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ORIGINAL ARTICLES

Development of a new easy complementation assay for DNA repair deficient human syndromes using cloned repair genes

Madeleine Carreau, Eric Eveno, Xavier Quilliet, Odile Chevalier-Lagente, Annie Benoit, Bianca Tanganelli[1], Miria Stefanini[1], Wim Vermeulen[2], Jan H.J.Hoeijmakers[2], Alain Sarasin and Mauro Mezzina[3]

Laboratory of Molecular Genetics, UPR 42 CNRS–IFC1 Institut de Recherches sur le Cancer, BP 8, 94801 Villejuif, France, [1]Consiglio Nationale delle Richerche, Instituto di Genetica Biochimica Evoluzionistica, Via Abbiategrasso 207, 27100 Pavia, Italy and [2]Department of Cell Biology and Genetics, Medical Genetics Center, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands

[3]To whom correspondence should be addressed

Nucleotide excision repair (NER)-deficient human cells have been assigned so far to a genetic complementation group by a somatic cell fusion assay and, more recently, by microinjection of cloned DNA repair genes. We describe a new technique, based on the host cell reactivation assay, for the rapid determination of the complementation group of NER-deficient xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and photosensitive trichothiodystrophy (TTD) human cells by cotransfection of a UV-irradiated reporter plasmid with a second vector containing a cloned repair gene. Expression of the reporter gene, either chloramphenicol acetyltransferase (CAT) or luciferase, reflects the DNA repair ability restored by the introduction of the appropriate repair gene. All genetically characterized XP, CS and TTD/XP-D cells tested failed to express the UV-irradiated reporter gene, this reflecting their NER defeciency whereas cotransfection with the repair plasmid expressing a gene specific for the given complementation group increased the enzyme activity to the level reached by normal cells. Selective recovery of both reporter enzyme activities was observed after cotransfection with the *XPC* gene for the XP17VI cells and with the *XPA* gene for both XP18VI and XP19VI cells. Using this method, we assigned three new NER-deficient human cells obtained from patients presenting clinical symptoms described as classical XP to either XP group A (XP18VI and XP19VI) and XP group C (XP17VI). Therefore, this technique increases the range of methods now available to determine the complementation group of new NER deficient patients with the advantage, unlike the somatic cell fusion assay or the microinjection procedure, of being simple, rapid, and inexpensive.

Introduction

Xeroderma pigmentosum (XP*), Cockayne's syndrome (CS) and trichothiodystrophy (TTD) are genetic diseases associated with specific defects in one of the major DNA repair pathways, nucleotide excison repair (NER) (1–6). These diseases present heterogeneous clinical symptoms as well as cellular responses to UV radiation. Each of these DNA repair deficiency diseases are divided into numerous complementation groups, which reflects the complexity of the nucleotide excision repair mechanism. Xeroderma pigmentosum is characterized by a high skin sensitivity to sunlight exposure resulting in a high frequency of cancer formation in the exposed area. Some XP patients also present neurological abnormalities (6). Genetic analysis of cells from XP individuals have shown seven complementation groups (designated XP-A to G), each corresponding to a specific gene defect in NER, plus one group (XP variant) with a defect in the post-replicative repair process (6,7). CS is characterized by mental and growth retardation in association with photosensitivity (8). CS patients have been assigned to two complementation groups (A and B). However, some CS individuals also present clinical symptoms of XP and they belong to complementation group B, D or G (9–11). The clinical hallmark of TTD is brittle hair linked to a reduced sulfur and cystine content, with sun sensitivity found in ~50% of all TTD cases described (12). Three complementation groups have recently been reported for the photosensitive TTD patients: TTD-A, TTD/XP-B and TTD/XP-D, the two latter having a NER deficiency genetically related to XP-B and XP-D respectively (13–15). Although CS and TTD share a common phenotype with XP (photosensitivity associated with NER deficiency), they do not exhibit cancer predisposition (5).

Cells from new patients are usually assigned to a given complementation group using the somatic cell fusion assay which measures the level of unscheduled DNA synthesis (UDS) in fused heterokaryons following UV irradiation (16). Classical XP cells have an impaired UV-induced UDS, whereas XP variant and CS cells are widely regarded as presenting normal UDS levels. Complementation analysis in CS is therefore carried out by measuring the recovery of RNA synthesis (RRS) instead of UDS (17). Functional complementation of NER-deficient cells can also be determined by an *in vitro* cell-free assay (18) or by microinjection of mRNA, proteins or cloned repair cDNA followed by UDS (19). Although these three techniques are reliable, they require multiple steps and time consuming procedures (somatic cell fusion assay), expensive equipment (microinjection), or complex protocols to obtain proper cell extracts and DNA substrate preparations (*in vitro* repair assay).

The host cell reactivation assay uses plasmids containing a reporter gene to monitor the DNA repair activity in NER-deficient cells (20–25). We have developed a rapid complementation technique which uses expression vectors containing a reporter gene, either luciferase or the chloramphenicol acetyltransferase (CAT), in a cotransfection experiment with a repair vector expressing a human DNA repair cDNA (*XPA*, *XPB*, *XPC*, *XPD*, and *CSB* previously known as *XPAC*, *ERCC3*, *XPCC*, *ERCC2*, and *ERCC6* respectively, see 26 for new nomenclature). We screened several genetically characterized XP, CS and TTD/XP-D cells for the ability of the repair genes

*Abbreviations: XP, xeroderma pigmatosum; CS, Cockayne's syndrome; TTD, trichothiodystrophy; NER, nucleotide excision repair; CAT, chloramphenicol acetyltransferase; CoA, coenzyme A.

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to reactivate the UV-irradiated pRSVCat and pRSVL reporter plasmids. Cotransfection of the UV-treated reporter plasmids with the repair vector specific for the given complementation group increased the enzyme activity to near normal level whereas cotransfection with the control vectors or other non-specific repair genes resulted in a reduced reporter enzyme activity in all NER-deficient cells tested. Therefore, recovery of luciferase or CAT activity depends on the correction ability of the specific DNA repair gene. Using this method, we assigned three new XP patients to XP complementation group A (XP18VI and XP19VI) and C (XP17VI). Confirmation of these findings were obtained by the somatic cell fusion and microinjection assays.

Materials and methods

Cells

Human primary diploid fibroblasts were obtained from skin biopsies of patients. Cell transformation with SV40 or pLas-wt (SV40 large T antigen) was performed as previously described (27). All normal and NER-deficient cells used are listed in Table I including three new NER-deficient cells, XP17VI, XP18VI and XP19VI from genetically uncharacterized XP patients. These three patients were clinically described as classical XP. All cells were grown in MEM medium supplemented with 15% FCS and 1% antibiotics (penicillin, streptomycin and fungizone).

Plasmids

Reporter plasmids. pRSVCat contains the cDNA encoding CAT, and pRSVL, contains the cDNA encoding luciferase, both of which are inserted between the Rous Sarcoma virus long terminal repeat and the Simian virus 40 polyadenylation sequences.

Repair expression plasmids. pXPC3, containing the cDNA for *XPC*, was generously provided by R.Legerski, Department of Molecular Genetics, M.D. Anderson Cancer Center; p2E-ER2, coding for *XPD*, was kindly provided by C.A.Weber, Biomedical Science Division, Lawrence Livermore National Laboratory; pSLME6 and pXPAC, containing the *CSB* and *XPA* repair gene, respectively, were generously provided by C.Troelstra, Department of Cell Biology and Genetics, Erasmus University, Rotterdam; and pCDER3, expressing the *XPB* (*ERCC3*) gene, was kindly provided by G.Weeda, Department of Cell Biology and Genetics, Erasmus University, Rotterdam. These repair genes are either controlled by SV40 or CMV promoters. The control plasmids used (pCDE, pSLM and pEBS7) correspond to the repair expression plasmids without the repair gene. All plasmids were grown in *E.coli* DH5α and purified by cesium chloride–ethidium bromide gradients. More than 90% of the recovered DNA was in the supercoiled form I conformation as determined by agarose gel electrophoresis. Plasmids were stored at 4°C in sterile TE solution (Tris–HCl 10 mM, EDTA 1 mM, pH 8).

UV irradiation

Prior to transfection, reporter plasmids at a concentration of 1 μg/μl in TE (10 mM Tris, 1 mM EDTA) were exposed to UV radiation at 254 nm from a germicidal lamp, at a dose rate of 3.5 W/m^2. UV doses used were either 500 or 1000 J/m^2 as indicated in each figure.

Cotransfection technique and cell extract preparation

One to 2×10^5 fibroblasts seeded in a 6-well plate were cotransfected with 1 to 5 μg of reporter plasmids, pRSVCat or pRSVL, with 1 to 5 μg of either the repair plasmid containing one of the repair genes (*XPA*, *XPB*, *XPC*, *XPD* and *CSB*) or the control plasmid (pSLM, pEBS7 and pCDE) by the calcium–phosphate coprecipitation technique, as previously described (28). The amount of DNA is determined for each cell line and is that producing the maximum of the enzyme activity 12–24 h after transfection. Cells were incubated with precipitated DNA for 5 to 16 h at 37°C and submitted to a 15% glycerol shock, then washed three times with PBS before adding fresh medium. For the CAT assay, cells were harvested 48 h after transfection, which corresponds to higher level activity of the enzyme, then washed in PBS and resuspended in 250 mM Tris–HCl, pH 7.8. Cell extracts were prepared by three freeze-thaw cycles. Cell debris were removed by centrifugation (15 min×12 000 rpm, 4°C). Cell extracts were further heated 10 min at 65°C and recentrifuged before CAT analysis. For the luciferase assay, cells were harvested 24 h after transfection which corresponds to the maximum of expression level, which is constant for most cells between 12 and 24 h. Cell extracts were prepared according to the Promega Luciferase Assay System. 96 or 24-well plates were used in which 0.5–2×10^4 cells/well were transfected with 0.3–1 μg of pRSVL DNA. Cell lysis was done directly in the plate allowing a minimum of steps in this procedure.

CAT and luciferase assay

CAT activity was measured using a non-chromatographic assay as described by Sleigh (29). Briefly, proteins (5 μg) were incubated at 37°C with the reaction mixture (1.6 mM of chloramphenicol, 90 μM of acetyl CoA and 2.5 nCi of [^{14}C]acetyl CoA) for 30 to 60 min depending on the cell line used. The labeled reaction was extracted with ethyl acetate and counted by liquid scintillation. CAT activity was quantified by the amount of [^{14}C]acetyl groups transfered from labeled coenzyme A to unlabeled chloramphenicol. CAT activity ranged from 10 to 80 pmol/mg of protein per minute. The luciferase assay was performed according to the Promega Luciferase Assay System by mixing the cell extracts (10 μl) with the reaction buffer (50 μl of 20 mM tricine, 1.07 mM $MgCO_3$, 2.57 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, 0.27 mM CoA, 0.47 mM luciferine, 0.53 mM ATP at pH 7.8). Luciferase activities were normalized in each sample for protein amount quantified by the Bradford technique (30). When multi-well plates are used, the luciferase activity can be measured in a three step procedure involving (i) cell lysis performed directly in the well, (ii) luciferase activity measurement and (iii) protein quantification. Relative reporter gene activity is expressed as the percent of activity in cells transfected with the UV-irradiated over the unirradiated plasmid. In these conditions of transfection and enzyme assay, low variation levels of the expression of reporter genes in independent transfection experiments were obtained, with standard error means (SEM) <15 %.

Somatic cell fusion assay and microinjection assay

Three days prior to the fusion assay, fibroblasts were labeled with latex beads (0.75 μm or 2 μm). Cell fusion was performed using polyethylene glycol. UDS was assayed as previously described (14, 19). Forty-eight hours after fusion, cells were UV irradiated at 20 J/m^2 and incubated with ^{3}H-thymidine for 3 h. The number of grains per nucleus was established by counting autoradiographic grains above nuclei. Microinjection of repair genes in XP cells was performed as previously described (19). Repair activity was determined after UV radiation at 15 J/m^2, ^{3}H-thymidine labeling (10 μCi/ml with specific activity of 50 Ci/mmol) and autoradiography.

Results

Luciferase activity in NER-deficient SV40-transformed and untransformed cell lines

Relative luciferase activity was measured in transformed and untransformed cells transfected with pRSVL UV-irradiated at

Table I. Cell lines used in this work

Phenotype	Cell line[a]	References/sources
Normal	MRC5V1	Brighton, UK
	C5ROLas	Rotterdam,
	198VI (untransformed)[b]	The Netherlands
		Villejuif, France
Xeroderma pigmentosum		
Group A	XP12ROSV	Rotterdam,
Group C	XP4PALas[b]	The Netherlands
		Villejuif, France
Group D	XP6BESV (GM08207B)	ATCC
	XP17PV (unstranformed)[b]	Pavia, Italy
Unknown	XP17VILas[b]	Villejuif, France
	XP17VI (unstranformed)[b]	
	XP18VI (unstranformed)[b]	
	XP18VILas[b]	Villejuif, France
	XP19VILas[b]	Villejuif, France
Cockayne's syndrome		
Group B	CS1ANSV	Brighton, UK
Trichothiodystrophy		
TTD/XP-D	TTD1VILas[b]	Villejuif, France
	TTD2VILas[b]	Villejuif, France
	TTD8PV (untransformed)	Pavia, Italy

[a]SV40-T antigen-transformed fibroblasts with the exception of the 5 indicated untransformed which are primary diploid fibroblasts.
[b]Cells obtained from skin biopsies established in our laboratory.

500 J/m^2 compared to unirradiated pRSVL in various NER-deficient cells: XP12ROSV (XP-A), XP4PALas (XP-C), XP6BESV (XP-D), CS1ANSV (CS-B), TTD2VILas and TTD8PV (both TTD/XP-D) and the uncharacterized XP17VI or XP17VILas, XP18VI and XP19VI and in normal MRC5V1, C5ROLas and 198VI cells (Figure 1). Relative activity was significantly reduced in all NER-deficient cells with activity ranging from 15 to 60% of normal cells. Results obtained in untransformed cells show that the luciferase assay is sensitive enough to monitor the repair activity directly in untransformed cells. A specific decrease in enzyme activity can be observed for each complementation group which corresponds to their relative NER deficiency. For example, XP group A cells (XP12ROSV) being the most UV-sensitive, as measured by cloning efficiency or UDS (data not shown), show a relative luciferase activity lower than the less sensitive XP-C, XP-D, CS-B or TTD cells which display intermediate levels of activity. The XP18VI and XP19VI cells assigned here to complementation group A, surprisingly, show higher relative luciferase activity than the XP-A XP12ROSV line. Each cell line showed classical UV dose-dependent decrease in luciferase activity, as previously observed in the host cell reactivation assay of damaged plasmids using XP cells (20–25) and similar time-course reactivation within 12–48 h post-transfection: extremely low reactivation extent for XP12ROSV cells and moderately low extent in XP18VI and XP19VI cells (not shown).

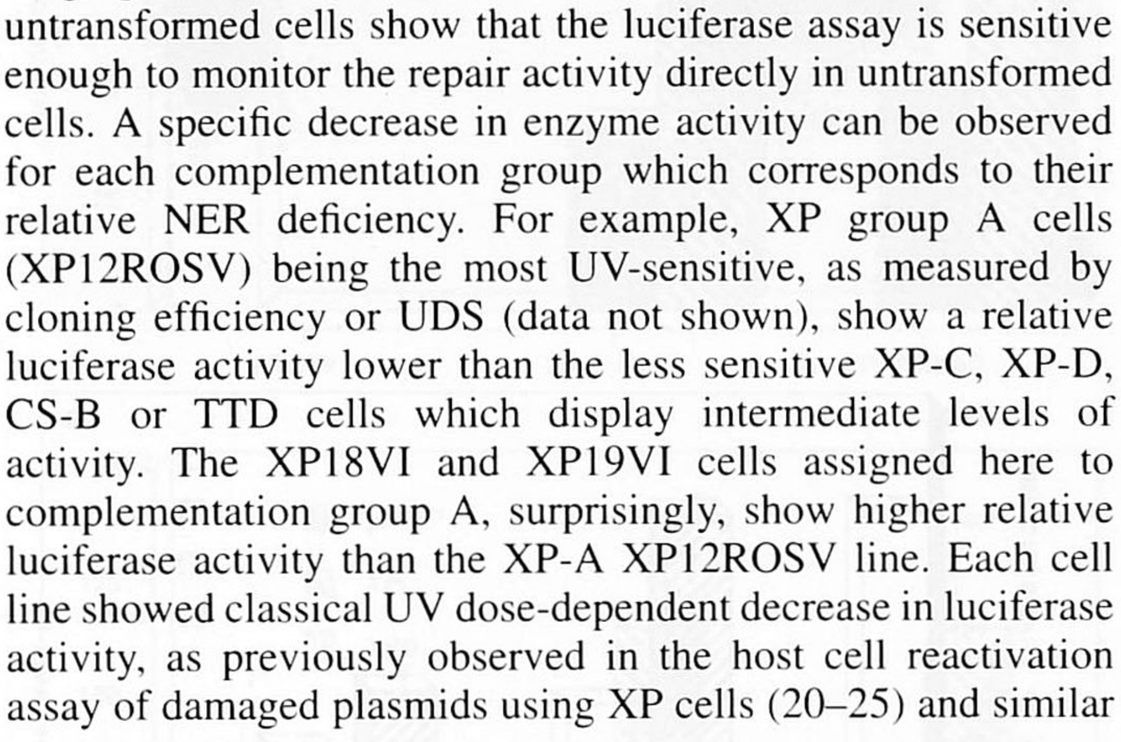

Fig. 1. Relative luciferase activity in NER-deficient cells. Relative luciferase activity was measured after transfection of pRSVL UV-irradiated at 500 J/m^2 in SV40-transformed (**A**) and untransformed (**B**) cells. Normal (wild type) : MRC5V1, C5ROLas, and 198VI, XP-A: XP12ROSV, XP-C: XP4PALas, XP-D: XP6BESV, XP17PV, CS-B: CS1ANSV, TTD/XP-D: TTD2VILas and TTD8PV, and the unknown XP cells untransformed (XP17VI, XP19VI) or transformed (XP17VILas, XP18VILas or XP19VILas). Bars represent the mean of two to three separate experiments where each was done in triplicate with SEM, with the exception of XP12ROSV, XP18VILas and XP19VILas in (A) and TTD8PV and XP19VI in (B) which are represented by one experiment.

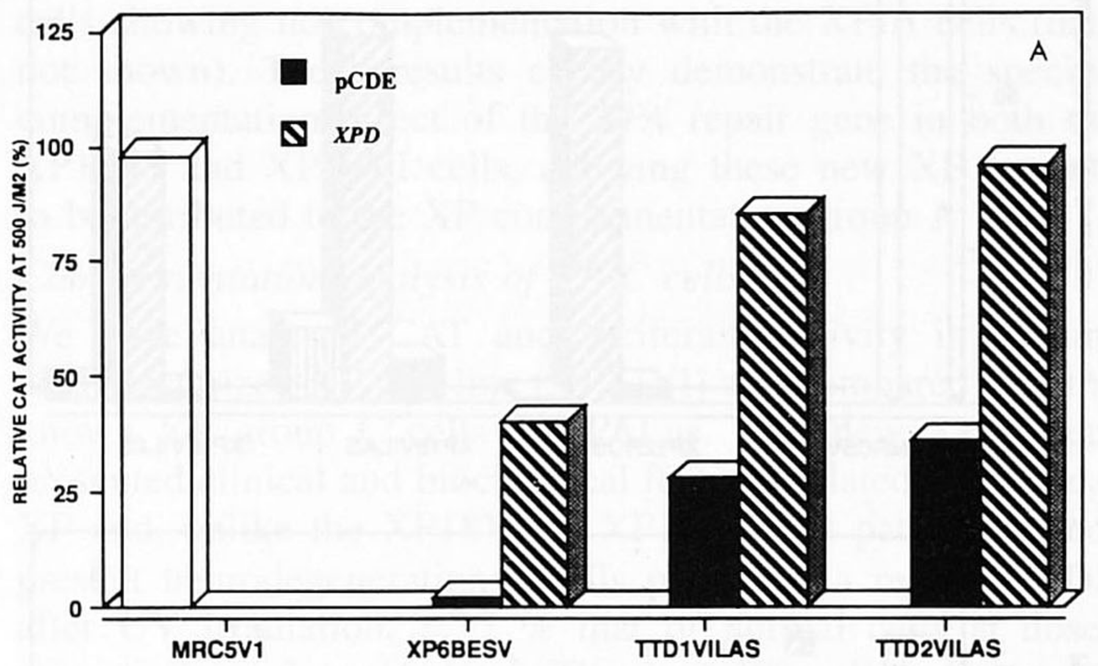

Fig. 2. Cotransfection assay in XP-D and TTD/XP-D cells. Relative CAT (**A**) and luciferase (**B**) activity was measured in XP-D (XP6BESV) and TTD/XP-D (TTD1VILas, TTD2VILas) cell lines and compared to wild type MRC5V1 cells. Reporter plasmids were UV-irradiated at 500 J/m^2 (A) or 1000 J/m^2 (B) and cotransfected with pCDE or pSLM (control), *XPA*, *XPB*, *XPC*, *XPD* and *CSB*. In the CAT assay (A) each point represents the mean of three separate experiments with SEM <15%. (B) Bars represent the mean of triplicate determinations with SEM.

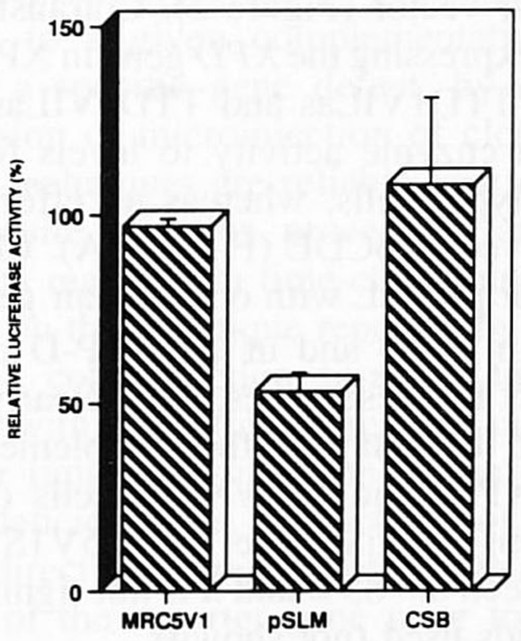

Fig. 3. Cotransfection assay in CS-B cells. Relative luciferase activity was measured 24 h after transfection of CS-B cells (CS1ANSV) with either pSLM or pSLME6 (*CSB*) plasmids compared to repair-proficient MRC5VI cells. The relative repair capacity was determined as the activity of UV-irradiated reporter plasmids pRSVL at 500 J/m^2 compared to the unirradiated one. Bars represent the mean of two separate experiments done each in triplicate with SEM.

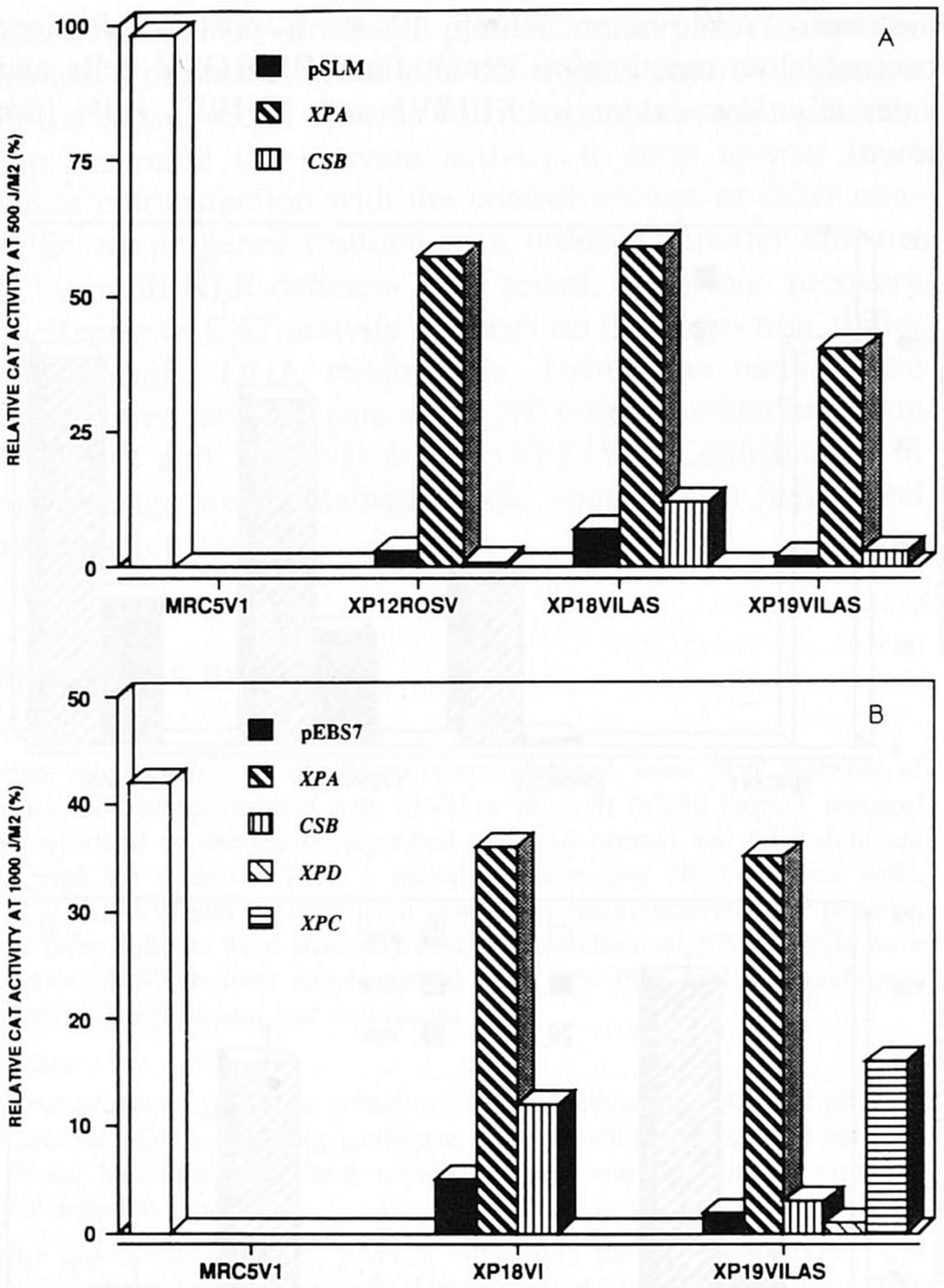

Fig. 4. Cotransfection assay in XP-A cells. Relative CAT activity was measured 48 h after cotransfection in XP-A cells (XP12ROSV) and in the new uncharacterized XP cells (untransformed XP18VI or transformed XP18VILas and XP19VILas) with pSLM (control), pXPAC (*XPA*) or pSLME6 (*CSB*) compared to repair proficient MRC5V1 cells. CAT activity was determined in transfected cells with UV-irradiated pRSVCat at 500 J/m^2 in (**A**) or 1000 J/m^2 in (**B**). Bars represent the mean of triplicate determinations with SEM <15%.

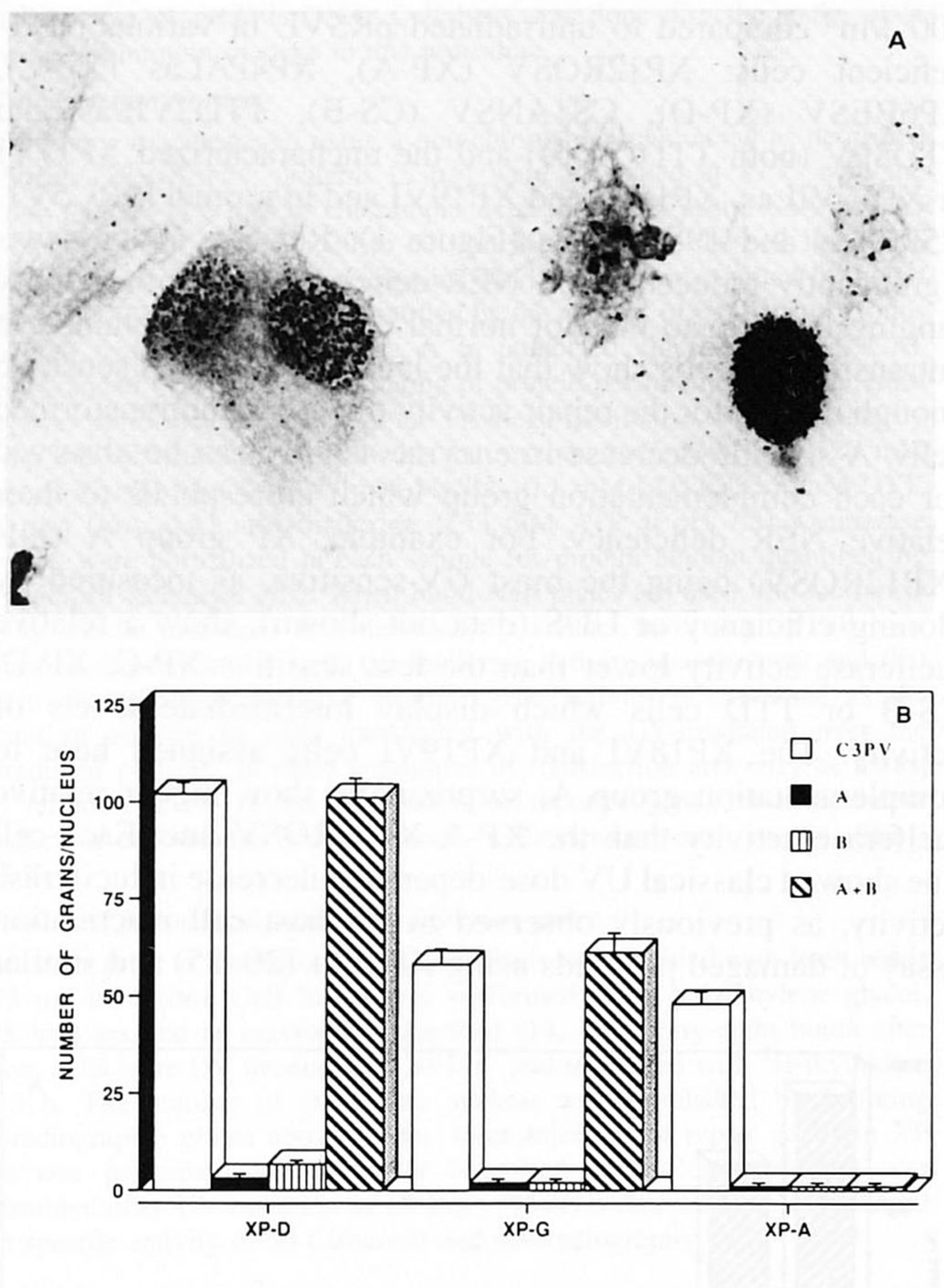

Fig. 5. Complementation of XP18VI cells by microinjection of *XPA* gene and by the somatic cell fusion assay. (**A**) pXPAC vector expressing the *XPA* gene was microinjected into one of the nuclei of homopolykaryons of XP18VI cells. The injected cells exhibit a wild type level of UV-induced UDS (dikaryon) compared to the non-injected surrounding cells showing a low residual level of UDS. The heavily labeled cell typically represents replicative DNA synthesis (S phase). (**B**) XP18VI fibroblasts were fused with the XP11PV (XP-A), TTD8PV (XP-D) and GM03021 (XP-G) cells. Fused cells were UV-irradiated at 20 J/m^2 and UDS was measured by autoradiography following ^{3}H-thymidine incorporation. Bars show the number of grains per nucleus with SEM of dikaryons compared to wild type C3PV cells. A is the XP18VI cells, B the known XP-D, XP-G or XP-A cells and A+B, the fused heterokaryons.

Complementation analysis of TTD/XP-D and XP-D cells

We measured luciferase and CAT activity in transformed XP-D and TTD/XP-D cells after cotransfection of either pRSVCat UV-irradiated at 500 J/m^2 or pRSVL UV-irradiated at 1000 J/m^2 with a repair vector (Figure 2). Cotransfection with the vector p2E-ER2 expressing the *XPD* gene in XP-D (XP6BESV) and TTD/XP-D (TTD1VILas and TTD2VILas) cells resulted in an increase in enzyme activity to levels found in normal MRC5V1 (wild type) cells, whereas no effect was observed with the control vector pCDE (Figure 2A). Cotransfection of the reporter vector pRSVL with other repair genes (*XPA*, *XPB*, *XPC* and *CSB*) in XP-D and in TTD/XP-D cells showed no reactivation effect by these genes, thus, clearly demonstrating the specificity of the assay by the complementation of *XPD* cDNA in both XP-D and TTD/XP-D cells (Figure 2B).The reactivation extent in wild type (MRC5V1SV or C5RILas) cells ranges between 65–85% and it is not significantly affected by any of plasmids used (not shown).

Complementation analysis of CS-B cells

Relative luciferase activity was measured in CS complementation group B cells (CS1ANSV) after cotransfection of UV-irradiated pRSVL (500 J/m^2) with the repair vector pSLME6 expressing the *CSB* gene or the control pSLM vector (Figure 3). Recovery of the luciferase activity to near normal levels was observed with pSLM6 expressing the *CSB* repair gene whereas cotransfection with the control vector pSLM resulted in a reduced luciferase activity. These results are in agreement with previous results establishing the involvement of *ERCC6 /CSB* in CS group B based on the use of microinjection or stably transfected CS1AN cells (31).

Complementation analysis of XP-A cells

XP-A cells and two cultures of uncharacterized XP cells were analysed with the new complementation assay. These new XP patients presented clinical features of XP including neurodegeneration, dwarfism and deafness. Detailed clinical description of these patients will be published elsewhere. Both cells (XP18VI and XP19VI) presented a severely reduced UDS following UV irradiation, <10% of normal cells at doses ranging from 5 to 15 J/m^2. These cells also presented a UV-survival curve similar to XP group A cells with a D_0 of 1 J/m^2 compared to 10 J/m^2 for normal MRC5V1 cells (data not shown). These preliminary results indicated the possibility

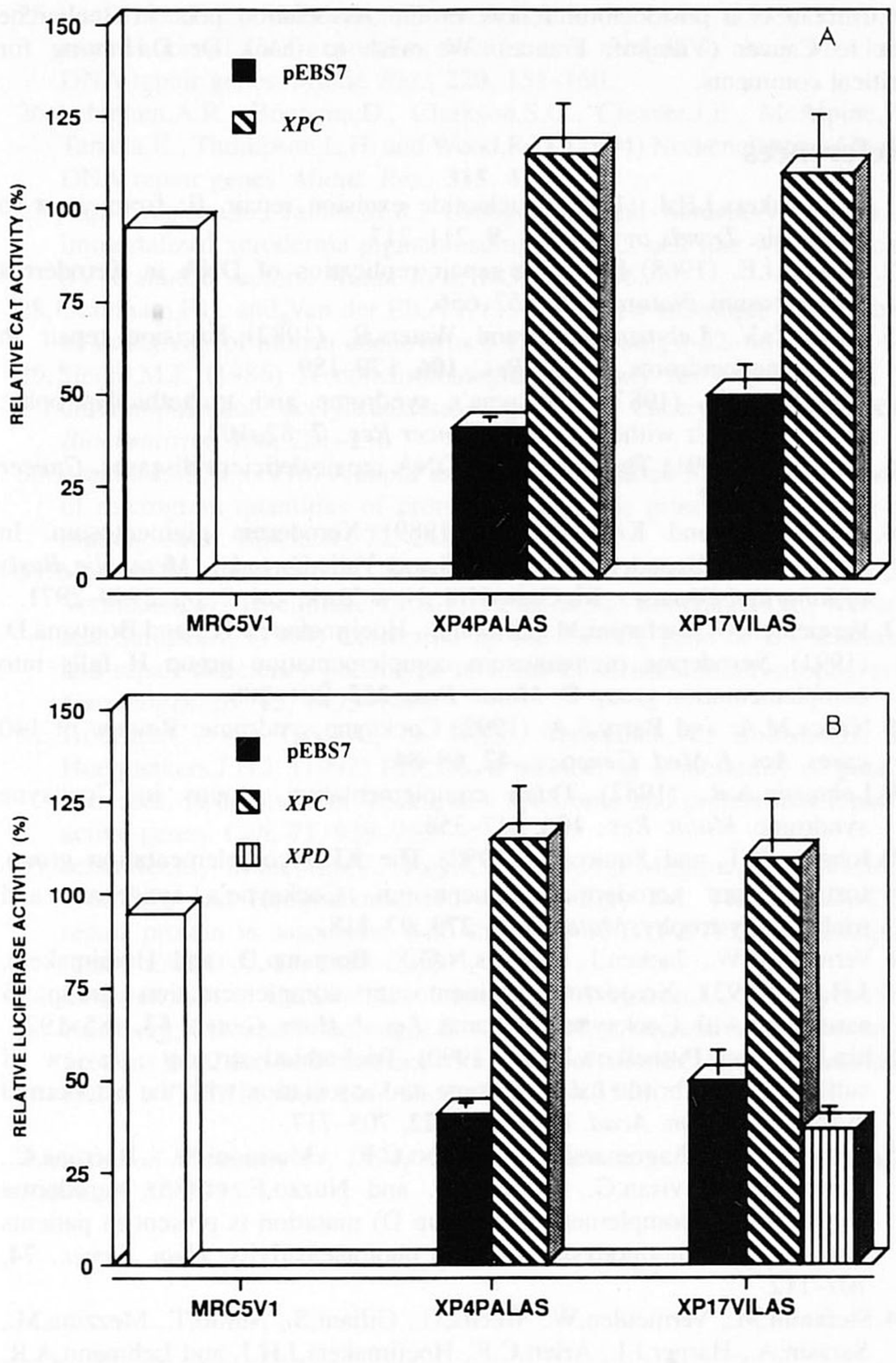

Fig. 6. Cotransfection assay in XP-C cells. Relative CAT (**A**) and luciferase (**B**) activity was measured after transfection of the reporter plasmids UV-irradiated at 500 J/m^2 in XP-C (XP4PALas) and the unknown XP cell line (XP17VILas) compared to the unirradiated one. In both CAT and luciferase assay XP17VILas was cotransfected with pEBS7 (control), pXPC3 (*XPC*) and only in B with p2E-E2 (*XPD*); XP4PALas was cotransfected also with pEBS7 and pXPC3 compared to normal MRC5V1 cells. In (A) bars represent the mean of at least two separate experiments with SEM. In
(B) bars represent the mean of triplicate determinations with SEM. In both (A) and (B) the SEM of wild type MRC5V1 cells being 1% is too low to appear in the graphics.

that both these patients belonged to XP complementation group A. We thus measured the DNA repair capacity of these uncharacterized cells in relation to the known XP-A cells (XP12ROSV), using the rapid complementation assay (Figure 4). The recovery of CAT activity in XP-A cells was observed after cotransfection with pXPAC vector expressing the *XPA* gene in XP12ROSV as well as in the uncharacterized XP18VILas and XP19VILas cells (Figure 4A and 4B) compared to the control vector pSLM or the *XPC*, *XPD* or *CSB* genes either at 500 J/m^2 (Figure 4A) or at 1000 J/m^2 (Figure 4B). Significant reactivation by the *XPA* gene was also observed with the luciferase assay (data not shown). Confirmation of these results was obtained using classical complementation analysis, either the somatic cell fusion assay or the microinjection of the *XPA* cDNA (Figure 5). Microinjection of the pXPAC plasmid expressing the *XPA* gene into XP18VI fibroblast showed wild type levels of UDS when compared to the non-injected surrounding cells (Figure 5A). Fusion of the XP18VI cells with XP-A, XP-D and XP-G are shown in Figure 5B. Complementation was observed with the XP-D and XP-G cells but not with the XP-A cells. Also, the somatic cell fusion assay gave similar results for the XP19VI cells showing no complementation with the XP-A cells (data not shown). These results clearly demonstrate the specific complementation effect of the *XPA* repair gene in both the XP18VI and XP19VI cells, allowing these new XP patients to be attributed to the XP complementation group A.

Complementation analysis of XP-C cells

We have analysed CAT and luciferase activity in a third uncharacterized XP cell line (XP17VI) and compared it to the known XP group C cells XP4PALas. This new XP patient presented clinical and biochemical features related to classical XP and, unlike the XP18VI or XP19VI, this patient did not present neurodegenerations. Cells presented a reduced UDS after UV irradiation, <15 % that of normal cells at doses ranging from 5 to 15 J/m^2. The new XP cells presented a UV-survival curve similar to XP group C cells with a D_0 of 5.5 J/m^2 and 4.5 J/m^2 for the XP-C cells XP4PASV compared to 10 J/m^2 for normal MRC5V1 cells (data not shown). Microinjection of *XPA*, *XPD* and *XPB* cDNA failed to correct the repair deficiency (data not shown). These preliminary results indicated the possibility that these cells belonged to XP complementation group C. Therefore, we determined the DNA repair capacity of XP17VILas transformed cells compared to the known XP group C XP4PALas cells using the new complementation assay (Figure 6). In both cells, cotransfection of the UV-irradiated pRSVL or pRSVCat with the *XPC* expression vector (pXPC3) resulted in restoration of the reporter enzyme activity to normal levels whereas cotransfection with the control vector pEBS7 or the *XPD* expression vector pE-ER2 resulted in a reduced enzyme activity similar to that found with the UV-irradiated reporter vector alone. These results clearly demonstrate the specificity of this complementation assay and suggest that the new XP patient belongs to the XP complementation group C. Confirmation of these results was obtained by somatic cell fusion assay (data not shown).

Discussion

NER-deficient cells from XP, CS or TTD patients have usually been attributed to a given complementation group, which corresponds to a specific gene defect, by employing either somatic cell fusion or microinjection of cloned repair genes. Although these techniques are reliable, one of the drawbacks is that they require complex procedures and/or specialized equipment which make them time-consuming and difficult to perform. Based on the technique reported by Henderson *et al.* (25), we used a cotransfection assay of luciferase or CAT reporter vector with a vector expressing one of the known repair genes for rapid determination of the complementation group of NER-deficient cells. The activity of the reporter gene product is an indirect measurement of the DNA repair capacity. UV irradiation of the reporter gene prior to transfection has been shown to inactivate the expression in NER-deficient cells as compared to normal cells, thus reflecting their reduced repair capacity. Complementation of the repair activity to wild type levels was obtained following cotransfection of the reporter vector with the appropriate repair gene. The reactivation effect of the *XPD* gene in both transient and stable

expression has been described in several XP-D and TTD cell lines (31). XP-D and TTD/XP-D cells presented reporter activity levels higher than the wild type MRC5V1 cells following the introduction of the *XPD* gene. This effect could result from the role of XPD in the transcription complex BTF2/TFIIH (33) thus promoting the over-transcription of the repaired pRSVL plasmid. On the other hand, the higher level of reactivation of TTD1VILas and TTD2VILas over XP6BESV cells (Figure 2A) could be due to higher expression efficiency of pRSVCat plasmid. Different expression efficiency could also explain the different level of reactivation in XP6BESV cells transfected with pRSVCat and PRSVL plasmids irradiated at 500 J/m^2 (Figures 1A and 2A, respectively), since, at this dose, an average of 37% of molecules resulting without UV-lesions in reporter genes. A possible transactivating effect was also observed with the *XPA* gene, when cotransfected into XP-D cells, giving increased reactivation levels (Figures 2B and 4B), and *XPC* gene when cotransfected in XP-C cells giving an increased reporter activity to levels higher than normal cells (Figure 6). This could result from some possible association to a transcription factor in a similar fashion as the yeast homologue Rad4 (34). Also, the XP-C cells showed a UV-dose dependent decrease in activity like cells from other XP. This suggests that the transcription-repair coupled process, which is still active in XP-C, is not sufficient to overcome the transcription inhibition by UV-lesions. Another possibility is that this subpathway could be specific for a certain chromatin structure and could take effect only when damage is induced while transcription is in progress. This is not the case here since our reporter vector is transfected following UV irradiation, thus supporting the implication of the transcription complex in preferential repair (for review see 26). On the other hand, CS cells showed a higher residual luciferase activity when cotransfected with a control vector, possibly reflecting their lower UV sensitivity and/or their ability to perform global genome repair. However, all results obtained with this complementation assay were gene specific for the complementation group thus allowing the assignment of three new uncharacterized XP patients (XP17VI, XP18VI and XP19VI). Relative reporter gene activity recovered in these cells after cotransfection with the repair vector expressing the *XPA* or the *XPC* gene linked the NER defect in the XP18VI and the XP19VI cells to the *XPA* gene and the XP17VI cells to the *XPC* gene. Confirmation of these results was observed by the somatic cell fusion assay and microinjection of the repair gene, thus allowing these patients to be attributed to XP complementation groups A and C.

In conclusion, we here demonstrate that cotransfection of the UV-irradiated pRSVL or pRSVCat with the appropriate repair expression vector results in the complementation of the NER defect in all XP, CS and TTD/XP-D cells analysed. Furthermore, the high sensitivity of the luciferase assay allows the use of small quantities of transformed or untransformed cells and plasmids and can be monitored without the use of a radioactive substrate.

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References

1. Hoeijmakers,J.H.J. (1993) Nucleotide excision repair. II: from yeast to mammals. *Trends in Genetics*, **9**, 211–217.
2. Cleaver,J.E. (1968) Defective repair replication of DNA in Xeroderma pigmentosum. *Nature*, **218**, 652–656.
3. Mayne,L.V., Lehmann,A.R. and Waters,R. (1982) Excision repair in Cockayne syndrome. *Mutat. Res.*, **106**, 179–189.
4. Lehmann,A.R. (1987) Cockayne's syndrome and trichothiodystrophy: defective repair without cancer. *Cancer Res.*, **7**, 82–103.
5. Sarasin,A. (1991) The paradox of DNA repair-deficient diseases. *Cancer J.*, **4**, 233–237.
6. Cleaver,J.E. and Kraemer,K.H. (1989) Xeroderma pigmentosum. In Scriver,C.R., Beaudet,A.L., Sly,W.S.and Valle,D. (eds), *Metabolic Basis of Inherited Diseases*. McGraw-Hill, New York, vol. 2, pp. 2949–2971.
7. Vermeulen,W., Stefanini,M., Giliani,S., Hoeijmakers,J.H.J.and Bootsma,D. (1991) Xeroderma pigmentosum complementation group H falls into complementation group D. *Mutat. Res.*, **255**, 201–208.
8. Nance,M.A. and Berry,S.A. (1992) Cockayne syndrome: Review of 140 cases. *Am. J. Med. Genetics.*, **42**, 68–84.
9. Lehmann,A.R. (1982) Three complementation groups in Cockayne syndrome. *Mutat. Res.*, **106**, 347–356.
10. Johnson,R.T. and Squires,S. (1992) The XPD complementation group. Insights into xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *Mutat. Res.*, **273**, 97–118.
11. Vermeulen,W., Jaeken,J., Jaspers,N.G.J., Bootsma,D. and Hoeijmakers, J.H.J. (1993) Xeroderma pigmentosum complementation group G associated with Cockayne syndrome. *Am. J. Hum. Genet.*, **53**, 185–192.
12. Itin,P.H. and Pitthelkow,M.R. (1990) Trichothiodystrophy : review of sulfur-deficient brittle hair syndrome and association with the ectodermal dysplasias. *J. Am. Acad. Dermatol.*, **22**, 705–717.
13. Stefanini,M., Lagomarsini,P., Arlett,C.F., Marinoni,S., Borrone,C., Crovato,F., Trevisan,G., Cordone,G. and Nuzzo,F. (1986) Xeroderma pigmentosum (complementation group D) mutation is present in patients affected by trichothiodystrophy with phototsensitivity. *Hum. Genet.*, **74**, 107–112.
14. Stefanini,M., Vermeulen,W., Weeda,G., Giliani,S., Nardo,T., Mezzina,M., Sarasin,A., Harper,J.I., Arlett,C.F., Hoeijmakers,J.H.J. and Lehmann,A.R. (1993) A new nucleotide-excision-repair-gene associated with the disorder trichothiodystrophy. *Am. J. Hum. Genet.*, **53**, 817–821.
15. Vermeulen,W. *et al.* (1994) Three unusual repair deficiencies associated with transcription factor BTF2 (TFIIH). Evidence for the existence of a transcription syndrome. *Cold Spring Harbor Quantitative Symposia* in press.
16. de Weerd-Kastelein,E.A., Keijzer,W. and Bootsma,D. (1972) Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. *Nature*, **238**, 80–83.
17. Mayne,L.V. and Lehmann,A.R. (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.
18. Wood,R.D., Robins,P. and Lindahl,T. (1988) Complementation of the Xeroderma pigmentosum DNA repair defect in cell-free extracts. *Cell*, **53**, 97–106.
19. Hoeijmakers,J.H.J. (1988) Use of microneedle injection to study DNA repair in mammalian cells. In Friedberg,E.C. and Hanawalt,P.C. (eds), *A Laboratory Manual of Research Procedures*. Marcel Dekker Inc. New York, vol. 3, pp. 133–150.
20. Day III,R.S., Ziolkowski,C.H.J. and DiMattina,M. (1981) Decrease host cell reactivation of UV-irradiated adenovirus 5 by fibroblasts from Cockayne's syndrome patients. *Photochem. Photobiol.*, **34**, 603–607.
21. Lehmann,A.R. and Oomen,A. (1985) Effects of DNA damage on the expression of the chloramphenicol acetyltransferase gene after transfection into diploid human fibroblasts. *Nucleic Acids Res.*, **13**, 2087–2095.
22. Protic-Sabljic,M. and Kraemer,K.H. (1985) One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells. *Proc. Natl Acad. Sci. USA*, **82**, 6622–6626.
23. Sheibani,N., Jennerwein,M.M. and Eastman,A. (1989) DNA repair in cells sensitive and resistant to *cis*-Diaminedichloroplatinum (II) : Host cell reactivation of damaged plasmid DNA. *Biochemistry*, **28**, 3120–3124.
24. Athas,W.F., Hedayati,M.A., Matanoski,G.M., Farmer,E.R. and Grossman,L. (1991) Development and field-test validation of an assay for DNA repair in circulating human lymphocytes. *Cancer Res.*, **51**, 5786–5793.

25. Henderson,E.E., Valerie,K., Green,A.P. and Riel,J.K. (1989) Host cell reactivation of CAT-expression vectors as a method to assay for cloned DNA-repair genes. *Mutat. Res.*, **220**, 151–160.
26. Lehmann,A.R., Bootsma,D., Clarkson,S.G., Cleaver,J.E., McAlpine,P.J., Tanaka,K., Thompson,L.H. and Wood,R.D. (1994) Nomenclature of human DNA repair genes. *Mutat. Res.*, **315**, 41–42.
27. Daja-Grosjean,L., James,M.R., Drougard,C. and Sarasin,A. (1987) An immortalized xeroderma pigmentosum, group C, cell line wich replicates SV40 shuttle vectors. *Mutat. Res.*, **183**, 185–196.
28. Gramham,F.L. and Van der Eb,A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology*, **52**, 481–485.
29. Sleigh,M.J. (1986) A nonchromatographic assay for expression of the chloramphenicol acetyltransferase gene in eucaryotic cells. *Anal. Biochemistry*, **156**, 251–256.
30. Bradford,M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.
31. Mezzina,M., Eveno,E., Chevalier-Lagente,O., Benoit,A., Carreau,M., Vermeulen,W., Hoeijmakers,J.H.J., Stefanini,M., Lehmann,A.R., Weber,C. and Sarasin,A. (1994) Correction by the *ERCC2* gene of UV-sensitivity and repair deficiency phenotype in a subset of trichothiodystrophy cells. *Carcinogenesis*, **15**, 1493–1498.
32. Troelstra,C., van Gool,A., de Wit,J., Vermeulen,W., Bootsma,D. and Hoeijmakers,J.H.J. (1992) ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell*, **71**, 939–953.
33. Schaeffer,L., Moncollin,V., Roy,R., Staub,A., Mezzina,M., Sarasin,A., Weeda,G. and Hoeijmakers,J.H.J. (1994) Egly JM. The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. *EMBO J.*, **13**, 2388–2392.
34. Bardwell,A.J., Bardwell,L., Iyer,N., Svejstrup,J.Q., Feaver,W.J., Kornberg,R.D. and Friedberg,E.C. (1994) Yeast nucleotide excision repair proteins Rad2 and Rad4 interact with RNA polymerase II basal transcription factor b (TFIIH). *Mol. Cell. Biol.*, **14**, 3569–3576.

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