraemer, 1983). 9 XP complementation groups have been identified (Fischer et al., 1985) that are all characterized by a defective incision step of the DNA excision repair pathway.

Interspecies complementation studies with UV-sensitive human and rodent DNA repair mutants, to determine whether they belong to identical complementation groups are incomplete and have not yet identified overlap. Stefanini et al. (1985) recently reported transient complementation of the repair defect in heterokaryons of 6 XP groups after fusion with UV-sensitive CHO12RO cells. However, this CHO mutant has not been assigned to any of the known CHO complementation groups. Thompson et al. (1985) have attempted complementation analysis between 2 CHO groups (1 and 4) and XP fibroblasts based on recovery of stable hybrid cells resistant to DNA damage. The low frequency of hybrid formation did not permit conclusions with respect to all XP groups and crosses of both CHO groups with XP-A, -C, -D, -F, and -G yielded only partial complementation (Thompson et al., 1985). Therefore, the question whether identical genes are affected in CHO and human repair mutants can probably be more efficiently addressed when the isolated genes are available.

The phenotypic similarity between CHO group 1 and human XP cells renders *ERCC-1* a potential candidate gene which could account for one of the genetic defects in this repair syndrome. In this report we have investigated this possibility. We conclude from our experiments that *ERCC-1* can most likely be excluded as the mutated gene in the XP complementation groups examined.

Materials and methods

Cell lines and media

The cell lines used in the experiments are listed in Table 1. The immortalized cells were cultured in DMEM/F10 medium supplemented with fetal calf serum (FCS) and antibiotics (penicillin, streptomycin). Primary fibroblasts were cultured in F10 medium with FCS and antibiotics. All cells were grown as monolayers in a 37°C incubator.

DNA transfection and cloning efficiency of transformants

On cosmid 43-34 the *ERCC-1* gene is flanked by the selectable marker genes coding for aminoglycosylphosphotransferase (*agpt*) and the *E. coli* guaninephosphoribosyltransferase (*Ecogpt*) (see Fig. 3) which allow selection for G418 and mycophenolic (MPA) resistance, respectively. Cosmid 43-34 DNA was transfected as a calcium phosphate precipitate into the different cell lines as previously described (Westerveld et al., 1984). 1–2 days after transfection selection was started by adding G418 to the medium. The concentration of G418 was dependent on the transfected cell line and ranged from 400 μg/ml to 800 μg/ml medium. After 10–14 days clones were counted

---

* At the recent UCLA meeting on 'Mechanisms and consequences of DNA damage processing' in Taos (January 1988) it was decided to rename CHO complementation groups 1 and 2 to 2 and 1 respectively in order to match the numbering with the complementing *ERCC* genes.

TABLE 1

CELL LINES USED IN THE DIFFERENT EXPERIMENTS OF THIS STUDY

Cell line [a]	Expts. [b]	Ref./source
HeLa	S N	
XP12RO-SV (A)	S N T	Veldhuizen (unpubl.)
XP2OS-SV (A)	T	Takebe et al., 1974
XP12BE-SV (A)	S	Camden cell bank
XP11BE (B)	S N M	Kraemer et al., 1975
XP4PA-SV (C)	S	Daya-Grosjean et al., 1987
XP8CA-SV (C)	N	Klein (unpubl.)
CW12 (C)	T	Wood et al., 1987
MH3XP (D)	N	Wood et al., 1987
XP1BR (D)	S	Keijzer (unpubl.)
HD2 (D)	S T	Johnson et al., 1985
CW3 (E)	N T	Wood et al., 1987
XP2RO (E)	S	De Weerd-Kastelein et al., 1973
XP2YO-SV (F)	N T	Yagi and Takebe, 1983
XP2BI (G)	M	Keijzer et al., 1979
XP3BR (G)	S	Arlett, 1980
XP3BR-SV (G)	N T	Klein (unpubl.)
XP2CS (H)	S N M	Moshell et al., 1983
XP3MA (I)	S M	Fischer et al., 1985
XP20MA (I)	N	Keijzer (unpubl.)

[a] The XP complementation group is given in parentheses.

[b] Southern analysis (S), Northern analysis (N), transfection (T) and/or microinjection (M).

and either isolated or grown into mass populations (mp's). In order to establish the cointegration of *Ecogpt* sequences in G418-resistant mp's, for each mp the cloning efficiency in medium with or without G418 and/or mycophenolic acid (MPA) was determined. For each cell line 3 independent mp's were tested in 3-fold. For XP3BR-SVmp 2000 cells/dish were plated and for the other tested lines 400–500 cells/dish were plated. In Table 2 the average relative cloning efficiency is given.

Southern blotting

Using routine procedures (Maniatis et al., 1982) genomic DNA from cell lines was digested with restriction enzymes and after size fractionation on agarose gels blotted to nitrocellulose filters which were subsequently hybridized to ^{32}P-labeled probes.

Northern blotting

Total RNA was isolated from cultured cells by the LiCl/urea procedure (Auffray and Rougeon, 1980) and size fractionated in 1.2% agarose gels containing formaldehyde. RNA was then transferred to nitrocellulose and hybridized in 50% formamide to *ERCC-1* probe EPv12 using standard procedures (Maniatis et al., 1982).

UV survival and unscheduled DNA synthesis (UDS)

The response of G418-resistant XP transformants to UV was measured by UV survival and UV-induced UDS as reported previously (Van Duin et al., 1988a).

Microinjection of ERCC-1 cDNA into XP primary fibroblasts

ERCC-1 cDNA constructs pcDE (Van Duin et al., 1986) and pSV3gpt (Mulligan and Berg, 1981) were mixed in a 1:1 molar weight ratio (0.15 μg/μl, final concentration) and coinjected into nuclei of XP homopolykaryons using procedures previously described (De Jonge et al., 1983). At 24 and 48 h post injection one part of the injected cells was stained for the expression of SV40 large T antigen encoded by pSV3gpt using a monoclonal antibody and an FITC-containing goat anti-mouse conjugate. In order to determine the effect on the repair potential the other half of the injected cells was at 24 and 48 h post injection

TABLE 2

CLONING EFFICIENCY OF G418-RESISTANT cos43-34 TRANSFORMANTS (mp's) IN NORMAL MEDIUM AND MEDIUM SUPPLEMENTED WITH G418, MPA OR G418 AND MPA EXPRESSED AS PERCENTAGE OF SEEDED CELLS WHICH FORMED COLONIES (see Materials and methods)

Each experiment was carried out in 3-fold. The average values for 3 independent mp's are given.

Cell line [a]	Cloning efficiency (%) in different media			
	Normal	+G418	+MPA	+G418/MPA
CW12mp (C)	35	34	28	27
HD2mp (D)	44	36	31	26
CW3mp (E)	27	24	25	20
XP3BR-SVmp (G)	5	5	0	0

[a] XP complementation group is given in parentheses.

irradiated with UV (15 J/m^2) and incubated for 2 h in medium containing [^{3}H]thymidine. Subsequently, UV-induced DNA synthesis was determined by autoradiography as described previously (Vermeulen et al., 1986).

Results

Analysis of the endogenous ERCC-1 gene in XP

To examine whether XP cells from all complementation groups harbor intact *ERCC-1* gene copies, the endogenous *ERCC-1* gene was analyzed by Southern blotting experiments. High molecular weight DNA was isolated from each of the cell lines and after digestion with various restriction endonucleases size fractionated on agarose gels and transferred to nitrocellulose filters that were subsequently hybridized to a ^{32}P-labeled *ERCC-1* cDNA probe. DNA from HeLa cells was used as a control since the *ERCC-1* gene was originally isolated from this cell line (Westerveld et al., 1984). The autoradiogram of a blot with TaqI-digested XP DNAs is shown in Fig. 1. All cell strains examined showed hybridization patterns similar to the HeLa control. Similar results were obtained with PvuII-digested DNAs (not shown). Therefore, as far as can be detected by this method, we conclude that *ERCC-1* is not deleted or grossly rearranged in the representative cell strains of the 9 XP complementation groups examined.

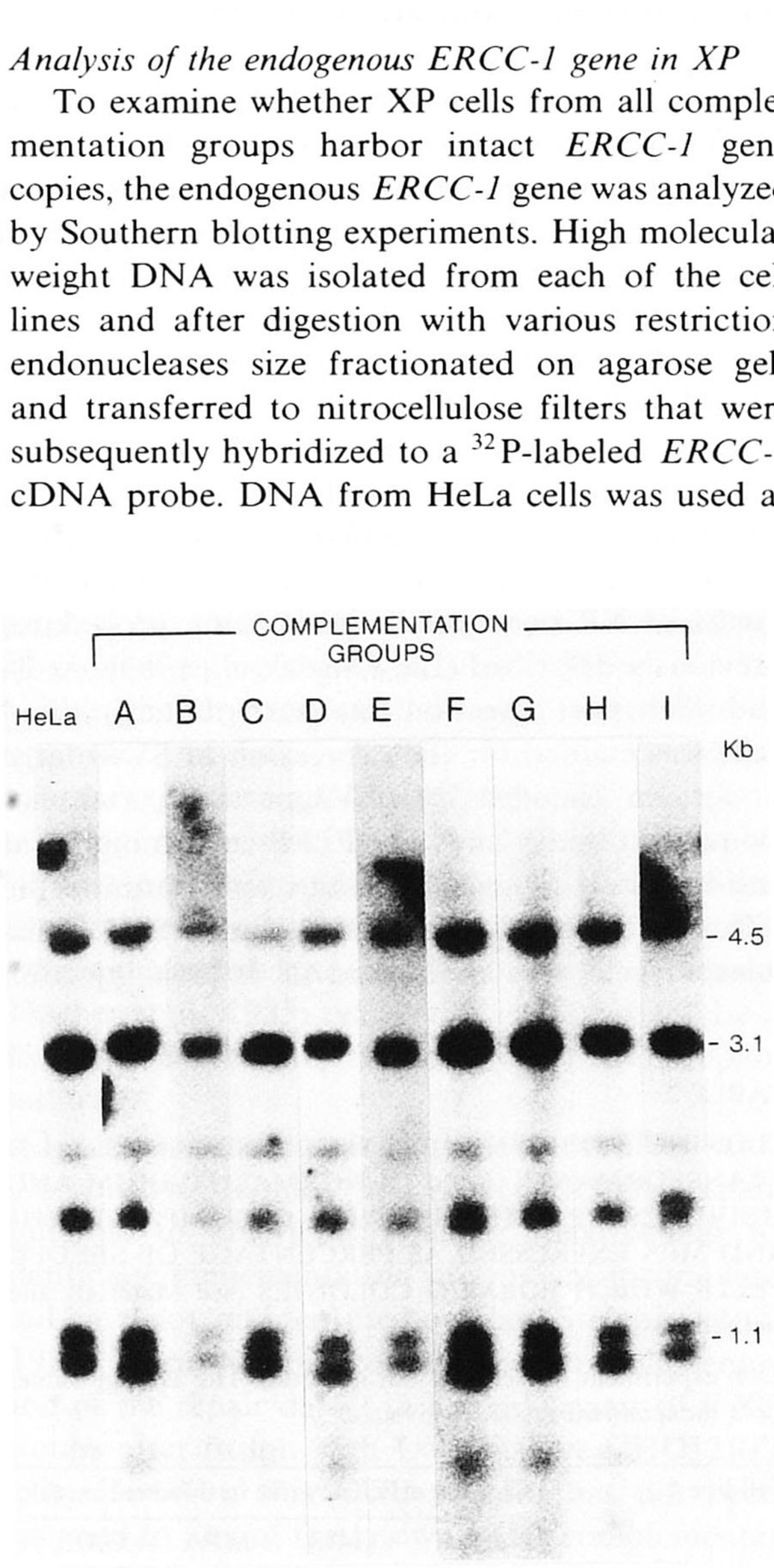

Fig. 1. Southern blot analysis of TaqI-digested DNA of all 9 XP complementation groups (A–I). HeLa DNA served as a control in this experiment. The blot was hybridized to *ERCC-1* cDNA probe EPv12 which recognizes all 10 *ERCC-1* exons. The molecular weight of the hybridizing bands is indicated on the right.

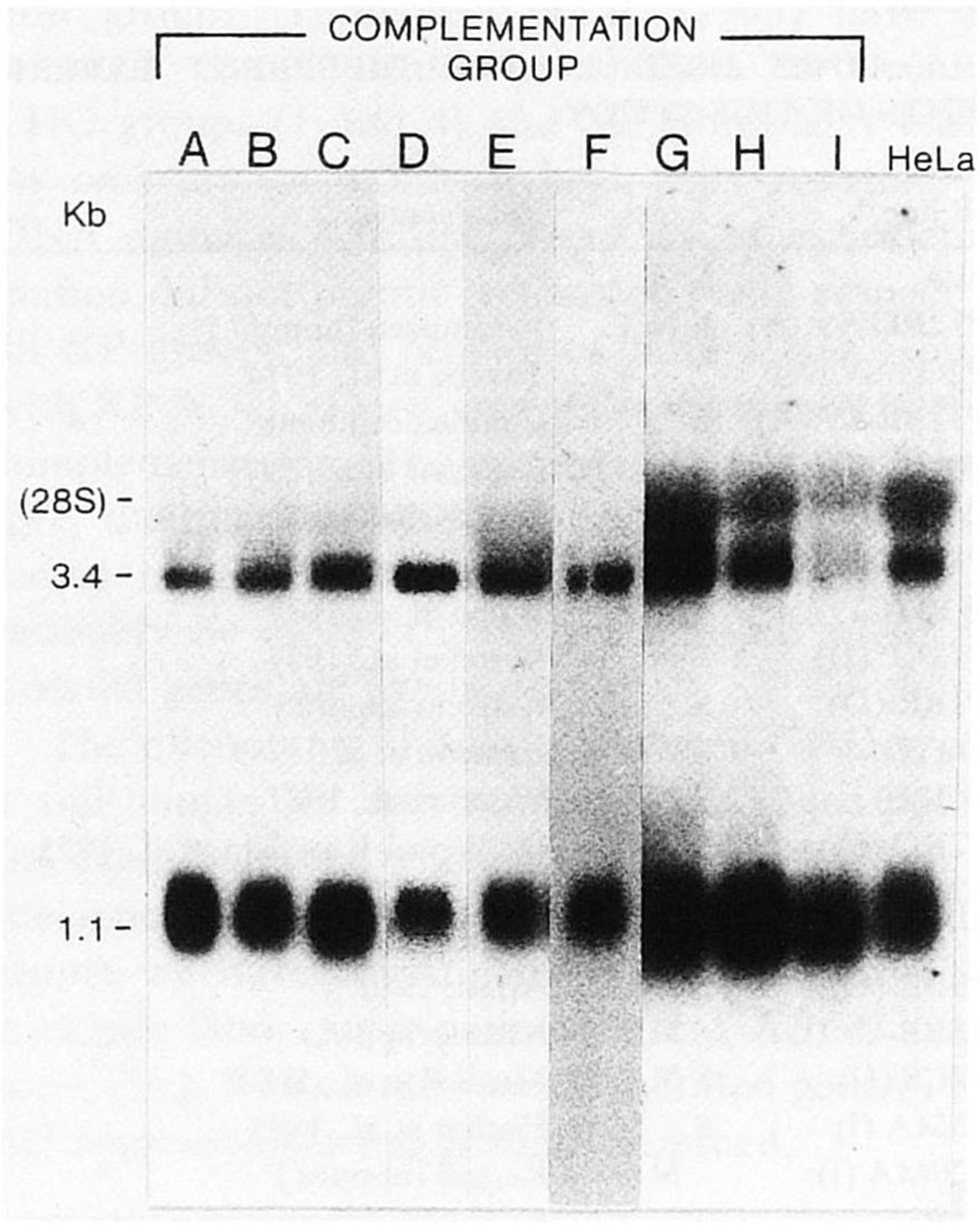

Fig. 2. Northern blot analysis of representative cell lines of all 9 XP complementation groups (A–I) and HeLa as a control. Per lane 15–30 μg total RNA was loaded. The EPv12 probe described in Fig. 1 was used for hybridization in 50% formamide at 42°C. Molecular weight of hybridizing bands is indicated on the left. In some lanes background labeling of ribosomal RNA (28S) is visible due to longer exposure times of the autoradiogram.

To investigate the expression of the endogenous *ERCC-1* gene in XP cells, total RNA was isolated and analyzed by Northern blot experiments using *ERCC-1* cDNA as a probe. The autoradiogram of this experiment is shown in Fig. 2. All 9 XP groups examined produced a correct *ERCC-1* transcript similar to that of HeLa cells.

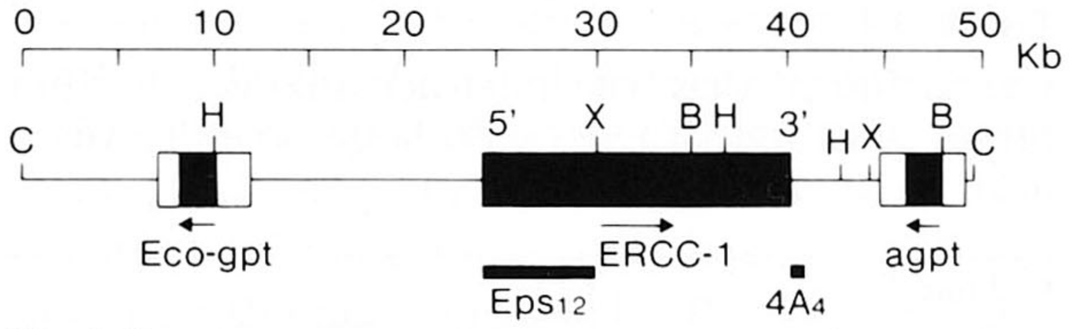

Fig. 3. Structure of cosmid 43-34. The position of the *ERCC-1* gene and *Ecogpt* and *agpt* dominant markers are shown in black. The open parts of the left- and right-hand boxes are pBR322 sequences. Only restriction sites are shown that are relevant for the Southern blot analysis in Fig. 4. Details of cosmid 43-34 have been described previously (Westerveld et al., 1984; Van Duin et al., 1987).

Transfection of ERCC-1 into immortalized XP cells

The Southern and Northern blot experiments presented above do not rule out the possibility that the *ERCC-1* gene is inactivated by point mutations or other small alterations. Therefore, the cloned functional *ERCC-1* gene was examined for its ability to correct the repair defect in XP cells. To that aim the cloned *ERCC-1* gene located on cosmid 43-34 (cos43-34), was transfected into established XP cells of 6 different complementation groups (see Materials and methods). Before transfection cos43-34 DNA was linearized with ClaI which generates 50-kb molecules in which the 5′ and 3′ *ERCC-1* ends are flanked by the dominant marker genes *Ecogpt* and *agpt* at a distance of approximately 10 and 4 kb of genomic DNA respectively (see Fig. 3). The transfected cells were selected in medium containing G418 and after 2 weeks mass populations (mp's) were generated which consisted of 20–200 independent transformants. In general, transfection efficiencies ranged from 10^{-4} to 10^{-5}. The G418-resistant transformants were subsequently examined for the presence of transfected *ERCC-1* DNA. Since on cos43-34 *ERCC-1* is flanked by the *agpt* and *Ecogpt* markers, cross-resistance of G418-resistant cells to mycophenolic acid (MPA), provided by the *Ecogpt* gene, would be indicative of the presence of *ERCC-1* sequences. The presence of the *Ecogpt* marker was determined by measuring the cloning efficiency of G418-resistant cos43-34 transformants in medium containing G418 and MPA. The results presented in Table 2 indicate that XP-C, -D and -E transformants had integrated both bacterial markers with a high efficiency. No MPA-resistant XP-G transformants were obtained. In an earlier report we showed that most of the XP-A and XP-F transformants (60–90%) had cointegrated *Ecogpt* sequences which was confirmed by Southern blot analysis (Hoeijmakers et al., 1987). The results of Southern blot analysis of the XP-C, -D and -E transformants are presented in Fig. 4. To discriminate between cos43-34-derived *ERCC-1* sequences and the endogenous gene the DNAs were digested

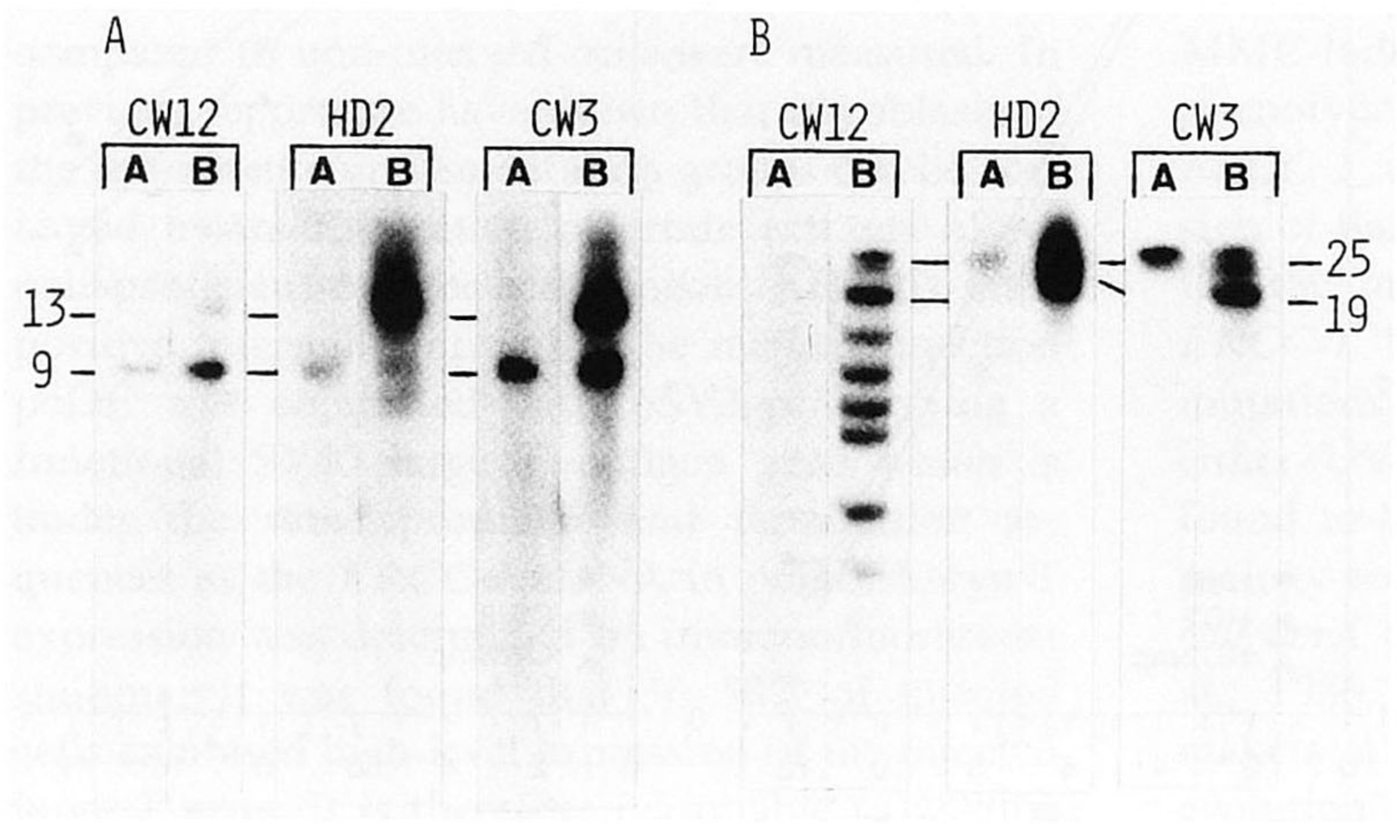

Fig. 4. Southern blot analysis of G418-resistant CW12 (XP-C), HD2 (XP-D) and CW3 (XP-E) cells that have been transfected with cos43-34. In panel A DNA of recipient (lanes A) and transfected cells (lanes B) were digested with BglII and hybridized to probe 4A4 located 3′ of *ERCC-1* (see Fig. 3). Panel B shows HindIII/XhoI double-digested DNA probed with *ERCC-1* cDNA probe EPs12 harboring the first 5 exons. On left and right side fragment sizes (kb) are indicated.

with HindIII/XhoI and BglII which generates cos43-34-specific *ERCC-1* bands when hybridized to 5′ and 3′ *ERCC-1* probes, respectively (see also Fig. 1). From a comparison between the signals of the endogenous and transfected *ERCC-1* gene it can be inferred that all transformed mass populations had integrated multiple copies of *ERCC-1* into their genome. The HD2 mass population has integrated considerably more DNA than CW12 cells in which much more degradation of the transfected DNA seems to have taken place (see Fig. 4). In contrast, integrated *agpt* sequences but not *ERCC-1* DNA or *Ecogpt* dominant marker could be detected in G418-resistant XP-G transformants (not shown) which is in agreement with the finding that these cells did not show cross-resistance to MPA.

Response of XP cos43-34 transformants to DNA damage

To test whether *ERCC-1* restores normal DNA repair characteristics in XP cells we determined

TABLE 3

UV-INDUCED UDS OF IMMORTALIZED XP CELL LINES AND G418-RESISTANT MASS POPULATIONS (mp)

Cell line [a]	UDS	
	Grains [b]	% of HeLa [c]
XP12RO-SV (A)	5 ± 2	13
XP12RO-SVmp (A)	5 ± 1	12
CW12 (C)	7 ± 1	23
CW12mp (C)	7 ± 1	23
HD2 (D)	16 ± 1	47
HD2mp (D)	15 ± 1	43
CW3 (E)	26 ± 1	88
CW3mp (E)	25 ± 1	86
XP2YO-SV (F)	5 ± 1	13
XP2YO-SVmp (F)	5 ± 1	13

[a] XP complementation group is given in parentheses.
[b] Number of grains (± SEM) per fixed square of non-S-phase nuclei.
[c] Percentage of UDS of HeLa, which was determined in each independent experiment.

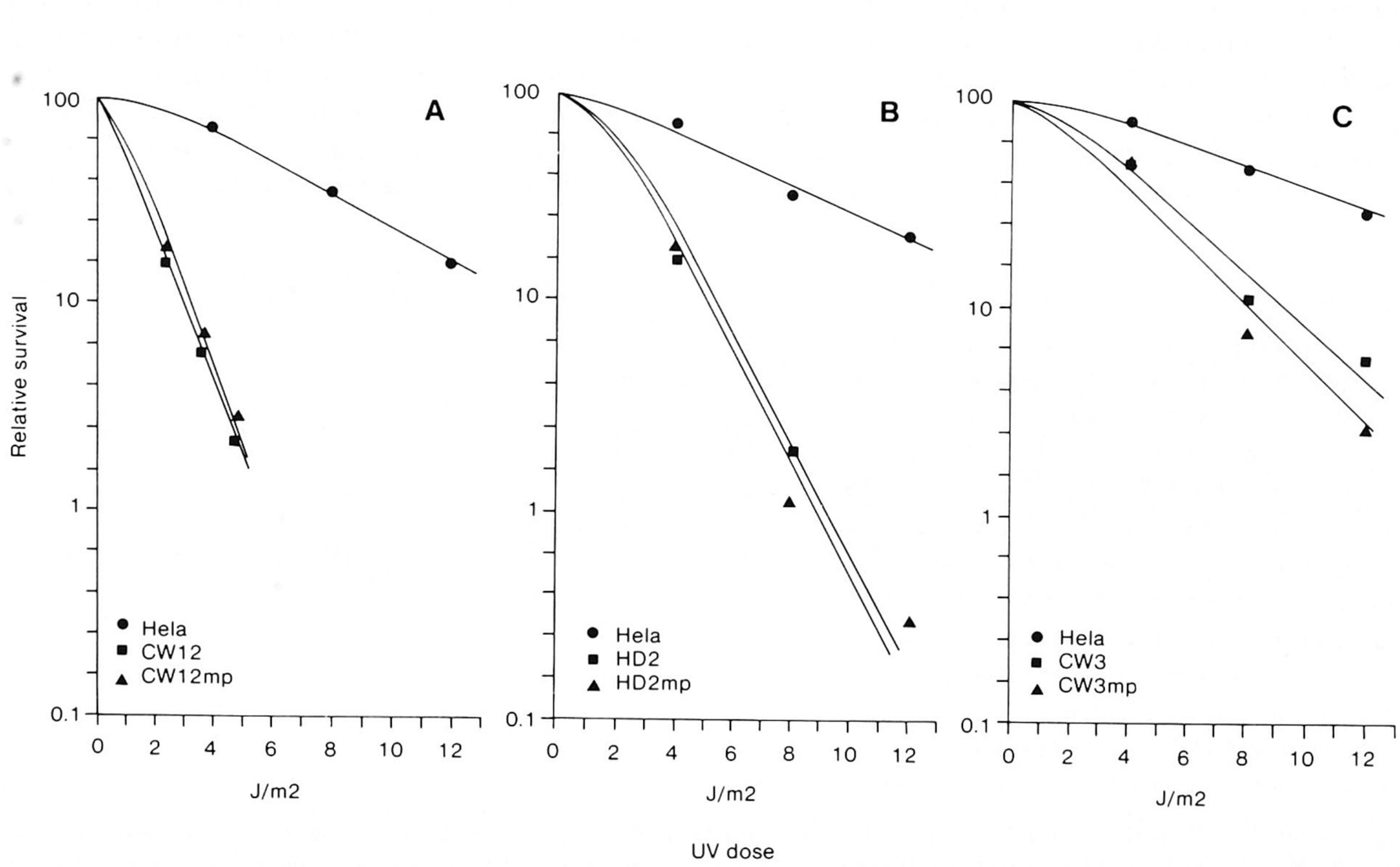

Fig. 5. UV survival plots of XP-C (CW12), XP-D (HD2) and XP-E (CW3) cells and cos43-34-transformed G418-resistant mass populations. HeLa cells served as wild-type controls.

UV survival and UV-induced unscheduled DNA synthesis (UDS) of the G418-resistant mass populations. UV survival plots are shown in Fig. 5 and UDS data are summarized in Table 3. All transformed XP cultures (XP-A and XP-F not shown) harboring transfected *ERCC-1* DNA displayed mutant UV survival phenotypes. The UDS levels of the transformants were comparable with those found in the various mutant cell lines and were in agreement with the survival data. With respect to the relatively high UDS values of the XP-E parental cells and transformants it should be noted that the residual UDS level of primary XP-E fibroblasts is up to 50% of that of repair-proficient cells (De Weerd-Kastelein et al., 1973; Fujiwara et al., 1985). Also HD2 and XP2YO-SV (XP-D and -F respectively) cells display higher UDS values than the original non-transformed diploid XP fibroblast cultures (Johnson et al., 1985; Yagi and Takebe, 1983).

Microinjection of ERCC-1 cDNA

XP complementation groups of which no immortalized cell lines suitable for transfection are available, were examined for *ERCC-1* correction by means of a microinjection assay. *ERCC-1* cDNA construct pcDE (Van Duin et al., 1986), harboring a functional *ERCC-1* cDNA driven by the SV40 early promoter, was injected into nuclei of XP homopolykaryons. After 24 and 48 h the levels of UV-induced DNA synthesis in injected compared to non-injected cells were measured. In previous reports we have shown that fibroblasts of the examined complementation groups can be corrected by microinjection of crude extracts of repair-proficient cells or heterologous XP cells. As a positive internal control for the nuclear injection pcDE was coinjected with pSV3gpt carrying a functional SV40 large T antigen gene which is under the same promoter and termination sequences as the *ERCC-1* cDNA in pcDE. Large T expression was determined by immunofluorescent staining. It was found that 30–50% of injected cells exhibited high-level expression of the injected large T gene. It is therefore reasonable to assume that the coinjected *ERCC-1* cDNA is correctly expressed in approximately the same fraction of cells. XP-B, -G, -H and -I homopolykaryons were subjected to this analysis, however, the introduction of the *ERCC-1* cDNA did not have a significant positive effect on UDS levels in any of these complementation groups (data not shown).

Discussion

The human *ERCC-1* gene was isolated by virtue of its ability to complement the DNA repair defect in CHO complementation group 1 cells. These CHO cells are sensitive to UV and MMC, indicating a role for *ERCC-1* in the excision repair of UV- and MMC-induced DNA damage. We have investigated the possibility that *ERCC-1* is the mutated gene in UV-sensitive XP cells of complementation groups A through I.

Cross-sensitivity to UV and MMC might be used to identify the human counterpart of CHO group 1 mutants. However, for many cell lines of human DNA repair syndromes the response to both agents is poorly documented and often limited to specific complementation groups. Fujiwara and coworkers have reported a normal response of XP-A cells to MMC (Fujiwara et al., 1977; Fujiwara, 1982) and also XP-C (Nagasawa and Little, 1983) and XP-F were reported to have wild-type repair of MMC-induced DNA damage (Plooy et al., 1985). As far as we know for the other complementation groups cross-sensitivity to MMC is not reported in the literature.

The biochemistry of repair of UV-induced DNA damage — primarily pyrimidine dimers — and MMC-induced DNA cross-links is unknown. The phenotype of CHO group 1 cells suggests that *ERCC-1* is involved in a common (pre)incision step of the removal of both types of DNA lesions. On the other hand, it cannot be excluded that *ERCC-1* has a dual function and that different mutations in *ERCC-1* can affect the response to either UV or MMC. The *ERCC-1* protein was found to have a 'mosaic' structure displaying homology to the yeast *RAD10* and parts of the *E. coli uvrA* and *uvrC* repair proteins (Van Duin et al., 1986, 1988b; Doolittle et al., 1986; Hoeijmakers et al., 1986). This could imply that during evolution multiple repair functions have been accommodated in the *ERCC-1* protein. Consequently, it is possible that some mutations in the *ERCC-1* gene might yield only a UV- and not a MMC-sensitive phenotype.

Southern and Northern blot analysis with *ERCC-1* probes did not give indications of altered *ERCC-1* genes in cell lines from all 9 XP complementation groups. These experiments, however, do not exclude point mutations or minor deletions in genomic *ERCC-1* sequences and do not allow any conclusion on the functionality of the *ERCC-1* protein in the examined XP cells.

Introduction of the cloned *ERCC-1* gene by DNA transfection is limited to immortalized cell lines. The experiments with XP3BR-SV (XP-G) cells show that even SV40-transformed lines are not always suitable for efficient DNA transfer. Although G418-resistant clones were obtained after transfection of cos43-34, no cross-resistance to MPA was found indicating that these cells had integrated very small amounts of DNA, which was confirmed by Southern blot analysis (not shown). These results are in accordance with our earlier observations that the quantity of integrated exogenous DNA is cell line specific and that human cells in general take up small amounts of DNA compared to some rodent cell lines (Hoeijmakers et al., 1987; Mayne et al., 1988a).

The *ERCC-1* gene was successfully transfected to XP-A, -C, -D, -E and -F cells whereas nuclear microinjection was used to introduce the *ERCC-1* cDNA in XP-B, -G, -H and -I fibroblasts. The presence of transfected *ERCC-1* sequences was confirmed by Southern blot analysis and double selection on G418 and MPA. Simultaneous injection of plasmid pSV3gpt expressing SV40 large T antigen indicated that in 30–50% of the injected cells the introduced *ERCC-1* cDNA should be properly expressed. *ERCC-1* had no effect on UV survival and UDS of mass populations of 20–200 independent G418-resistant transformants. Likewise in the transient microinjection assay *ERCC-1* cDNA did not elevate UV-induced DNA repair synthesis.

Although we have not demonstrated that the transfected and microinjected *ERCC-1* gene is correctly expressed into functional protein, we believe that our negative results are significant. Firstly, the *ERCC-1* constructs used in the experiments are able to correct the defect in CHO group 1 mutants with a very high efficiency (Westerveld et al., 1984; Van Duin et al., 1986) indicating that they encode functional *ERCC-1* protein and are correctly expressed when introduced into mammalian cells. Secondly, control experiments (i.e. cross-resistance to MPA, Southern blot analysis of the transfected human gene and coinjection with plasmid pSV3gpt) verified that intact *ERCC-1* copies were present in at least a detectable fraction of the various XP cells into which *ERCC-1* was introduced. Therefore, we anticipate that correction of any of the XP groups by *ERCC-1* when present would not have escaped our attention. Hence, it is unlikely that mutations in the *ERCC-1* gene will account for this complex human genetic disorder. Thompson and coworkers (1985) have reported that fusion of CHO group 1 cells, harboring a mutated *ERCC-1* gene, with XP cells of various complementation groups yields (partial) correction of the mutual repair defect, strongly suggesting that these XP groups are not defective in *ERCC-1*. This is in agreement with our conclusion.

Cell lines from patients with Cockayne's syndrome and Fanconi's anemia display a cellular hypersensitivity to UV and MMC, respectively (Kraemer, 1983). Nothing is known about the DNA repair defect in FA. For CS complementation groups A and B it has recently been demonstrated that these cells are deficient in the preferential repair of active genes (Mayne et al., 1988b), which explains their characteristic absence of RNA synthesis recovery after UV exposure (Mayne and Lehmann, 1982). Southern blot analysis indicated normal *ERCC-1* copies in all 3 CS complementation groups and both FA groups (not shown). Furthermore, transfection of *ERCC-1* to CS-A and CS-B cells did not correct the defect in RNA synthesis recovery and survival after UV irradiation (not shown).

The experiments thusfar show that a defective *ERCC-1* gene is most likely not represented in patients from 9 XP excision-deficient complementation groups. This implies that at least 10 genes (9 XP loci and *ERCC-1*) are involved in the incision step of the excision repair mechanism, further stressing the biochemical complexity of this repair process. Furthermore, the fact that an *ERCC-1* defect is not found in XP and possibly also not in CS and FA suggests that mutations in the *ERCC-1* gene may be incompatible with life. Alternatively, it is not excluded that patients with

a defect in *ERCC-1* exist but have not been identified yet.

Acknowledgements

We thank Drs. R.T. Johnson, R.E. Moses, A. Sarasin and H. Takebe for generously providing us with the different immortalized XP and FA cells, N. van Damme, J. de Geus, J. van den Tol and R.C.A. van Ham for technical assistance and C. van Dijk and M. Kuit for help in preparing the manuscript.

This work was financially supported by MEDIGON, Foundation of Medical and Health Research in The Netherlands, Contract No. 900-501-091 and EURATOM, Contract No. BJ6-141-NL.

References

Arlett, C.F. (1980) Mutagenesis in repair deficient human cell strains, in: M. Alacevic (Ed.), Progress in Environmental Mutagenesis, Elsevier, Amsterdam, pp. 162–174.

Auffray, C., and F. Rougeon (1980) Purification of mouse immunoglobulin heavy-chain messenger RNA's from total myeloma tumor RNA, Eur. J. Biochem., 107, 303–314.

Daya-Grosjean, L., M.R. James, C. Drougard and A. Sarasin (1987) An immortalized xeroderma pigmentosum, group C, cell line which replicates SV40 shuttle vectors, Mutation Res., 183, 185–196.

De Jonge, A.J.R., W. Vermeulen, B. Klein and J.H.J. Hoeijmakers (1983) Microinjection of human cell extracts corrects xeroderma pigmentosum defect, EMBO J., 2, 637–641.

De Weerd-Kastelein, E.A., W. Keijzer and D. Bootsma (1973) A third complementation group in xeroderma pigmentosum, Mutation Res., 22, 87–91.

Doolittle, R.F., M.S. Johnson, J. Husain, B. Van Houten, D.C. Thomas and A. Sancar (1986) Domainal evolution of a prokaryotic DNA repair protein and its relationship to active transport proteins, Nature (London), 323, 451–453.

Fischer, E., W. Keijzer, H.W. Thielmann, O. Popanda, E. Bohnert, L. Edler, E.G. Jung and D. Bootsma (1985) A ninth complementation group in xeroderma pigmentosum, XP-I, Mutation Res., 145, 217–225.

Fujiwara, Y. (1982) Defective repair of mitomycin-C crosslinks in Fanconi's anemia and loss in confluent normal human and xeroderma pigmentosum cells, Biochim. Biophys. Acta, 699, 217–225.

Fujiwara, Y., M. Tatsumi and M.S. Sasaki (1977) Cross-link repair in human cells and its possible defect in Fanconi's anemia cells, J. Mol. Biol., 113, 635–649.

Fujiwara, Y., Y. Uehara, M. Ishihashi, Y. Yamamoto and K. Nishioka (1985) Assignment of 2 patients with xeroderma pigmentosum to complementation group E, Mutation Res., 145, 55–61.

Hoeijmakers, J.H.J., M. Van Duin, A. Westerveld, A. Yasui and D. Bootsma (1986) Identification of DNA repair genes in the human genome, Cold Spring Harbor Symp. Quant. Biol., LI, 91–101.

Hoeijmakers, J.H.J., H. Odijk and A. Westerveld (1987) Differences between rodent and human cell lines in the amount of integrated DNA after transfection, Exp. Cell Res., 169, 111–119

Hoy, C.A., L.H. Thompson, C.L. Mooney and E.P. Salazar (1985) Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents, Cancer Res., 45, 1737–1743.

Johnson, R.T., S. Squires, G.C. Elliott, G.L.E. Koch and A.J. Rainbow (1985) Xeroderma pigmentosum D–HeLa hybrids with low and high ultraviolet sensitivity associated with normal and diminished DNA repair ability, respectively. J. Cell. Sci., 76, 115–133.

Keijzer, W., N.G.J. Jaspers, P.J. Abrahams, A.M.R. Taylor, C.F. Arlett, B. Zelle, H. Takebe, P.D.S. Kinmont and D. Bootsma (1979) A seventh complementation group in excision deficient xeroderma pigmentosum, Mutation Res., 62, 183–190.

Kraemer, K.H. (1983) Heritable diseases with increased sensitivity to cellular injury, in: T.B. Fitzpatrick, A.Z. Eisen, K. Wolff, I.M. Freedberg and K.F. Austen (Eds.), Update: Dermatology in General Medicine, McGraw-Hill, New York, pp. 113–141.

Kraemer, K.H., E.A. de Weerd-Kastelein, J.H. Robbins, W. Keijzer, S.F. Barrett, R.A. Pettinga and D. Bootsma (1975) Five complementation groups in xeroderma pigmentosum, Mutation Res., 33, 327–340.

Maniatis, T., E.F. Fritsch and J. Sambrook (1982) In: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mayne, L.V., and A.R. Lehmann (1982) Failure of RNA synthesis to recover after UV-irradiation; An early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum, Cancer Res., 42, 1473–1478.

Mayne, L.V., T. Jones, S.W. Dean, S.A. Harcourt, J.E. Lowe, A. Priestley, H. Steingrimsdottir, H. Sykes, M.H.L. Green and A.R. Lehmann (1988a) SV40-transformed normal and DNA-repair-deficient human fibroblasts can be transfected with high frequency but retain only limited amounts of integrated DNA, Gene, 66, 65–76.

Mayne, L.V., L.H.F. Mullenders and A.A. van Zeeland (1988b) Cockayne's syndrome: a UV-sensitive disorder with a defect in the repair of transcribing DNA but normal overall excision repair, in: E.C. Friedberg and P. Hanawalt (Eds.), Mechanisms and Consequences of DNA Damage Processing, UCLA Symposia on Molecular and Cellular Biology, New Series Vol. 83, in press.

Moshell, W.N., M.B. Ganges, M.A. Lutzner, H.G. Coon, S.F. Barett, J.M. Dupuy and J.H. Robbins (1983) A new patient with both xeroderma pigmentosum and Cockayne's syndrome establishes the new xeroderma pigmentosum complementation group H, in: E.C. Friedberg and B. Bridges (Eds.) Cellular Response to DNA Damage, Liss, New York, pp. 209–213.

Mulligan, R.C., and P. Berg (1981) Selection for animal cells that express the *Escherichia coli* gene coding for xanthine–guanine phosphoribosyltransferase, Proc. Natl. Acad. Sci. (U.S.A.), 78, 2072–2076.

Nagasawa, H., and B. Little (1983) Suppression of cytotoxic effect of mitomycin-C by superoxide dismutase in Fanconi's anemia and dyskeratosis congenita fibroblasts, Carcinogenesis, 4, 795–799.

Plooy, A.C.M., M. Van Dijk, F. Berends and P.H.M. Lohman (1985) Formation and repair of DNA interstrand cross-links in relation to cytotoxicity and unscheduled DNA synthesis induced in control and mutant human cells treated with *cis*-diamminedichloroplatinum(II), Cancer Res., 45, 4178–4184.

Stefanini, M., W. Keijzer, A. Westerveld and D. Bootsma (1985) Interspecies complementation analysis of xeroderma pigmentosum and UV-sensitive Chinese hamster cells, Exp. Cell Res., 161, 373–380.

Takebe, H., S. Nii, M. Jida and H. Utsumi (1974) Comparative studies of host-cell reactivation, colony forming ability and excision repair after UV-irradiation of xeroderma pigmentosum, normal human and some other mammalian cells, Mutation Res., 25, 383–390.

Thompson, L.H., D.B. Busch, K.W. Brookman, C.L. Mooney and P.A. Glaser (1981) Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells, Proc. Natl. Acad. Sci. (U.S.A.), 78, 3734–3737.

Thompson, L.H., K.W. Brookman, L.E. Dillehay, C.L. Mooney and A.V. Carrano (1982) Hypersensitivity to mutation and sister-chromatid-exchange induction in CHO cell mutants defective in incising DNA containing UV lesions, Somat. Cell Genet., 8, 759–773.

Thompson, L.H., C.L. Mooney and K.W. Brookman (1985) Genetic complementation between UV-sensitive CHO mutants and xeroderma pigmentosum fibroblasts, Mutation Res., 150, 423–429.

Thompson, L.H., E.P. Salazar, K.W. Brookman, C.C. Collins, S.A. Stewart, D.B. Busch and C.A. Weber (1987) Recent progress with the DNA repair mutants of Chinese hamster ovary cells, J. Cell Sci., Suppl. 6, 97–110.

Van Duin, M., J. de Wit, H. Odijk, A. Westerveld, A. Yasui, M.H.M. Koken, J.H.J. Hoeijmakers and D. Bootsma (1986) Molecular characterization of the human excision repair gene *ERCC-1*: cDNA cloning and amino acid homology with the yeast DNA repair gene *RAD10*, Cell, 44, 913–923.

Van Duin, M., M.H.M. Koken, J. van den Tol, P. Ten Dijke, H. Odijk, A. Westerveld, D. Bootsma and J.H.J. Hoeijmakers (1987) Genomic characterization of the human excision repair gene *ERCC-1*, Nucleic Acids Res., 22, 9195–9213.

Van Duin, M., J.H. Janssen, J. de Wit, J.H.J. Hoeijmakers, L.H. Thompson, D. Bootsma and A. Westerveld (1988a) Transfection of the cloned human excision repair gene *ERCC-1* to UV-sensitive CHO mutants only corrects the repair defect in complementation group 2 mutants, Mutation Res., 193, 123–130.

Van Duin, M., J. van den Tol, P. Warmerdam, H. Odijk, D. Meijer, A. Westerveld, D. Bootsma and J.H.J. Hoeijmakers (1988b) Evolution and mutagenesis of the mammalian excision repair gene *ERCC-1*, Nucleic Acids Res., 16 (12), Nucl. Acids Res., 16, 5305–5322.

Vermeulen, W., P. Osseweyer, A.J.R. de Jonge and J.H.J. Hoeijmakers (1986) Transient correction of excision repair defects in fibroblasts of 9 xeroderma pigmentosum complementation groups by microinjection of crude human extracts, Mutation Res., 165, 199–206.

Weber, C.A., E.P. Salazar, S.A. Stewart and L.H. Thompson (1988) Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells, Mol. Cell. Biol., 8, 1137–1146.

Westerveld, A., J.H.J. Hoeijmakers, M. Van Duin, J. De Wit, H. Odijk, A. Pastink, R.D. Wood and D. Bootsma (1984) Molecular cloning of a human DNA repair gene, Nature (London), 310, 425–429.

Wood, C.M., T.L. Timme, M.N. Hurt, B.R. Brinkley, D.H. Ledbetter and R.E. Moses (1987) Transformation of DNA repair-deficient human diploid fibroblasts with Simian virus 40 plasmid, Exp. Cell Res., 169, 543–553.

Yagi, T., and H. Takebe (1983) Establishment by SV40 transformation and characteristics of a cell line of xeroderma pigmentosum belonging to complementation group F, Mutation Res., 112, 59–66.

Zdzienicka, M.Z., L. Rosa, A. Westerveld, D. Bootsma and J.W.I.M. Simons (1987) Biological and biochemical consequences of the human *ERCC-1* repair gene after transfection into a repair deficient CHO cell line, Mutation Res., 183, 69–74.

Zdzienicka, M.Z., G.P. Van der Schans and J.W.I.M. Simons (1988) Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells, Mutation Res., 194, 165–170.