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DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation to mRNA levels in primary lymphocytes

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This paper is dedicated to the memory of Gerda Frentz who died on 9 November 1998.

Abstract

We have previously shown that high DNA repair capacity protects psoriasis patients against chemically induced basal cell carcinoma [Dybdahl et al. Mutat. Res. 433 (1999) 15–22]. We have used the same study persons to investigate the correlation between expression of eight genes involved in nucleotide excision repair and DNA repair capacity. mRNA levels of *XPA, XPB, XPC, XPD, XPF, XPG, CSB* and *ERCC1* in primary lymphocytes from 33 individuals were quantified by dot-blots and normalized to β-actin. *ERCC1* and *XPD* mRNA quantities were highly correlated ($r = 0.89$; $P < 10^{-11}$) while *XPA, XPB, XPC, XPG, XPF*and *CSB* mRNAs were moderately correlated ($r = 0.2$–0.7). Thus, the mRNA expressions seem to fall in at least two groups. There was a three to sevenfold variation in the expression levels of the mRNAs. This is in contrast to the more than a hundredfold variation in mRNA levels reported in cancer patients.

DNA repair capacity was measured in a host cell reactivation assay, where primary lymphocytes were transfected with an UV-irradiated plasmid encoding firefly-luciferase. Only *ERCC1* and *XPD* mRNA levels correlated with the DNA repair capacity ($P < 0.03$). In order to see if *ERCC1* or *XPD* activity was limiting for DNA repair, we cotransfected with plasmids encoding NER genes, thus over-expressing either *XPB, XPC, XPD, CSB* or *ERCC1* in the host cell reactivation assay. Only *XPB* over-expression increased DNA repair capacity. Thus, there is no indication that neither *XPD* nor *ERCC1* limits the DNA repair capacity. However, our results indicate that *ERCC1* and *XPD* mRNA levels may be used as a proxy for DNA repair capacity in lymphocytes.

Abbreviations: NER: nucleotide excision repair; HCR: host cell reactivation; PHA: phytohemagglutinin; PBS: phosphate buffered saline

Keywords: Nucleotide excision repair; *XPA*; *XPB*; *XPC*; *XPD*; *XPF*; *XPG*; *CSB*; *ERCC1*; Host cell reactivation

1. Introduction

DNA repair is an important aspect of cancer prevention. This is illustrated by the fact that human beings with the rare disease xeroderma pigmentosum, being defective in one of the genes involved in nucleotide excision repair, have more than a 1000-fold increased

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risk of basal cell carcinoma [1]. Also less dramatic changes in DNA repair have been associated with increased risk of getting cancer [2–5]. These studies are all in case-control studies, where decreased DNA repair capacity was associated with increased risk of either lung cancer or basal cell carcinoma. DNA repair is also important in cancer treatment because chemotherapy is genotoxic, and some chemotherapy-resistant tumors have been shown to have increased expression of genes involved in DNA repair [6–8].

Nucleotide excision repair (NER) is the main repair pathway responsible for removing bulky adducts and helix-distorting lesions from DNA. The process is catalyzed by 13–16 polypeptides working in enzyme complexes [9]. The expression of NER genes has mostly been studied in cancer cell lines, immortalized cell lines, or in seriously ill cancer patients [10–14]. At present, little is known about the kinetics of the molecular process or about the regulation of NER gene expression in healthy humans. One other study [15] have quantified the mRNA levels of five NER genes in 12 healthy individuals.

We have previously described an association between high DNA repair and protection against chemically induced basal cell carcinoma in psoriasis patients [4]. We have now used lymphocytes from this previously established study group of psoriasis patients and controls with or without basal cell carcinoma to study gene expression. One purpose was to attempt to identify a limiting factor of DNA repair in human cells. A second purpose was to attempt to find a molecular proxy for DNA repair capacity measurements. DNA repair capacity can be measured by host cell reactivation (HCR). This assay requires a large number of viable cells, and it can be associated with considerable day-to-day variation. If a limiting factor in the DNA repair capacity measured in host cell reactivation assay was identified it would be easier to find a reliable substitute for HCR. Two different approaches were used: (1) we determined the relative abundance of NER mRNAs to see whose abundance correlated with DNA repair capacity measured by (HCR) and (2) we measured HCR while over-expressing selected DNA repair proteins to determine if the DNA repair capacity was increased.

The mRNA levels of eight genes involved in NER; namely *XPA, XPB, XPC, XPD, XPF, XPG, CSB,* and *ERCC1* were quantified. XPA and XPC are involved in recognition of damaged DNA [16]. XPB and XPD are both helicases that unwind the DNA, while CSB is a putative helicase. XPG and ERCC1 are endonucleases, and ERCC1 and XPF forms a dimer in vivo [16]. We here report that the mRNA levels of *ERCC1* and *XPD* correlate with DNA repair capacity. However, when we measured the DNA repair capacity in cells cotransfected with plasmids expressing *ERCC1, XPB, XPC, XPD* or *CSB*, only over-expression of *XPB* increased DNA repair capacity.

2. Materials and methods

2.1. Cells

A human Epstein–Barr virus immortalised lymphoblastoid cell line GM 00131A was obtained from the National Institute of General Medical Sciences Human Mutant Cell Repository, Camden, NJ. The cells were cultured in RPMI 1640, 20% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin (all from Gibco BRL, Life Technologies).

2.2. Plasmids

The pE12-12, pcDX-X, pSLM-XPD, and pcBlsSE6, encoding cDNAs from *ERCC1, XPB, XPD,* and *CSB,* respectively, were generous gifts of Dr. Geert Weeda, Erasmus University, Rotterdam. pBS-XPC was generously provided by Dr. Fumio Hanaoka, Osaka University, Osaka. Dr. Teit E. Johansen generously provided pTEJ4. pUV60 is pGL3-basic (Promega) with a *SalI-HindIII* fragment from pCEP4 (Invitogen) containing the promoter of the immediate early gene of human cytomegalovirus inserted between *XhoI* and *HindIII* sites. Thus, pUV60 encodes the firefly gene luciferase gene under transcriptional control of the promoter of the immediate early gene of human cytomegalovirus.

2.3. Probes

The β-actin: A 0.6 kb fragment was amplified by RT-PCR from RNA isolated from GM00131, and confirmed by restriction analysis. The primers have been described previously [17]. *ERCC1*: An *EcoRI-HindIII*-fragment containing the entire coding region of 1.1 kb from pE12-12. *XPA:* The entire coding

region of 0.8 kb was amplified by RT-PCR from RNA isolated from GM00131 and confirmed by restriction analysis. The primers were 5′-AGC TAG GTC CTC GGA GTG and 5′-AAA CAG GTC ACT GAA CTA AA-3′. *XPB:* An *EcoRI*-fragment containing 2.8 kb of the coding region was isolated from pcDX-X. *XPC*: 0.29 kb of the coding region was amplified by RT-PCR from RNA isolated from GM00131 and confirmed by restriction analysis. The primers were 5′-CAG ACT ACA TTG GAA AGG AGA T-3′ and 5′-CCT TAG CAA AGG TTT CCT CTT G-3′. *XPD:* A 2.2 kb *EcoRI*-fragment containing the entire coding region was isolated from pSLM-XPD. *XPF*: 0.75 kb of the coding region was amplified by RT-PCR from RNA isolated from GM00131 and confirmed by restriction analysis. The primers were 5′-TTT CTC GAC GAT ATC CTT CCT CG-3′ and 5′-TAT CTG GAT CCT TTG TGG CAC CA-3′. *XPG*: 0.63 kb of the coding region was amplified by RT-PCR from RNA isolated from GM00131 and confirmed by restriction analysis. The primers were 5′-ATG GAT ATC TGG CTG TTT GGA G-3′ and 5′-CAT ACA TGT CAC AGC TCT GTT T-3′. *CSB:* A *PstI*-fragment containing 2.8 kb of the coding region was isolated from pcBlsSE6. DNA probes were labeled with [^{32}P]-dCTP (6000 Ci/mmol) (Amersham Pharmacia) using Prime-it random primer labeling kit (Stratagene). The probes gave specific hybridization to a band of the appropriate size on Northern blots using total RNA isolated from the lymphoblastoid cell line GM00131 indicating that the hybridization was specific under the conditions used (results not shown).

2.4. Blood collection

All of the subjects gave written informed consent. The study was conducted in accordance with the Helsinki declaration and was approved by the local medical ethical committee. Sixty-four milliliter of venous blood was collected from each person and lymphocytes were isolated using CPT tubes (Becton and Dickinson) and frozen in 50% fetal calf serum, 10% DMSO, 40% RPMI 1640 with 2 mM Glutamax-I. Northern blots were performed as described [18].

2.5. Dot-blots

Aliquots of 2×10^7 lymphocytes from each person were thawed and grown for 3 days in RPMI 1640, 20% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin (all from Gibco), and 20 ug/ml phytohemagglutinin (PHA, Difco Laboratories, Detroit, MI) as described by Dybdahl et al. [4]. Viability was on an average 88%. The blastogenic rate was on average 0.85 ± 0.43. However, control experiments showed that identical mRNA levels were obtained from the same batch of lymphocytes with independent PHA stimulations, where very different blastogenic rates were obtained. The cells were harvested and mRNA was isolated using Oligotex direct mRNA mini kit (Qiagen). The mRNA was eluted in 200 μl 5 mM Tris–HCl, pH 7.5 and stored at -80°C.

The RNA was dotted on Gene Screen Plus membrane in100 μl aliquots containing 20 μl RNA, 6 μl formaldehyde and 50 μl formamide using a Bio-Rad dot-blot apparatus. Ten parallel filters were made. After air drying, the membranes were pre-hybridized in Quick hyb hybridization solution (Stratagene), and hybridized overnight to the ^{32}P-labeled probes. After washing, the dot-blots were subjected to autoradiography and the dots in the dot-blot were subsequently cut out as 5 mm diameter circles and quantified by liquid scintillation counting. The amount of radioactivity in each dot hybridized to *XPA, XPB, XPC, XPD, XPF, XPG, CSB* or *ERCC1* probes was normalized to the amount of radioactivity in the dot-blot hybridized to β-actin. Samples with hybridization signals at or below background levels were excluded. Thus seven *CSB* samples, and four *XPC* samples were excluded.

For reproducibility, two independent experiments including cell thawing, PHA stimulation and mRNA extractions were performed for two persons, and the RNA was blotted on the same filters. The normalized amount of *XPA, XPB, XPC, XPD, CSB* and *ERCC1* mRNA from one experiment was plotted versus the value for the second experiment (Fig. 2). As shown the correlation between the two determinations was very good.

2.6. Host cell reactivation

The assay was performed as described [4,19] except that luciferase was used as reporter gene. pUV60 was isolated from *Escherichia coli* DH5a using Qiagen maxi plasmid isolation kit and the plasmid preparation was phenol/chloroform extracted once, chloroform

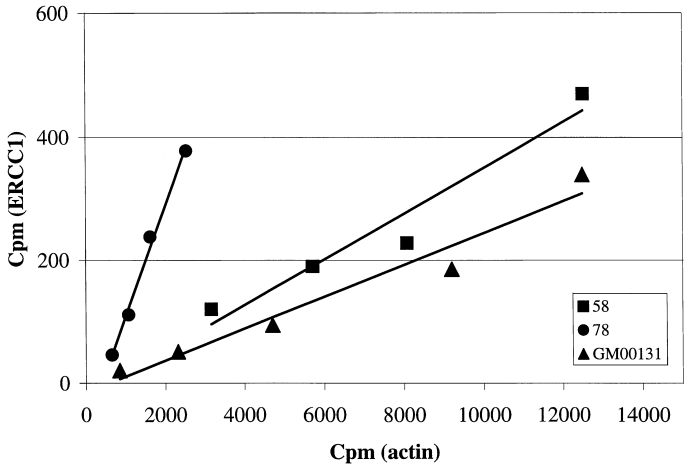

Fig. 1. Control experiment showing that normalization to *β-actin* corrected for loading differences. Different amounts of mRNA isolated from PHA stimulated lymphocytes from two study persons (filled squares and filled circles) and mRNA from the lymphoid cell line GM00131 (filled triangles) were immobilized on filters and hybridized to *ERCC1* and *β-actin* probes. The ratio between *ERCC1* and *β-actin* was constant and the ratio differs in the examples showed, indicating that *ERCC1* mRNA abundance varied from sample to sample.

extracted twice, ethanol precipitated and dissolved in TE-buffer prior to transfection at 200 mg/ml. The plasmid was UV-irradiated as described [4] and used for transfection the same day. UV-doses were 0, 250, 500 and 750 J/m^2.

Aliquots of 3×10^7–4×10^7 cells were thawed and grown as described above. After 3 days of growth, the cells were used for transfection. Transfections were carried out in a volume of 250 μl containing 10^6 cells and 1 μg DNA in 100 μg/ml DEAE-dextran for

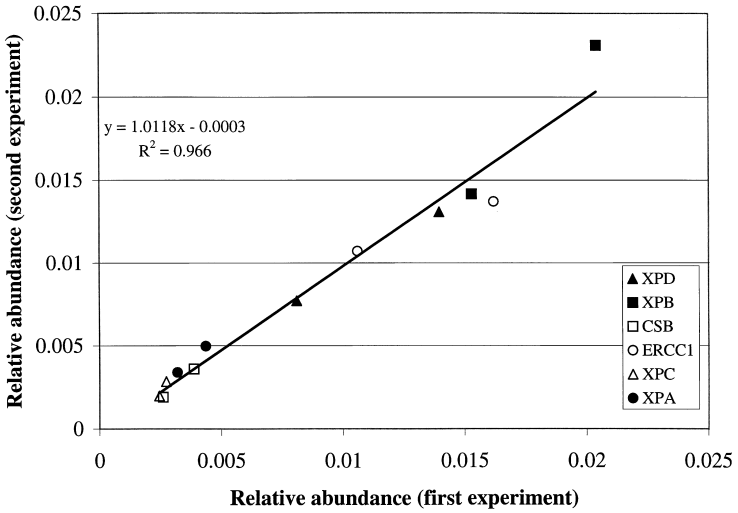

Fig. 2. Correlation between two independent determinations of the normalized mRNA values for *XPA, XPB, XPC, XPD, CSB* and *ERCC1* for two study persons. Lymphocytes from two study persons were thawed, grown for 3 days in two independent assays and mRNA was purified and hybridized to radioactively labeled *XPA*-, *XPB*-, *XPC*-, *XPD*- ,*CSB*-, *ERCC1*- and *β-actin* probes. The hybridization signal was quantified by scintillation counting and normalized to the cpm value for *β-actin*. The normalized values from the two determinations were plotted against each other.

15 min. Transfections were performed in triplicate. Following transfection, the cells were washed and resuspended in 1 ml conditioned medium and returned to the incubator for 44–46 h. The cells were then washed in phosphate saline buffer containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS), and resuspended in 100 μl PBS. Luciferase activity was assayed by adding 100 μl Luclite substrate (Packard Instruments), and luminescence was quantified by scintillation counting using single photon mode. Generally, the luciferase activity of the 0 J/m^2 points was 50 fold over background activity. The cell line GM00131 was included as internal control to check the performance of the HCR assay.

In our assay, the log-transformed luciferase activity showed a strong linear relationship when plotted versus UV-dose (Fig. 5). DNA repair capacity was, therefore, expressed as D_0, which is defined as the dose of a damaging agent required to reduce the percentage of luciferase activity to 37% along the log-linear dose-response curve [4,19,20]. D_0 did not correlate with PHA stimulation or cell survival. We have previously reported that in repeated measurements of DNA repair capacity ca 10% of the measurements were flawed [4]. In order to increase the fidelity of the slopes, we here used four UV-doses instead of three in the slope determination.

2.7. Cotransfection assay

The cDNAs encoding *XPB, XPD, ERCC1* and *CSB* were inserted into the expression vector pTEJ4 under transcriptional control of the human ubiquitin promoter [21]. *XPC* cDNA was inserted into pCEP4 (Invitrogen, Groningen, Netherlands) under control of the promoter of the immediate early gene of human cytomegalovirus. Each expression vector was able to complement lymphoblast cells defective in the gene in question in a complementation assay as described [22]. There are no lymphoblasts available that are defective in *ERCC1*. Therefore, the CHO cell-lines UV-5 and UV-20 were used to confirm the functional activity of pERCC1.

Host cell reactivation assay was performed as described above, except that 0.5 μg pUV60 and 0.5 μg NER-gene-containing plasmid or control plasmid was used in each transfection. Transfections with control and gene-containing plasmid, which were compared, were always performed in parallel on the same day.

3. Results

The study persons are members of a previously described set [4,23,24] consisting of 4 × 20 persons matched on age and sex with and without psoriasis and with and without basal cell carcinoma. Of the initial 80 persons, 33 were selected in three groups on the basis of their DNA repair capacity: 11 with ‘high repair capacity’, 11 with ‘medium repair capacity’ and 11 with ‘low repair capacity’, regardless of their psoriasis and skin cancer status. Because several of the samples had been used up in previous studies, all 33 subjects donated a new blood sample, which was used in this study.

Host cell reactivation assay was performed with lymphocytes, which had been grown for 3 days in the presence of PHA, which stimulates the growth of T-lymphocytes. In order to make the cell populations in the HCR assay and in mRNA extraction as comparable as possible, lymphocytes for mRNA extraction were also grown for 3 days in the presence of PHA.

Northern blotting using the probes for the various NER genes was performed to ensure specific hybridization (Results not shown). However, in order to be able to quantify the amount of hybridization obtained in each sample, we chose to perform dot blots because these can be quantified by scintillation counting, which is accurate even for weak signals.

Pilot experiments had indicated that Gene Screen Plus filters had a maximal binding capacity of about 2 mg RNA pr dot (7 mm^2). We, therefore, decided to isolate mRNA from the lymphocytes instead of total RNA to ensure that enough target mRNA could be bound to the membranes. By isolating only mRNA, more than 90% of the RNA (rRNA) is removed, thus increasing the relative abundance of target mRNA. However, the small amount of purified mRNA (expected yield from 10^7 cells is 1–2 mg mRNA) made it impossibly to quantify the amount of mRNA present in each sample prior to loading. Furthermore, a control experiment (Fig. 1) showed that the normalization to actin mRNA levels gave an adequate correction for

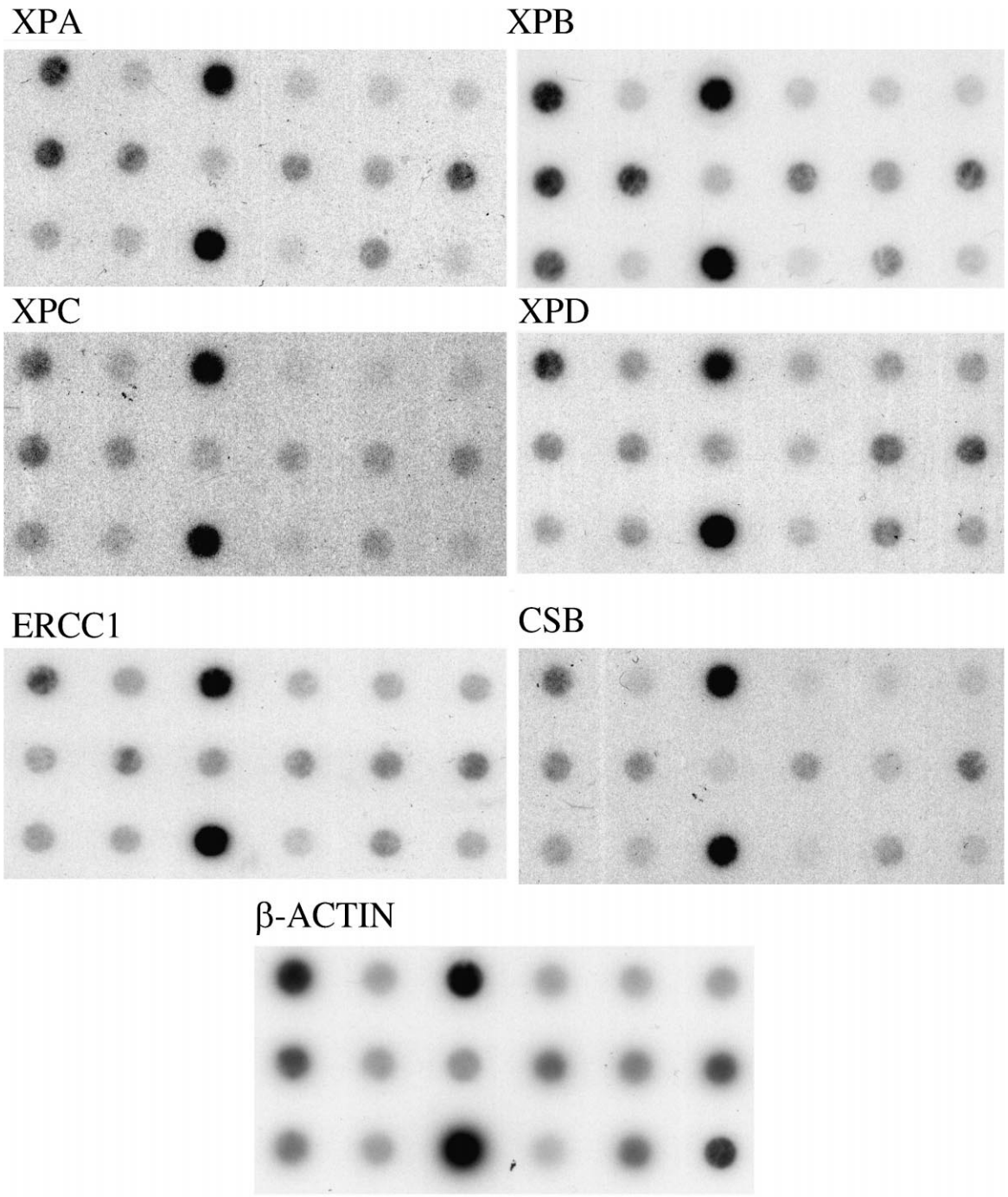

Fig. 3. Examples of autoradiograms of the dot-blots using different probes. RNA was immobilized to the membrane, and hybridized to radioactively labeled probe. The films were exposed for two days, except for the *β-actin*-blot, which was exposed for 2 h.

loading, even though very different amounts of mRNA were loaded in the different samples. We, therefore, chose not to ensure that equal amounts of mRNA were loaded in each sample.

The mRNA levels were determined for eight genes involved in nucleotide excision repair, namely *XPA, XPB, XPC, XPD, XPF, XPG, ERCC1* and *CSB*. mRNA was purified from 1×10^7–2×10^7 cells and aliquots of 1/10 of the mRNA were blotted on 10 different membranes. Each membrane was hybridized to one of the NER probes or to β-actin probe, and subsequently subjected to autoradiography (Fig. 3). The hybridization signals were quantified by cutting out the dot-blots as 5 mm diameter circles and subjecting these to liquid scintillation counting. The amount of radioactivity hybridized to each dot was normalized to the hybridization signal obtained from the β-actin dot-blots. The reproducibility of the mRNA level determination was estimated by making two independent mRNA isolations from two study subjects. On separate occasions lymphocytes were thawed, PHA stimulated and mRNA was isolated. The viability and growth yield of the two independent cultures of the same lymphocyte preparation varied. The mRNA levels of *XPA, XPB, XPC, XPD, CSB* and *ERCC1* were determined, and the mRNA levels from day-1 were correlated with the mRNA levels from day-2. The correlation between the mRNA levels was very good (Fig. 2), indicating that the person to person variation in mRNA levels is not due to differences in cell viability or PHA stimulation but in fact due to person to person differences.

The mean average abundance of NER mRNAs relative to actin mRNA levels is shown in Table 1. The cpm value from the dot-blots of mRNA isolated from each individual hybridized to the eight different NER gene probes was normalized to the cpm value of the β-actin dot-blot. For each gene, the mean mRNA level from the 33 individuals is listed in Table 1. The NER mRNAs constitute from 0.2 to 1.5% of the β-actin mRNA level (Table 1). We found a three- to sevenfold variation in the mRNA levels of all eight NER genes between individuals. When the mRNA levels of different genes were plotted against each other, some of them correlated. Examples of these correlations are shown in Fig. 4. Pearson's correlation coefficient was used as a measure of the correlation between the abundance of two mRNAs. The correlation coefficients for statistically significant correlations between different NER gene mRNA levels are listed in Table 2.

Table 1
mRNA abundance of NER genes relative to β-actin with standard deviations[a]

Gene	Mean relative mRNA abundance ± S.D.	Interval
XPA	0.003278 ± 0.001183	0.00095–0.0061
XPB	0.015141 ± 0.005619	0.0075–0.030
XPC	0.002076 ± 0.000739	0.00090–0.0036
XPD	0.008702 ± 0.003315	0.0032–0.019
XPF	0.004555 ± 0.00156	0.00052–0.0088
XPG	0.012673 ± 0.004385	0.0040–0.025
ERCC1	0.013084 ± 0.004213	0.0049–0.025
CSB	0.004059 ± 0.001331	0.0019–0.0072

[a] The hybridization signal of each dot-blot of the eight NER genes was normalized to the hybridization signal of the corresponding β-actin dotblot. Each mRNA level was measured once. The mean values of relative mRNA levels of the eight NER genes from the 33 study persons are summarized.

Several statistically significant correlations between NER mRNA levels were found. The amounts of *ERCC1* mRNA and *XPD* mRNAs were highly correlated (Fig. 4(A) and Table 2). The tight correlation between *XPD* and *ERCC1* mRNA levels has also been documented in brain tissue and in bone marrow [10,13].

The amounts of *XPA, XPB, XPC, XPF, XPG* and *CSB* mRNA correlated to each other with some exceptions (Fig. 4 and Table 2). Especially *XPG* and *XPB* mRNA levels correlated closely (Fig. 4(B) and Table 2). Thus it seems that the genes can be divided into at least two groups on the basis of the correlation of the mRNA levels: *ERCC1*and *XPD* in one group and *XPA, XPB, XPC, XPG, XPF* and *CSB* in the other group.

DNA repair capacity was measured in lymphocytes from 29 of the 33 individuals using the host cell reactivation assay. In this assay, the lymphocytes are transfected with UV-damaged plasmid encoding luciferase (pUV60) and the ability to repair the UV-damaged DNA and express luciferase was measured as function of the UV-dose. The DNA repair capacity was determined as the UV-dose required to reduce the remaining luciferase activity to 37%, denoted D_0. Examples of the dose-response curves are shown (Fig. 5).

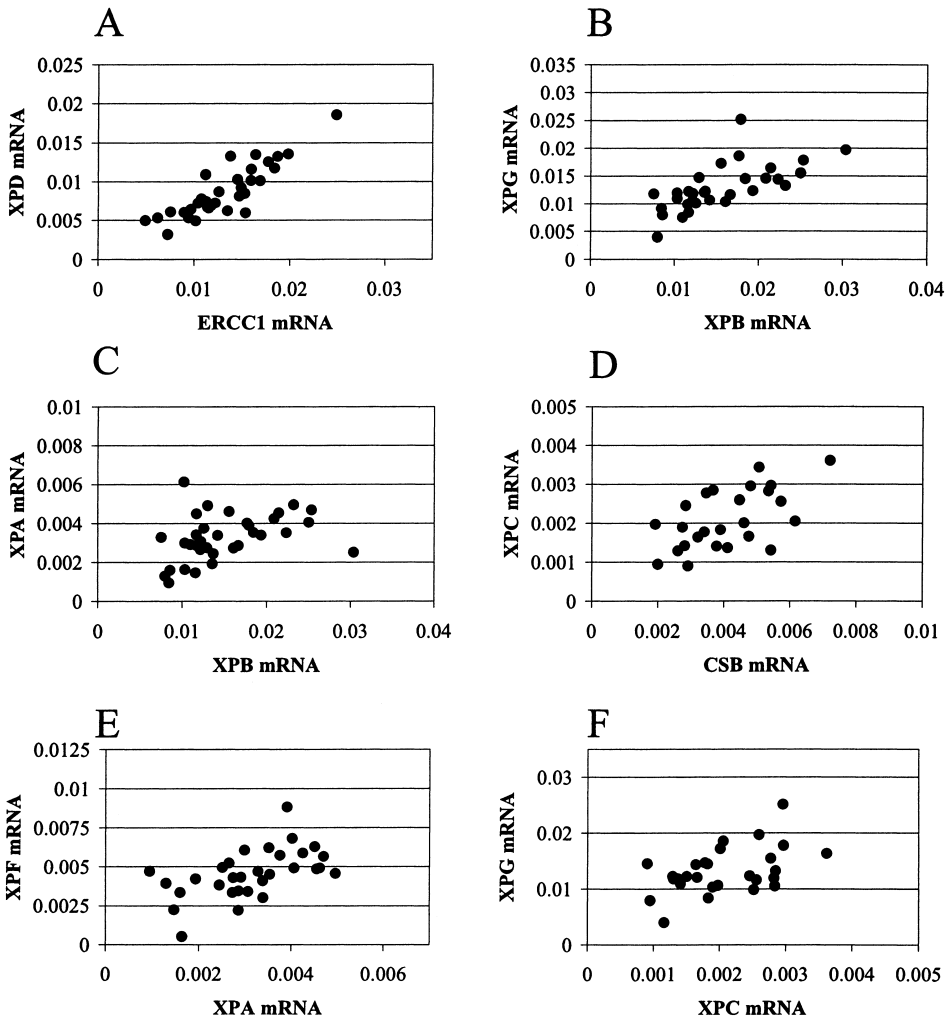

Fig. 4. Correlation between normalized mRNA levels of different genes. mRNA levels of each gene were normalized to the *β-actin* mRNA level. (A) *XPD* mRNA plotted vs. *ERCC1* mRNA; (B) *XPG* mRNA plotted vs. *XPB* mRNA; (C) *XPA* mRNA plotted vs. *XPB* mRNA; (D) *XPC* mRNA plotted vs. *CSB* mRNA; (E) *XPF* mRNA vs. *XPA* mRNA; (F) *XPG* mRNA vs. *XPC* mRNA.

When the correlation between DNA repair capacity (D_0) and steady state levels of the eight mRNAs was examined, a statistically significant positive correlation was found between DNA repair capacity and *ERCC1* mRNA levels and DNA repair capacity and *XPD* mRNA levels (Fig. 6 and Table 3). This could indicate that the amount of ERCC1 or XPD protein is rate limiting for nucleotide excision repair in the host cell reactivation assay in primary lymphocytes.

A second approach was also used to determine rate-limiting steps in DNA repair in the host cell reactivation assay. Nucleotide excision repair is a complex repair process involving many steps and many participating enzymes. One way of approaching an understanding of its regulation is to see if the repair capacity can be increased by over-expression of one or more of the enzymes involved in the process. Over-expression of an enzyme can only lead to increased activity, if the enzyme activity is limiting for a (part of a) reaction, or if the gene/enzyme in question has some regulatory role in the repair process.

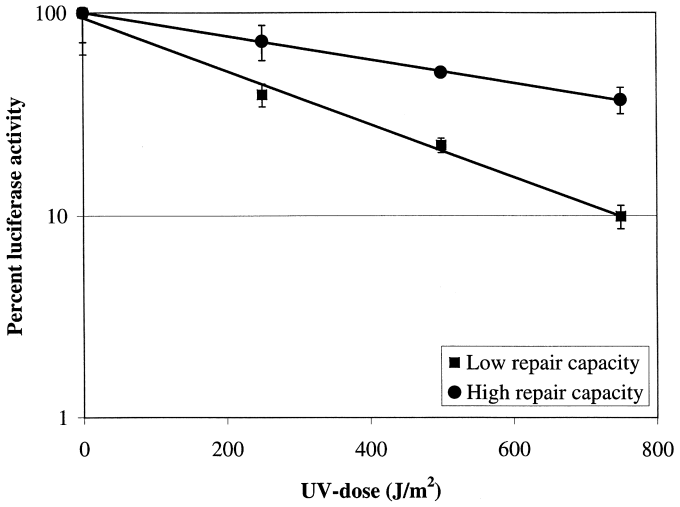

Fig. 5. Two examples of dose-response curves in the host cell reactivation assay. The assay was performed with four dose-points in triplicate.

Table 2
Correlation of the relative mRNA levels of eight NER genes[a]

mRNA levels that are correlated[b]	Correlation coefficient[c]	Two-tailed *P*-value[d]
XPA and *XPB*	0.515	0.0018
XPA and *XPC*	0.447	0.012
XPA and *XPF*	0.543	0.0013
XPA and *XPG*	0.583	0.00047
XPA and *CSB*	0.368	0.059
XPB and *XPC*	0.459	0.0095
XPB and *XPD*	−0.335	0.053
XPB and *XPG*	0.680	1.8×10^{-5}
XPB and *ERCC1*	−0.426	0.012
XPC and *XPG*	0.474	0.0093
XPC and *CSB*	0.579	0.0015
XPD and *XPG*	−0.348	0.074
XPD and *ERCC1*	0.892	1.4×10^{-12}
XPG and *XPF*	0.594	0.00034
XPG and *CSB*	0.455	0.022
XPG and *ERCC1*	−0.342	0.055

[a] The relative mRNA levels of the eight NER genes in the 33 study persons are correlated to each other two-by-two. Correlation coefficients and the corresponding *P*-value are listed for the correlation of various pairs of mRNAs.

[b] Only statistically significant or nearly significant correlations are listed.

[c] Pearson's correlation coefficient, *r*.

[d] $t(p) = r^*\text{sqrt}(n-2)/\text{sqrt}(1-r\hat{}2)$, where $t(p)$ follows the t-distribution with $(n-2)$ degrees of freedom, and n is the number of observations.

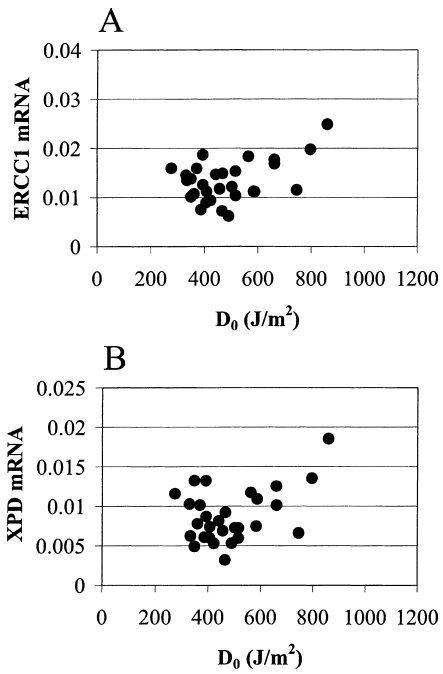

Fig. 6. Normalized *ERCC1* (A) and *XPD* (B) mRNA levels plotted vs. DNA repair capacity (D_0) measured by host cell reactivation. The normalized mRNA value of ERCC1 and XPD from each individual is plotted against the DNA repair capacity measured as D_0. D_0 is calculated as described in materials and methods.

Table 3
Correlation between DNA repair capacity measured by host cell reactivation and relative NER gene mRNA abundance for genes involved in nucleotide excision repair[a]

NER gene	Correlation coefficient[b]	Two-tailed *P*-value[c]
XPA	−0.021	> 0.1
XPB	−0.169	> 0.1
XPC	0.156	> 0.1
XPD	0.407	0.028
XPF	−0.0007	> 0.1
XPG	−0.115	> 0.1
ERCC1	0.450	0.014
CSB	0.049	> 0.1

[a] The DNA repair capacity was correlated with the relative mRNA level of each of the eight NER genes as shown in Fig. 6. The degree of correlation is given as the correlation coefficient.

[b] Pearson's correlation coefficient, *r*.

[c] $t(p) = r^*\text{sqrt}(n-2)/\text{sqrt}(1-r\hat{}2)$, where $t(p)$ follows the t-distribution with $(n-2)$ degrees of freedom, and n is the number of observations.

cDNAs encoding *XPB*, *XPD*, *CSB* and *ERCC1* were inserted into pTEJ4 [21], where the cDNAs are under transcriptional control of the human ubiquitin promoter. *XPC* cDNA was inserted into pCEP4, where it is under transcriptional control of the promoter of the immediate early gene of human cytomegalovirus. The two promoters were equally strong in lymphocytes (results not shown). The plasmid expressing XPB is called pXPB, the plasmid expressing XPC is called pXPC etc. Each expression plasmid was able to complement DNA repair activity in lymphobast cell lines deficient in the appropriate gene (results not shown) except for pERCC1 which was shown to complement the ERCC1 deficient CHO cell line UV20 (results not shown). Furthermore control experiments (Fig. 7) shows that transient transfections with the expression plasmids pXPB, pXPC, pXPD, pCSB, and pERCC1 lead to increased hybridization to XPB,XPC,XPD,CSB and ERCC1 probes, respectively, on dot-blots. This indicates that the genes encoded by the plasmids are transcribed in primary lymphocytes under the conditions used for determination of the DNA repair capacity.

Lymphocytes from eight different donors were co-transfected with UV-irradiated pUV60 and an expression plasmid expressing *XPB*, *XPD*, *XPC*, *CSB* or *ERCC1*. When *XPB* was over-expressed in the host cell reactivation, D_0 increased in the average with 59% (Table 4). The effect of XPB over-expression could be divided into three categories of response: No effect (study person 13), moderate (25–60%) increase in the DNA repair capacity (study persons 17, 58, 60, LB4, and LB5) or large increase (126%) in the DNA repair capacity (study persons 5 and 21). In contrast, over-expression of *ERCC1*, *XPD*, *XPC* or *CSB* did not increase the DNA repair capacity. The effect of

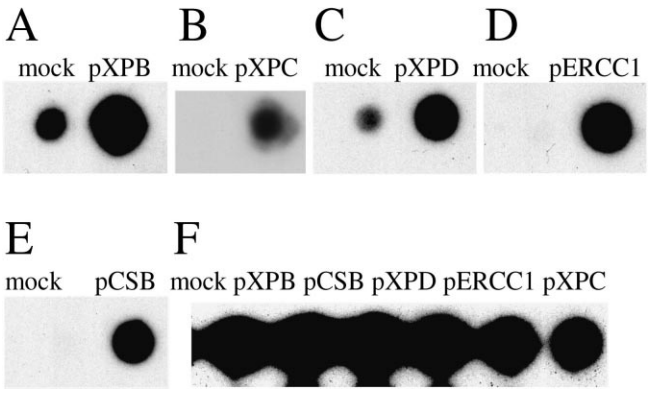

Fig. 7. Plasmid encoded NER genes were transcribed during the transient transfections. Primary lymphocytes from one study-person were PHA stimulated and transfected with the plasmids indicated as described for host cell reactivation assay. After 24 h, total RNA was isolated and used for dot-blot analysis, 3 mg was loaded on each dot. Mock transfected lymphocytes were transfected with pTEJ4 that contains no insert. The other lymphocytes were transfected with the plasmid indicated. Dot-blots were hybridized to the following probes: (A) *XPB*; (B) *XPC*; (C) *XPD*; (D) *ERCC1*; (E) *CSB*; (F) β-actin. The experiment was repeated with lymphocytes from another study person with similar results.

Table 4
Comparison of the DNA repair capacity (measured as D_0 values in host cell reactivation assay) with and without co-transfection with a plasmid over-expressing *XPB*[a]

Co-transfection plasmid	DNA repair capacity D_0 (J/m^2)	
	None	pXPB
Study person		
5	703	1592
13	447	464
17	456	606
21	463	1051
58	663	883
60	446	582
LB4	886	1412
LB3	401	503
Average	558	886
Two-tailed *p*-value[b]		0.018

[a] The DNA repair capacity was measured in the presence or absence of over-expression of *XPB* in lymphocytes isolated from different study persons.

[b] Paired t-test compared to D_0 without over-expression of DNA repair genes.

over-expression of *XPB, XPD* and *ERCC1* in lymphocytes from one study person is shown in Fig. 8.

4. Discussion

We have measured the mRNA levels of eight genes involved in NER, namely *XPA, XPB, XPC, XPD, XPF, XPG, ERCC1* and *CSB* in 33 individuals. The study persons were 45–55 years old, and selected from a set of psoriasis patients and healthy controls with and without basal cell carcinoma. The study persons were, therefore, without life-threatening diseases.

mRNA levels were found to vary three to sevenfold for all eight genes studied. The observed variation is not likely to reflect differences in cell growth, as two independent mRNA isolations from each of two different donors gave very good reproducibility. Likewise, the variation is probably not due to background hybridization, since there is no correlation between the relative NER mRNA level and the amount of RNA dotted on the filters (results not shown). If unspecific hybridization was an issue, we would expect all NER mRNA to correlate with each other, and not fall into two distinct groups, or we would expect the related sequences (for instance, XPB and XPD) to correlate.

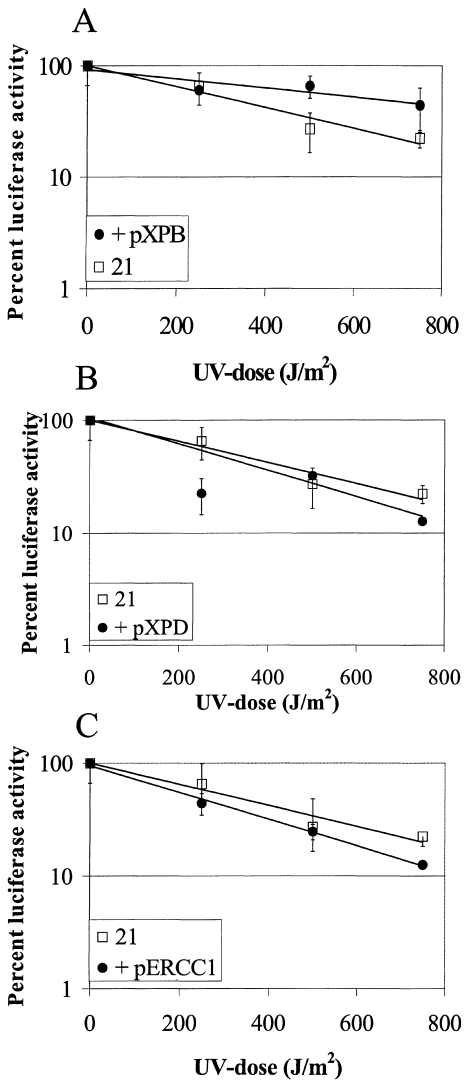

Fig. 8. Host cell reactivation assay with (closed circles) or without (open squares) over-expression of *XPB* (A), *XPD* (B) and *ERCC1* (C). Lymphocytes from study person no. 21 were PHA stimulated and the DNA repair capacity was measured in the presence and in the absence of plasmids over-expressing NER genes as described in materials and methods. The data points for the DNA repair capacity without over-expression of NER genes is the same in all three panels. Similar results were obtained with lymphocytes isolated from seven other study persons. The data point at 250 J/m^2 with over-expression of *XPD* was considered to be an out-lier.

mRNA levels of *ERCC1* and *XPD* were found to be very tightly correlated with each other. The tight coordination of the transcription and the fact that the two genes are separated by less than 250 kb [25] suggest

that transcription of the two genes is controlled by the same regulatory elements. Furthermore, the mRNA levels of *XPB, XPA, XPC, XPF, XPG* and *CSB* were found to correlate to each other, but not to the levels of *XPD* and *ERCC1*. The observed correlation between mRNA levels of eight NER genes which enzyme products catalyze very different steps in two independent NER pathways could reflect that NER mRNA transcript levels in living organisms were jointly regulated in response to exogenous DNA damaging agents such as sun light exposure or diet. Apparently, the NER genes are not controlled as one coordinated block in lymphocytes, but at least as two units.

In these experiments, *ERCC1* and *XPD* mRNA levels both correlated moderately with DNA repair capacity. The positive correlation between *ERCC1* mRNA levels and DNA repair capacity has been reported by others [10,12,13,17]. Thus, *XPD* and *ERCC1* mRNA levels can apparently be used as a proxy for DNA repair capacity when live cells are not available. It was recently shown by semi-quantitative PCR that the mRNA level of *XPB*, *XPC*, *XPG*, *ERCC1* and *CSB* were similar in PHA stimulated and unstimulated lymphocytes [15]. It is, therefore, possible that the mRNA levels in PHA stimulated lymphocytes actually will be similar to mRNA levels found in full blood. Such a correlation could be useful in molecular epidemiological studies.

When *ERCC1* and *XPD* were overproduced in the host cell reactivation assay, neither of them increased DNA repair indicating that DNA repair is not normally limited by *ERCC1* or *XPD* availability. We have only measured the mRNA levels of eight of 13–16 genes involved in NER. It is entirely possibly that the mRNA levels of *ERCC1* and *XPD* are co-regulated with mRNA levels of other genes, which influence the DNA repair capacity.

ERCC1 forms a complex with XPF (also called ERCC4) in vivo. The mRNA levels of XPF and ERCC1 did not correlate to each other (results not shown), which is perhaps surprising. Recently, it has been shown that *ERCC1* over-expression only leads to accumulation of ERCC1 protein when XPF is also over-expressed [26]. Thus, ERCC1 may not accumulate when over-produced without XPF, and that this could be the reason why *ERCC1* over-production does not increase DNA repair capacity. On the other hand, since the mRNA levels of *XPF* and *ERCC1* do not correlate to each other, ERCC1 protein availability could limit ERCC1-XPF dimer formation in some cells, where subsequent over-expression of ERCC1 would lead to increased formation ERCC1-XPF dimers.

There are several studies suggesting that a subset of cancer patients have vastly increased NER mRNA levels [11–14]. It seems that the normal expression level of these NER genes vary three to sixfold, and that the very high expression level of NER genes seen in cancer patients, may be a result of their disease or of the cancer treatment. Cheng et al. [15] has quantified the mRNA levels of five NER in 12 healthy volunteers by semi-quantitative PCR and found 25% standard deviation [15], where we found 33% (Table 1). In the above-mentioned study, *ERCC1, XPB, XPC, XPG* and *CSB* mRNA levels were found to be similar to the mRNA level of β-actin, whereas we find that they constitute 0.2–1.5% of the β-actin mRNA level. Using dot-blot quantification, the relative abundance of a mRNA is dependent on a variety of factors including the specific activity of the probe, probe length and the washing procedure. The length of the used probes varied from 0.3 to 2.8 kb, whereas the β-actin probe was 0.8 kb. The specific activity of the probes varied less than a factor of two (not shown), and the washing procedure was the same for all the dot-blots.

Over-expression of *XPB* increased DNA repair capacity in the host cell reactivation assay. The DNA repair capacity was on an average increased by 60%, which is much less than the three fold variation observed among the study subjects. However, it is important to realize that all host cell reactivation belonging to an experiment were done on the same day. Within day variation of the host cell reactivation assay is considerably smaller than 60%. The bothersome issue of day-to-day variation of this assay, therefore, does not influence our results. Over-expression of *XPB* in lymphoblastoid cells also increased DNA repair capacity ca twofold (to be described elsewhere).

In the study persons, the effect of over-expression fell into three categories: no effect (one person), moderate effect (five persons) and large effect (two persons). This could indicate that the effect of *XPB* over-expression is dependent on the other factors in the cell, probably the availability of the other subunits of TFIIH. *XPB* is not the main factor responsible for the inter-personal variation in DNA repair capacity

since the effect of *XPB* over-expression is less than the person-to-person variation found. On the other hand, the maximal DNA repair capacity measured without *XPB* over-expression was ca 860 J/m^2 (Fig. 6) whereas the maximal DNA repair capacity with *XPB* over-expression was ca 1600 J/m^2 (Table 4). This indicates that XPB protein level is an important determinant for the DNA repair capacity. In another study, over-expression of *XPA* in a human cell line was found to cause a 50–100 percent increase in survival after UV-radiation and an increase in DNA repair capacity similar to what we have found [27]. XPA participate in damage recognition and recruitment of TFIIH (including XPB) to form the pre-incision complex [28].

Host cell reactivation involves live cells and allows for a complete set of cellular response mechanisms over a period of 44 h. It is, therefore, also possible that XPB over-production does not simply complement the available XPB enzyme level, but modifies some regulatory function in NER. XPB is a sub-unit in the transcription factor complex TFIIH [29], and XPB in TFIIH has been shown to interact with the tumor suppressor p53 in vivo [30]. Although the biological significance of this interaction is unclear, it is possible that NER activity is regulated by the XPB-p53 interaction, and that this regulation is altered when XPB is overproduced.

We find that NER mRNAs are regulated in at least two independent groups in normal persons. However, in studies including cancer patients, co-ordinated over-expression has been reported for all NER genes examined suggesting that coordinated transcriptional induction can occur [6,7]. Whether this only occurs under extreme circumstances or is a more general phenomenon remains to be seen.

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References

[1] K.H. Kraemer, M.M. Lee, J. Scotto, Xeroderma pigmentosum: cutaneous, ocular, and neurologic abnormalities in 830 cases, Arch. Dermatol. 123 (1987) 241–250.
[2] Q. Wei, L. Cheng, W.K. Hong, M.R. Spitz, Reduced DNA repair capacity in lung cancer patients, Cancer Res. 56 (1996) 4103–4107.
[3] Q. Wei, G.M. Matanoski, E.R. Farmer, M.A. Hedayati, L. Grossman, DNA repair and susceptibility to basal cell carcinoma. A case-control study, Am. J. Epidemiol. 140 (1994) 598–607.
[4] M. Dybdahl, G. Frentz, U. Vogel, H. Wallin, B.A. Nexø, Low DNA repair is a risk factor in skin carcinogenesis: a study of basal cell carcinoma in psoriasis patients, Mutat. Res. 433 (1999) 15–22.
[5] L. Cheng, S.A. Eicher, Z. Guo, W.K. Hong, M.R. Spitz, Q. Wei, Reduced DNA repair capacity in head and neck cancer patients, Cancer Epidemiol. Biomarkers Prev. 7 (1998) 465–468.
[6] Q. Li, L. Ding, J.J. Yu, C. Mu, B. Tsang, F. Bostick-Bruton, E. Reed, Cisplatin and phorbol ester independently induce ERCC-1 protein in human ovarian carcinoma cells, Int. J. Oncol. 13 (1998) 987–992.
[7] Q. Li, K. Gardner, L. Zhang, B. Tsang, F. Bostick-Bruton, E. Reed, Cisplatin induction of ERCC-1 mRNA expression in A2780/CP70 human ovarian cancer cells, J. Biol. Chem. 36 (1998) 23419–23425.
[8] R.J. Parker, A. Eastman, F. Bostick-Bruton, E. Reed, Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation, J. Clin. Invest. 87 (1991) 772–777.
[9] A. Sancar, DNA excision repair, Ann. Rev. Biochem. 65 (1996) 43–81.
[10] M. Dabholkar, M. Berger, J. Vionnet, C. Egwuagu, J.R. Silber, J.J. Yu, E. Reed, Malignant and nonmalignant brain tissues differ in their messenger RNA expression patterns for *ERCC1* and *ERCC2*, Cancer Res. 55 (1995) 1261–1266.
[11] M. Dabholkar, M. Berger, J. Vionnet, L. Overton, C. Thompson, F. Bostick-Bruton, J.J. Yu, J.R. Silber, E. Reed, Comparative analyses of relative ERCC3 and ERCC6 mRNA levels in gliomas and adjacent non-neoplastic brain, Mol. Carcinog. 17 (1996) 1–7.
[12] M. Dabholkar, F. Bostick-Bruton, C. Weber, V.A. Bohr, C. Egwuagu, E. Reed, ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients, J. Natl. Cancer Inst. 19 (1992) 1512–1517.
[13] M. Dabholkar, F. Bostick-Bruton, C. Weber, C. Egwuagu, V.A. Bohr, E. Reed, Expression of excision repair genes in non-malignant bone marrow from cancer patients, Mutat. Res. 293 (1993) 151–160.
[14] M. Dabholkar, J. Vionnet, F. Bostick-Bruton, J.J. Yu, E. Reed, Messenger RNA levels of XPAC and ERCC1 in ovarian

cancer tissue correlate with response to platinum-based chemotherapy, J. Clin. Invest. 94 (1994) 703–708.
[15] L. Cheng, Y. Guan, L. Li, R.J. Legerski, J. Einspahr, J. Bangert, D.S. Alberts, Q. Wei, Expression in normal human tissues of five nucleotide excision repair genes measured simultaneously by multiplex reverse transcription-polymerase chain reaction, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 801–807.
[16] R.D. Wood, Nucleotide excision repair in mammalian cells, J. Biol. Chem. 272 (1999) 23465–23468.
[17] Q. Wei, X. Xu, L. Cheng, R. Legerski, R. Ali-Osman, Simultaneous amplification of four DNA repair genes and β-actin in human lymphocytes by multiplex reverse transcriptase-PCR, Cancer Res. 55 (1995) 5025–5029.
[18] R. Pellé, N. Murphy, Northern Hybridisation: rapid and simple electrophoretic conditions, Nucleic Acid Res. 21 (1993) 2783–2784.
[19] W.F. Athas, M.A. Hedayati, G.M. Matanoski, E.R. Farmer, L. Grossman, Development and field-test validation of an assay for DNA repair in circulating human lymphocytes, Cancer Res. 51 (1991) 5786–5793.
[20] M. Protíc-Sabljic, K.H. Kraemer, One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells, Proc. Natl. Acad. Sci. USA 82 (1985) 6622–6626.
[21] T.E. Johansen, M. Schøller, S. Tolstoy, T.W. Schwartz, Biosynthesis of peptide precursors and protease inhibitors using new constitutive and inducible eukaryotic expression vectors, FEBS Lett. 267 (1990) 289–294.
[22] M. Carreau, E. Eveno, X. Quilliet, O. Chevalier-Lagente, A. Benoit, B. Tanganelli, M. Stefanini, W. Vermeulen, J.H.J. Hoeijmarkers, A. Sarasin, M. Mezzina, Development of an new easy complementation assay for DNA repair deficient human syndromes using cloned repair genes, Carcinogenesis 16 (1995) 1003–1009.
[23] M. Dybdahl, U. Vogel, G. Frentz, H. Wallin, B.A. Nexø, Polymorphism in the DNA repair gene XPD: Correlations with risk and age at onset of basal cell carcinoma, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 77–81.
[24] P. Møller, L.E. Knudsen, G. Frentz, M. Dybdahl, H. Wallin, B.A. Nexø, Seasonal variation of DNA damage and repair in patients with non-melanoma skin cancer and referents with and without psoriasis, Mutat. Res. 407 (1998) 25–34.
[25] H.W. Mohrenweiser, A.V. Carrano, A. Fertitta, B. Perry, L.H. Thompson, J. D Tucker, C.A. Weber, Refined mapping of the three DNA repair genes ERCC1 ERCC2 and XRCC1 on chromosome 19, Cytogenet. Cell Genet. 52 (1989) 11–14.
[26] T. Yagi, A. Katsuya, A. Koyano, H. Takebe, Sensitivity of group F xeroderma pigmentosum cells to UV and mitomycin C relative to levels of XPF and ERCC1 overexpression, Mutagenesis 13 (1998) 595–599.
[27] J.E. Cleaver, W.C. Charles, M.L. McDowell, W.J. Sadinski, D.L. Mitchell, Overexpression of the XPA repair gene increases resistance to ultraviolet radiation in human cells by selective repair of DNA damage, Cancer Res. 55 (15-12-1995) 6152–6160.
[28] D.P. Batty, R.D. Wood, Damage recognition in nucleotide excision repair of DNA, Gene 241 (2000) 193–204.
[29] R. Drapkin, A. Sancar, D. Reinberg, Where transcription meets repair, Cell 77 (1994) 9–12.
[30] X.W. Wang, H. Yeh, L. Schaeffer, R. Roy, V. Moncollin, J.-M. Egly, Z. Wang, E.C. Friedberg, M.K. Evans, B.G. Taffe, V.A. Bohr, G. Weeda, J.H.J. Hoeijmarkers, K. Forrester, C.C. Harris, p53 modulation of TFIIH-associated nucleotide excision repair activity, Nat. Genet. 10 (1995) 188–195.