# c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF

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## ABSTRACT

**Cells deficient in c-Fos are hypersensitive to ultraviolet (UV-C) light. Here we demonstrate that mouse embryonic fibroblasts lacking c-Fos (*fos*−/−) are defective in the repair of UV-C induced DNA lesions. They show a decreased rate of sealing of repair-mediated DNA strand breaks and are unable to remove cyclobutane pyrimidine dimers from DNA. A search for genes responsible for the DNA repair defect revealed that upon UV-C treatment the level of *xpf* and *xpg* mRNA declined but, in contrast to the wild type (wt), did not recover in *fos*−/− cells. The observed decline in xpf and xpg mRNA is due to impaired re-synthesis, as shown by experiments using actinomycin D. Block of *xpf* transcription resulted in a lack of XPF protein after irradiation of *fos*−/− cells, whereas the XPF level normalized quickly in the wt. Although the *xpg* mRNA level was reduced, the amount of XPG protein was not altered in c-Fos-deficient cells after UV-C, due to higher stability of the XPG protein. The data suggest a new role for c-Fos in cells exposed to genotoxic stress. Being part of the transcription factor AP-1, c-Fos stimulates NER via the upregulation of xpf and thus plays a central role in the recovery of cells from UV light induced DNA damage.**

## INTRODUCTION

The genome is permanently harmed by endogenous and exogenous insults. The dose of exposure, however, inducing DNA damage is variable, depending on life style and numerous endogenous and environmental factors. Therefore, DNA repair might be supposed to be highly regulated, adapted to the level of the genotoxic insult. In fact, promoters of several DNA repair genes are subject to modulation by genotoxins, indicating that fine-tuned mechanisms of regulation of DNA repair have been evolved. For example, ultraviolet (UV-C) light increases the expression of the DNA repair proteins DDB2, XPC, Pol I, Lig1 and Fen1 (1–5).

There are two important players that are thought to be involved in the regulation of DNA repair: p53 and c-Fos. Both transcription factors are induced by many types of genotoxic stress and implicated in maintaining genomic stability and cell survival. Thus, mouse embryonic fibroblasts (MEFs) deficient in p53 are more sensitive to UV-C light than the corresponding wild type (wt) (6). This was also found for MEFs that are deficient in c-Fos (7). Whereas for p53-deficient cells hypersensitivity is ascribed to be due to the abolition of $G_1$/S checkpoint control (8,9), impaired base excision repair (10,11) and nucleotide excision repair (12), the hypersensitivity of c-Fos-deficient cells remained up to now enigmatic.

Our initial finding that MEFs derived from *c-fos* knockout mice are hypersensitive to UV-C light was explained on the basis of an impaired recovery of the cells from the UV-C induced block of DNA replication (7). Hypersensitivity of c-Fos-deficient cells was confirmed by determining apoptosis and chromosomal aberrations in both established and primary MEFs treated with UV light and various chemical genotoxins (13,14). The c-Fos protein together with a member of the Jun family or ATF1 forms the heterodimeric activator protein AP-1 (15,16) that stimulates a broad spectrum of genes harbouring AP-1 sites in the promoter. *c-fos* is immediate-early inducible upon transcriptional activation by growth factors (17), heavy metals (18), UV light (19), alkylating agents (20) and other forms of genotoxic stress (21). The fact that cells lacking c-Fos are hypersensitive to genotoxins, responding with an increased frequency of cell death and chromosomal aberrations, suggests that c-Fos plays an important role in the cellular defence against DNA damaging agents. On the other hand, c-Fos overexpression drives malignant transformation (22,23), which might explain why c-Fos is expressed at high level in several human tumors (24,25). c-Fos overexpression was also shown to provoke resistance to chemotherapy by protecting cells against the anticancer drug cisplatin (26,27).

Here we examined the role for c-Fos in the regulation of DNA repair. Comparing the expression of ∼130 DNA repair genes (by means of a DNA repair microarray) in wt and *c-fos* knockout (*fos*−/−) cells after UV-C exposure, we found the

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*NER* genes *xpf* and *xpg* to be differentially expressed. Whereas normal cells recover quickly from *xpf* and *xpg* mRNA downregulation, in *fos*−/− cells sustained depression of *xpf* and *xpg* gene activity was observed. This results in reduced repair protein level, notably XPF, decreased repair of UV-C induced pyrimidine dimers (CPDs) and persistence of NER intermediate DNA single-strand breaks (SSB). Thus, c-Fos appears to be involved in the recovery from transcriptional inhibition leading to reconstitution of the original gene activity that was depressed upon genotoxic treatment. Based on the findings, we propose a novel concept for the biological function of the 'classical' cellular immediate-early genotoxic response: stimulation of re-expression of DNA repair genes (notably *xpf*) upon DNA damage and fast restoration of normal DNA repair capacity.

## MATERIALS AND METHODS

### Cell lines

The cell lines used (fos+/+1-98M designated as wt, fos−/−7-98M designated as *fos*−/−) were described previously (6,14). The cells were grown in DMEM containing 10% fetal bovine serum (FBS), in 7% $CO_2$ at 37°C.

### UV-C treatment

Growth medium was removed and cells were irradiated with UV-C light at a dose rate of 1 J/m$^2$ per second with a radium NSE 11-270 low pressure UV-C lamp (Philips). Thereafter, the removed medium was returned and cells were incubated at 37°C for the appropriate time periods.

### Preparation of cell extracts and western blot analysis

Nuclear extracts were prepared as described previously (28). Samples of 25 μg protein extract were separated by 10% SDS–PAGE and electroblotted onto nitrocellulose membranes, which were then incubated with antibodies as described previously (29). Monoclonal anti-p53 and c-fos antibodies (sc-99, sc-52; Santa Cruz Biotechnology) were diluted 1:500 in 5% non-fat dry milk, 0.2% Tween/ phosphate-buffered saline (PBS) and incubated overnight at 4°C. Monoclonal anti-XPF antibody (MS1385-PO; Neomarkers) was diluted 1:1000 in 5% non-fat dry milk, 0.1% Tween/ PBS and incubated overnight at 4°C. Polyclonal anti-ERK2 and polyclonal anti-XPG antibodies (sc-154, sc-12558; Santa Cruz Biotechnology) was diluted 1:3000 and incubated overnight at RT. The protein–antibody complexes were visualized by ECL (Amersham).

### Preparation of RNA, RT–PCR and real-time RT–PCR

Total RNA was isolated using the RNA II Isolation Kit from Machery and Nagel. An aliquot of 2 μg RNA was transcribed into cDNA by Superscript II (Invitrogen) in a volume of 40 μl and 3 μl was subjected to RT–PCR. RT–PCR was performed by the use of specific primers (MWG Biotechnology) and Red-Taq Ready Mix (Sigma-Aldrich). The PCR program used was as follows: 1.5 min, 94°C [denaturation: 45 s, 94°C; annealing: 1 min, 56–62°C; elongation: 1 min, 72°C, 25 cycles], 10 min, 72°C. Real-time PCR was performed using the LightCycler FastStart DNA Master SYBR Green

I Kit (Roche Diagnostics) and the light cycler of Roche Diagnostics.

### Southwestern analysis

Genomic DNA was isolated from subconfluent grown cells by the use of the QIA(amp) blood mini kit (Qiagen). DNA (0.5 μg) was transferred to a positively charged nylon membrane (Hybond plus; Amersham) by vacuum slot-blotting, denatured with 0.3 M NaOH, neutralized with 5× SSC and fixed by baking the membrane for 2 h at 80°C. Monoclonal antibodies specific for thymine dimers (Kamiya Biomedical Company) were used at a dilution of 1:100. The additional western blot procedure and detection was performed as described above.

### Measurement of RNA synthesis

Transcription blockage upon UV-C exposure was checked by the incorporation of [$^3$H]uridine. Cells were exposed to UV-C and post-incubated for 1–8 h. One hour before the end of the post-incubation period 0.5 μCi/ml [5,6-$^3$H]uridine was added to the medium. Thereafter, cells were washed twice with PBS and 6% trichloroacetic acid (TCA) to remove unincorporated [$^3$H]uridine. Cells lysis was performed by adding 2 ml of 0.1 N NaOH to the cells and overnight incubation. An aliquot of 0.5 ml of the lysate was mixed with 4 ml scintillation cocktail and counted in a liquid scintillation counter. The incorporated radioactivity in cells not exposed to UV-C was set to 100%.

### BrdU incorporation

Cells were cultured in DMEM (10% FBS) and, after exposure to UV-C, the thymidine analogue BrdU (10 μM) was added to the medium. After 1 h of incubation, the incorporation was analysed using a BrdU Incorporation Kit (Roche) in a microplate reader.

### Cloning and transfection of mouse XPF cDNA

The XPF cDNA from *fos*+/+1-98M MEFs was amplified by RT–PCR using specific primers and cloned in the vector pcDNA3.1/V5-His-TOPO to generate the vector pcDNAtopo-mXPF. This vector was utilized for transient transfection of MEFs using the Fugene HD system from La Roche.

### Single-cell gel electrophoresis (SCGE, comet assay)

Exponentially growing cells were exposed to UV-C and, after the indicated time periods, trypsinized and washed with ice-cold PBS. Alkaline cell lysis and electrophoresis was essentially performed as described previously (30).

### Chromatin immunoprecipitation assay (ChIP)

Cellular genomic DNA and proteins were cross-linked within the cells by the addition of 190 μl of 37% formaldehyde to the medium (7 ml) for 10 min in a 10 ml dish. The reaction was stopped by the addition of 700 μl of 1.25 M glycine. After 5 min incubation medium was removed, cells were washed twice with PBS, collected and resuspended in 1 ml PBS containing 1 μl phenylmethylsulfonyl fluoride (PMSF). Genomic DNA was fragmented by sonication to a fragment size between 500 and 1500 bp. Sodium lauroyl sulphate was added to a final concentration of 0.5%. After 20 min mixing,

membrane fragments were removed by centrifugation (10 min, 10 000 *g*). Equal amounts of fragmented DNA were subjected to immunoprecipitation (IP) using a c-Fos-specific antibody (sc-52; Santa Cruz) and protein G–Sepharose. Immunoprecipitated proteins cross-linked to DNA were washed four times in 1 ml wash buffer (1% Triton X-100, 0.1% SDS, 150 mM NaCl, 2 mM EDTA 8.0, 20 mM Tris–HCl, pH 8.0 and protease inhibitors). After the final washing step the immunoprecipitate was resuspended in 1 ml washing buffer containing 500 mM NaCl. After an

additional centrifugation step, the immunoprecipitate was resuspended in 400 µl elution buffer (1% SDS and 100 mM NaHCO$_3$) and 500 µg/ml proteinase K and RNase I were added and incubated 30 min at 37°C. Cross-links were reversed by heating 65°C overnight. The probe was adjusted to 200 mM NaCl, proteins were removed by phenol–chloroform extraction, DNA was recovered by ethanol precipitation and resuspended in 50 µl aqua bidest. PCR was performed using specific primers for the AP-1-binding site of XPF and, as negative control, β-actin.
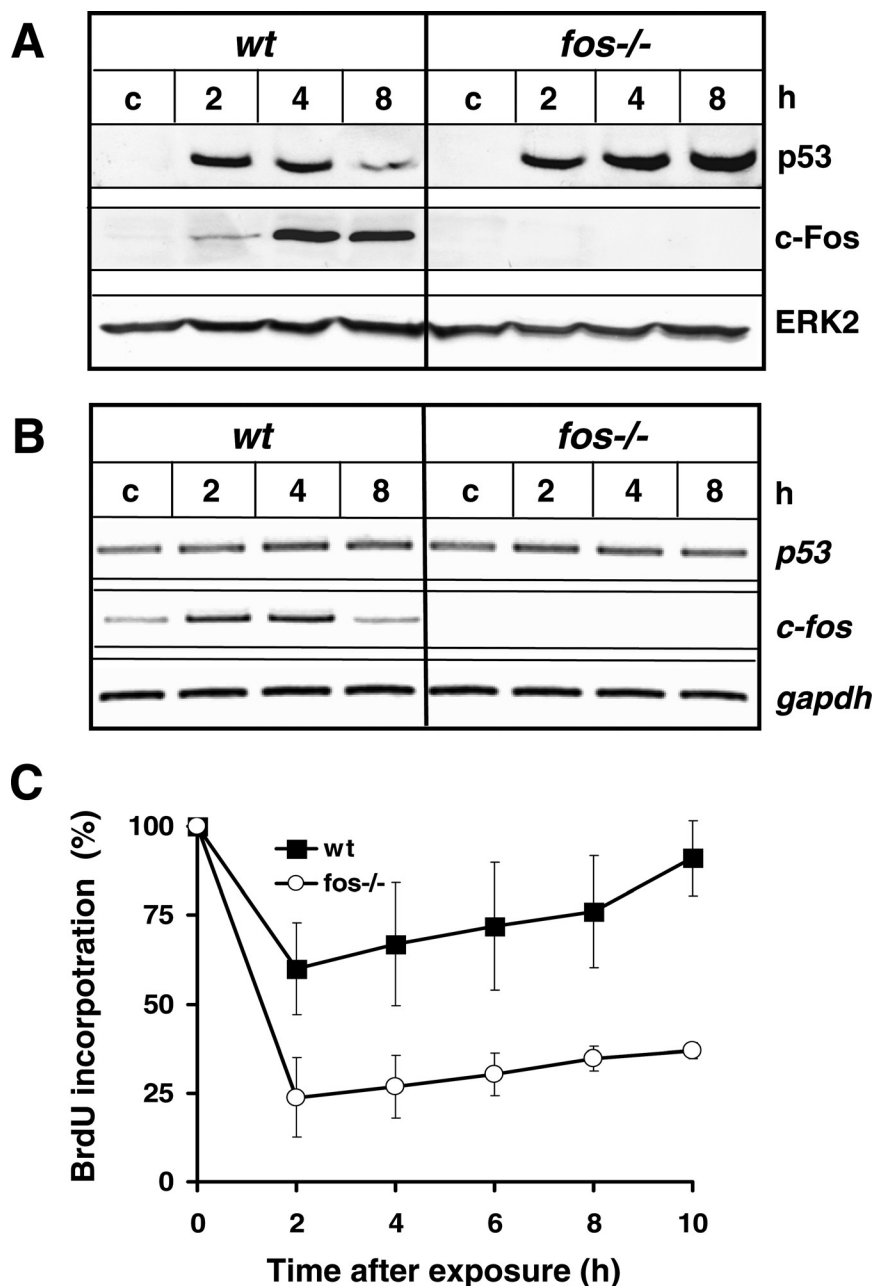


**Figure 1.** Characterization of wt and *fos*−/− cells. (**A**) To analyse the c-Fos and p53 status, wt and *fos*−/− cells were exposed to 20 J/m$^2$. At different times after exposure, cells were harvested, nuclear extracts were prepared and 25 µg were subjected to western blot analysis (c, non-exposed control). The filter was incubated with p53, c-Fos and, for loading control, ERK2-specific antibody. (**B**) To analyse the *c-fos* and *p53* mRNA status, wt and *fos*−/− cells were exposed to 20 J/m$^2$. At different time points after exposure, cells were harvested and total RNA was isolated. Two micrograms were subjected to cDNA synthesis, followed by RT–PCR with specific primers (c, non-exposed control). As internal control, *gapdh* was amplified. (**C**) DNA replication in wt and *fos*−/− fibroblasts as a function of time after UV-C treatment (7.5 J/m$^2$ UV-C), as measured by BrdU incorporation. Data are the mean of at least three independent experiments.

## RESULTS

### Characterization of wt and c-Fos-deficient cell lines

We used isogenic embryonal mouse fibroblasts (MEFs) that are wt and knockout for *c-fos* (*fos*−/−). Both cell lines are 'wt' for p53. Because p53 frequently mutates in mouse fibroblasts, the maintenance of the p53 status was carefully checked before each experimental series. As shown in Figure 1A, wt and *fos*−/− cells display no detectable basal level of p53 protein, but a clear p53 accumulation 2–8 h after UV-C treatment (20 J/m$^2$). (The sustained activation of p53 in *fos*−/− cells 8 h after UV exposure could be indicative of remaining DNA lesions; see below.) In contrast to the p53 protein, the corresponding mRNA increased only slightly (Figure 1B). This is in line with the p53 accumulation upon genotoxic stress to be due to stabilization and nuclear translocation. The expression of the c-Fos protein (Figure 1A) and mRNA (Figure 1B) was also enhanced upon UV-C treatment of wt cells. As expected, it was not expressed in *fos*−/− cells.

### c-Fos-deficient cells are impaired in the removal of pyrimidine dimers

UV-C light induces the formation of CPDs that inhibit DNA replication (31). DNA replication is much stronger inhibited and less efficiently restored in c-Fos-deficient cells than in the wt (Figure 1C), which was taken to indicate that *fos*−/− cells suffer from a DNA repair defect. To analyse the repair capacity of *fos*−/− cells, we determined the induction and repair of CPDs. As demonstrated in Figure 2A (showing a representative blot) and Figure 2B (quantification of three

independent experiments), CPDs were detectable already 5 min after treatment with UV-C (7.5 and 20 J/m$^2$) to a similar level in wt and *fos*−/− cells. Within the 12 h post-incubation period, CPDs were significantly removed from DNA in the wt cells (by ∼60%). This did not occur in *fos*−/− cells, in which repair of CPDs was much less efficient (∼20% removal). Repair of CPDs upon treatment with a higher dose of UV-C (20 J/m$^2$) was found only in wt cells whereas in *fos*−/− cells CPDs were not removed at all (Figure 2A and B, right panel). For control, dilution experiments with [$^{14}$C]thymidine pre-labelled cells were performed (data not shown), excluding an impact of DNA replication on the CPD level determined in the post-incubation period.

### c-Fos lacking cells are deficient in the processing of single-strand breaks

To elucidate which step in the repair of CPDs is disturbed in c-Fos lacking cells, alkaline SCGE was conducted. Cells were exposed to different doses of UV-C and harvested 6 h later for analysis. As shown in Figure 3A, *fos*−/− cells showed a significant increased accumulation of DNA SSBs. To analyse whether the increased amount of DNA SSBs upon UV-C is due to increased formation of CPDs or lack of repair in *fos*−/− cells, time course experiments were performed. As shown in Figure 3B, 2 h after UV-C exposure both wt and *fos*−/− cells displayed a transient induction of DNA SSBs. These are considered to be repair intermediates. Whereas in the wt repair of these breaks occurred during the 8 h post-incubation period, *fos*−/− cells did not return to control level. They rather showed an increase in DNA breaks
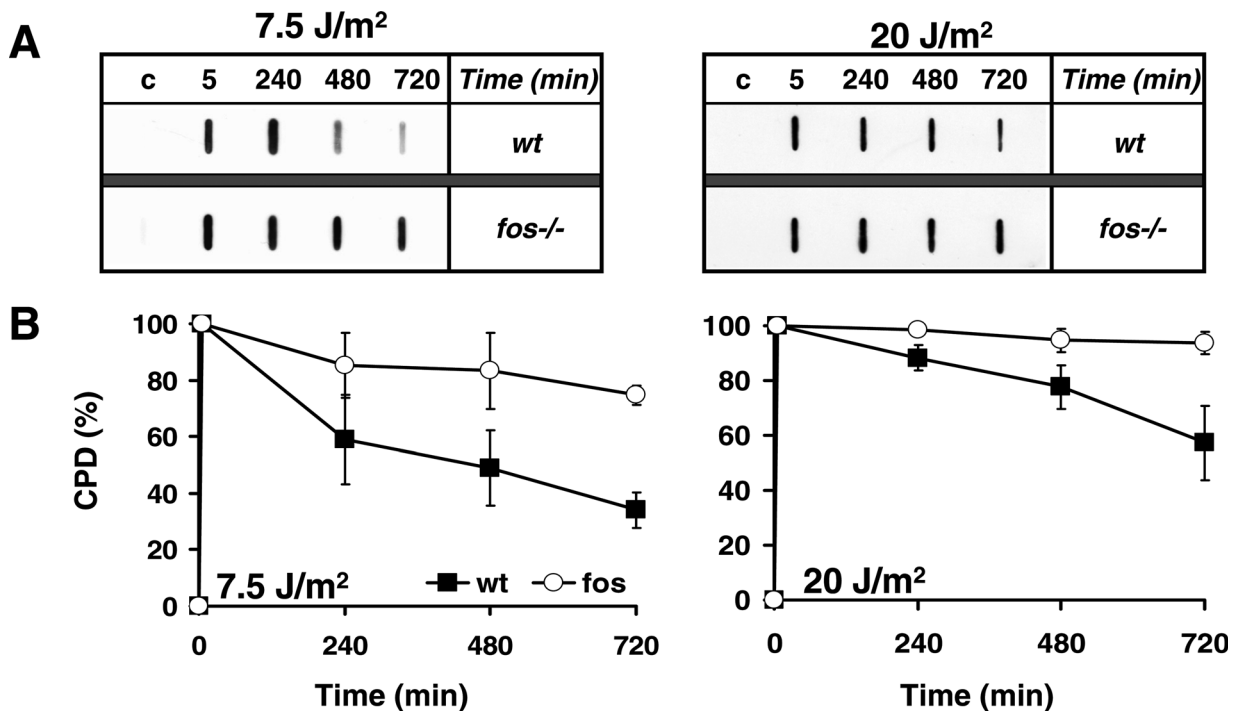


**Figure 2.** Removal of CPDs from DNA in wt and *fos*−/− cells. Induction and repair of UV-C lesions in wt and *fos*−/− MEFs was determined by southwestern analysis. Cells were exposed to 7.5 (left panel) or 20 (right panel) J/m$^2$ UV-C. At different time points following irradiation, genomic DNA was isolated, equal amounts of DNA were blotted and subjected to incubation with anti-CPD antibodies (c, non-exposed control). (**A**) Presentation of representative blots. (**B**) For quantification, the CPD signal measured 5 min after treatment was set to 100%. Data are the mean of three independent experiments ± SD.
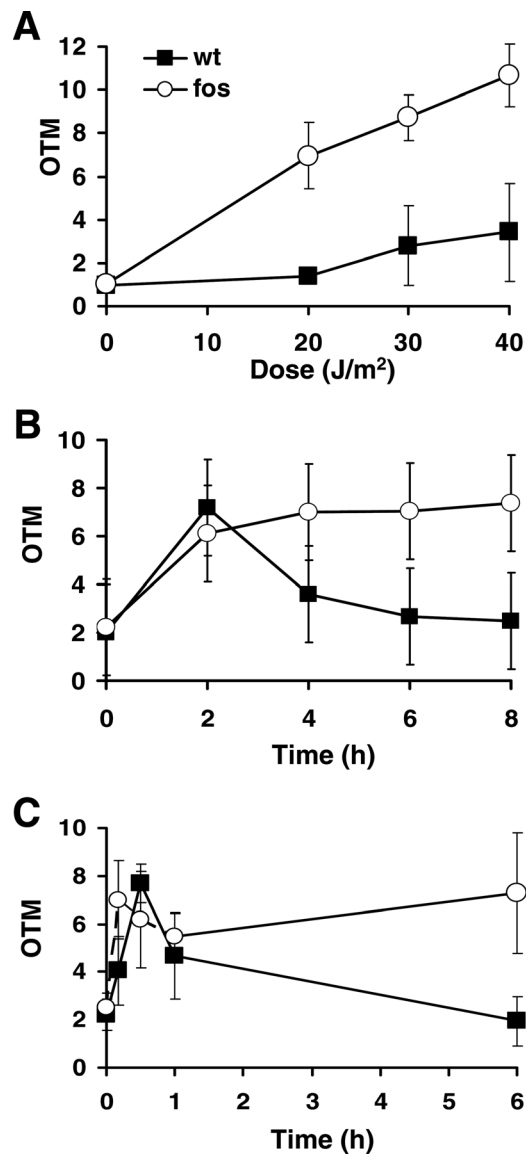
**Figure 3.** Formation and repair of DNA SSBs. To analyse the NER-mediated formation and repair of DNA SSBs, alkaline single-cell gel electrophoresis (SCGE, comet assay) was performed. (**A**) wt and *fos*−/− cells were exposed to different UV-C doses and harvested after 6 h. (**B**) Cells were exposed to 20 J/m² and subjected to SCGE after incubation times of 2, 4, 6 and 8 h and (**C**) after short incubation times between 0 and 1 and after 6 h. Data (OTM = Olive tail moment) of three independent experiments ± inter-experimental SD are shown.

during the post-incubation period, indicating a defect in the late step of NER in which repair intermediates are sealed. Therefore, the NER incision step appears to be unaffected. This was again shown using shorter incubation times after UV-C treatment. In both cell lines, the formation of DNA strand breaks occurred already 10 min after UV-C exposure, reaching similar maximum levels 30 min after irradiation (Figure 3C).

### Expression profile of DNA repair genes

To determine whether the c-Fos-related phenotype, i.e. lack of CPD removal and defective sealing of repair intermediates,

is due to impaired regulation of DNA repair gene(s), the expression of genes involved in DNA repair and translesion synthesis was studied by microarray analysis followed by confirmative RT–PCR. Comparing wt and *fos*−/− cells treated with UV-C, we observed no differential expression of most of the genes involved in NER (*xpa*, *xpb*, *xpc*, *xpd*, *ddb1*, *ddb2*, *ercc1*, *csa*, *csb* and *lig1*) or translesion synthesis (*polH*, *polI*, *polK*, *polL* and *polM*) (data not shown). Interestingly, however, microarray analysis revealed that the *xpf* level was slightly enhanced 6 h after UV-C exposure in wt cells, whereas in *fos*−/− cells it was clearly reduced. The mRNA level of *xpg* was significantly enhanced in wt cells, which was not found in *fos*−/− cells (data not shown).

### Expression of NER endonucleases upon UV-C exposure

To substantiate the findings obtained by microarray analysis, quantitative real-time PCR was performed. The compiled data obtained by three independent experiments are shown in Figure 4A. Treatment of wt cells with 7.5 J/m² increased the mRNA expression of *xpf* and, more pronounced, *xpg*. Upon treatment with 20 J/m² the *xpf* mRNA level dropped below 50% control level, whereas the *xpg* expression was still enhanced above the control. In *fos*−/− cells exposure to 7.5 J/m² reduced the *xpf* and *xpg* mRNA level to 50 and 90%, compared with the unirradiated control. With 20 J/m² the expression level of *xpf* and *xpg* dropped to 20 and 50% of control level, respectively. Comparable results were obtained by semi-quantitative RT–PCR, which was performed to verify the specificity of the products quantified by real-time RT–PCR and, moreover, to examine the time course of *xpf* and *xpg* mRNA re-synthesis in more detail. As shown in Figure 4B, the level of *xpf* mRNA was not significantly reduced in the wt, whereas in *fos*−/− cells it declined immediately after UV-C treatment and was nearly not detectable up to 12 h after irradiation. It recovered only 16 h later. The *xpg* mRNA level in the wt slightly dropped 1–2 h after irradiation and recovered 4–6 h later, whereas in *fos*−/− cells recovery did not occur within this period. In contrast to *xpf* and *xpg*, the mRNA level of the NER endonuclease ERCC1 did not show significant variation upon UV-C treatment (Figure 4B).

### Decrease in *xpf* and *xpg* mRNA level is related to high-mRNA instability

To identify the mechanism responsible for the decrease in *xpf* and *xpg* mRNA expression upon UV-C, the stability of the mRNA was determined. To this end, wt cells were exposed to actinomycin D or α-amanitin for 3 or 6 h. Thereafter, RNA was extracted and the expression of *xpg*, *xpf*, *ercc1* and *gapdh* was analysed by RT–PCR. As shown in Figure 5A, treatment with actinomycin D or α-amanitin reduced dramatically the *xpf* and *xpg* mRNA level. In contrast, the expression level of *ercc1* (and *gapdh* included for control) mRNA was not altered by the inhibitors. The data demonstrate low stability of *xpf* and *xpg* mRNA and high stability of *ercc1* (and *gapdh*) mRNA. As shown in Figure 5B, the same was found after exposure to actinomycin D for 1 or 2 h. Here we also show that actinomycin D reduces the *xpf* and *xpg* level to comparable amounts in wt and *fos*−/−
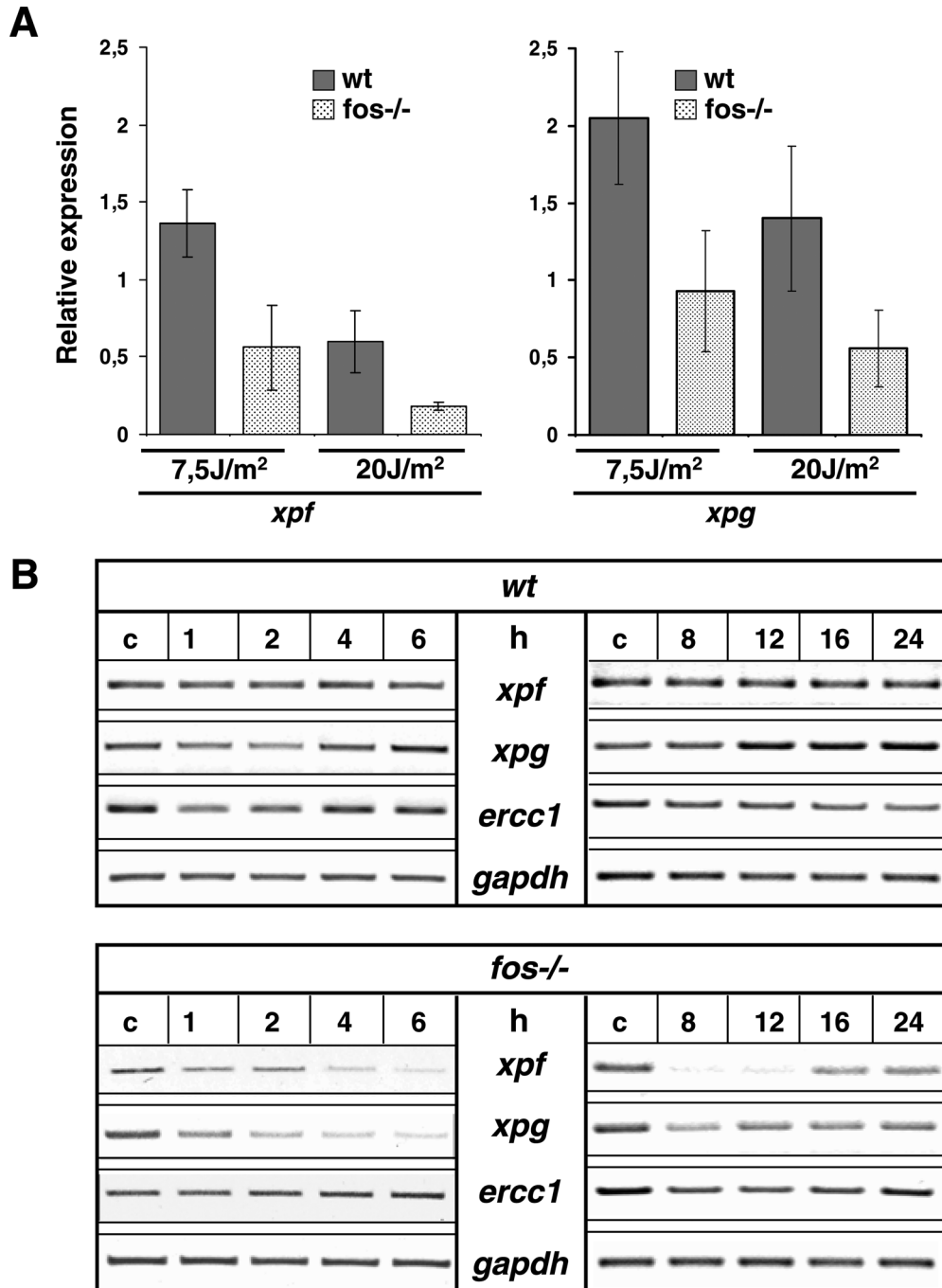
**A**



**B**



**Figure 4.** RNA expression of *xpf*, *xpg* and *ercc1*. (**A**) Exponentially growing wt and *fos−/−* MEFs were exposed to 7.5 or 20 J/m$^2$ UV-C for 6 h. Total RNA was isolated and real-time RT–PCR was performed using *xpf*, *xpg* or, as positive control, *gapdh* specific primers. For quantification, the expression was normalized with *gapdh* and the untreated control was set to 1. Data are the mean of three independent experiments ± SD. (**B**). In a different set of experiment, wt and *fos−/−* cells were exposed to 20 J/m$^2$ UV-C for different time points, total RNA was isolated and semi-quantitative RT–PCR was performed using *xpf*, *xpg*, *ercc1* or, as positive control, *gapdh* specific primers (c, non-exposed control).

cells, indicating no difference in the stability of the mRNA species between the two cell lines. Taken together, the data revealed that lack of re-synthesis and low stability of *xpf* and *xpg* mRNA are responsible for the observed decrease in the mRNA levels observed in c-Fos-deficient cells after UV-C exposure. It might be speculated that mRNA synthesis is generally blocked in *fos−/−* cells compared with the wt.

This however was not the case. As shown in Figure 5C, treatment with 20 J/m$^2$ UV-C reduced the overall transcription up to 40% control level both in wt and *fos−/−* cells within a 12 h post-incubation period. Therefore, the stimulatory effect of c-Fos on mRNA re-synthesis upon UV-C appears to be related to a specific subset of blocked genes, notably the repair genes *xpf* and *xpg*.
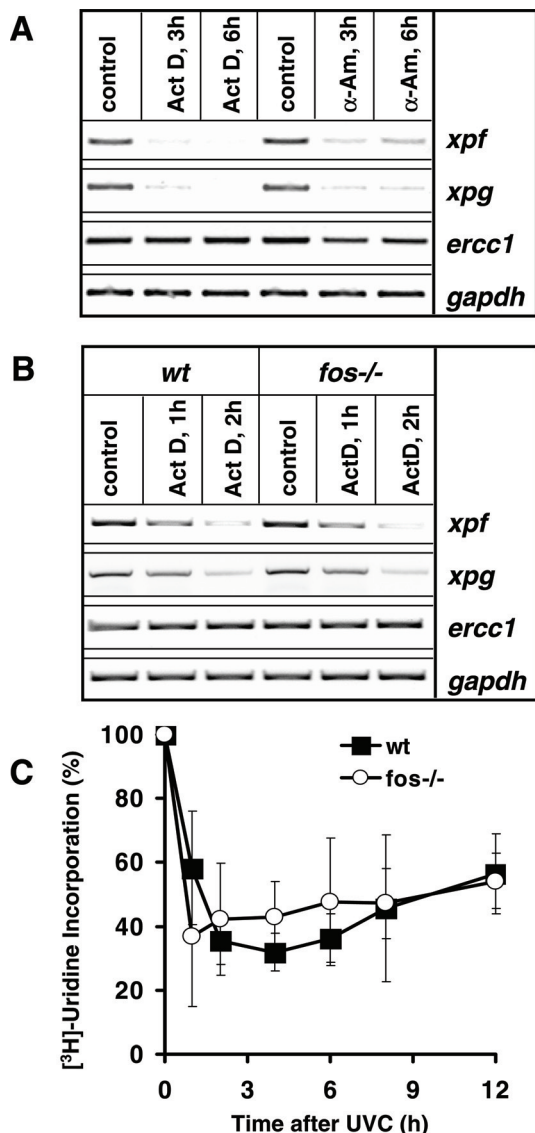
**Figure 5.** Impact of transcription blockage on *xpf and xpg* mRNA expression. (**A**) Exponentially growing wt cells were exposed to 1 μM actinomycin D or 0.1 μM α-amanitin for 3 or 6 h and total RNA was isolated. Two micrograms were subjected to cDNA synthesis, followed by RT–PCR with *xpf*, *xpg* or *ercc1* specific primers. As internal control, *gapdh* was amplified. (**B**) Exponentially growing wt and *fos−/−* cells were exposed to 1 μM actinomycin D for 1 or 2 h and total RNA was isolated. Two micrograms were subjected to cDNA synthesis, followed by RT–PCR with *xpf*, *xpg* or *ercc1* specific primers. As internal control, *gapdh* was amplified. (**C**) Transcription blockage upon UV-C exposure was checked by incorporation of [$^3$H]uridine. Cells were exposed or not exposed to 20 J/m$^2$ UV-C for different time points and the incorporated radioactivity was determined as described in Materials and Methods. The incorporated radioactivity of the non-UV-C exposed probe (0 h) was set to 100%. Data are the mean of three independent experiments ± SD.

### Deficient *xpf* re-synthesis leads to lack of XPF protein

Does impaired *xpf* and *xpg* mRNA synthesis lead to reduced protein levels? The expression level of XPF and XPG was determined and shown in Figure 6. UV-C did not influence the XPF level in wt cells. It led, however, to a strong reduction in *fos−/−* cells 6–18 h after treatment (Figure 6A). This is in line with the observed decline and lack of re-synthesis in the *xpf* mRNA level in *fos−/−* cells. In contrast, the expression of XPG protein was enhanced 6–12 h after irradiation in the wt and unaltered in *fos−/−* cells (Figure 6B).

### XPF complementation partially restores CPD removal

To proof the importance of XPF expression for CPD removal, we cloned the murine *xpf* cDNA in the vector pcDNAtopomXPF. The vector was used for transient transfection of *fos−/−* cells. Transfection efficiency was ∼60%, as measured by parallel transfection using a GFP construct. The transfection was also checked by western blotting, detecting the XPF protein (data not shown). As demonstrated in Figure 6C, XPF transfection provoked removal of CPDs in *fos−/−* cells. Quantification revealed that the complemented cells removed up to 40% of the CPDs 12–16 h after UV-C, whereas in mock transfected cells no repair was observed.

### Importance of AP-1 for XPF re-synthesis

To further substantiate the role of c-Fos (AP-1) in the regulation of re-synthesis of XPF, inhibitor experiments were performed. As shown in Figure 7A and B, down-modulation of *xpf* mRNA and XPF protein can also be achieved in UV-C irradiated wt cells by pre-treatment with SP600125, a specific JNK inhibitor that attenuates AP-1-mediated gene activation (32). This is opposed to *fos−/−* cells in which SP600125 did not further reduce the XPF level. This supports the hypothesis that c-Fos/AP-1 is involved in controlling the re-synthesis of XPF upon DNA damage. A computer-based study revealed a AP-1 consensus binding site in the putative promoter region of the *xpf* gene. To analyze if the *xpf* promoter is recognized by c-Fos/AP-1 under *in vivo* conditions, ChIP experiments were performed using a c-Fos-specific antibody. As shown in Figure 7C, c-Fos was found to bind to the *xpf* promoter. This binding was clearly enhanced in wt cells treated with UV-C (Figure 7C, lanes 5 and 6). c-Fos did not recognize the β-actin promoter, which was used as negative control.

## DISCUSSION

Previously we showed that MEFs deficient for c-Fos are hypersensitive to the cytotoxic, apoptosis-inducing and clastogenic effects of UV-C light (6,7,14) and other genotoxins (13). The reason for the hypersensitivity of c-Fos-deficient cells remained enigmatic. Cells lacking c-Fos display a defect in the recovery from UV-C induced DNA replication inhibition, indicating a defect in the repair or processing of UV-C induced DNA lesions rather than impaired signalling. To establish a role for c-Fos in DNA repair, we compared the DNA repair efficiency of isogenic wt and c-Fos lacking mouse fibroblasts upon UV-C exposure. Here we report for the first time a defect of c-Fos-deficient cells in the repair of these lesions. Whereas wt cells removed most of the CPDs within a post-incubation period of 12 h, *fos−/−* cells were unable to repair CPDs. It is important to emphasize that the cell lines used in our study are phenotypically p53 wt, which was confirmed by the induction of p53 target genes (*p21* and *gadd45*) in both cell lines (data not shown). Therefore, the molecular mechanism underlying the repair
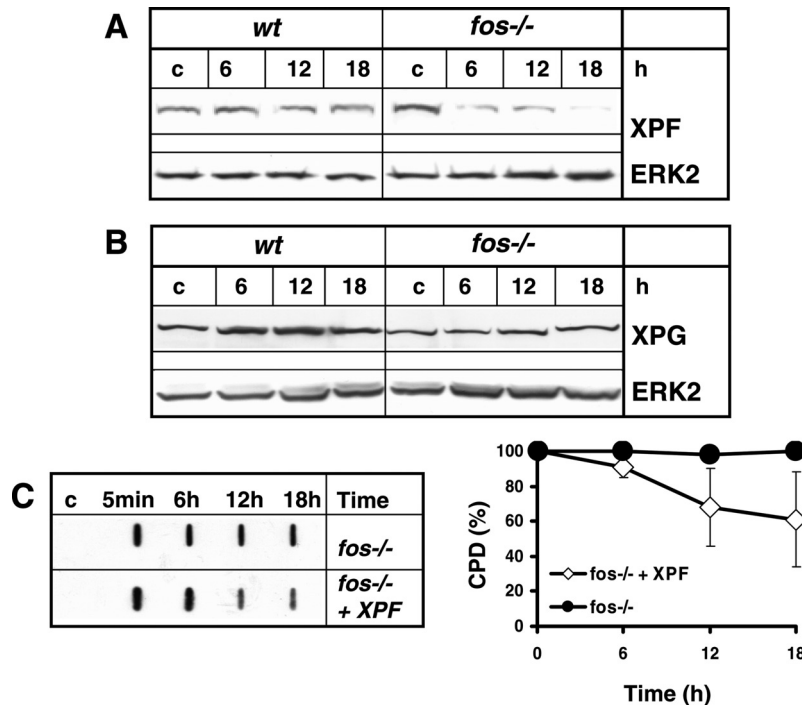
**Figure 6.** Expression of XPF and XPG protein and impact of XPF transfection on CPD removal. (**A** and **B**) Exponentially growing wt cells were exposed to 20 J/m$^2$ UV-C for the indicated times. Protein extracts were prepared and 25 μg were subjected to western blot analysis. The filter was incubated with XPF (A) or XPG (B) specific antibodies. For loading control, ERK2 was detected. (**C**) Exponentially growing *fos−/−* cells were transfected with an empty vector or the vector pcDNAtopo-mXPF. Twelve hours after transfection, cells were exposed to UV-C light and were harvested after 0–18 h of recovery. Genomic DNA was isolated, equal amounts of DNA were blotted and subjected to incubation with anti-CPD antibodies. For quantification, the CPD signal 5 min after treatment was set to 1. Data are the mean of three independent experiments ± SD (right panel) from which a representative experiment is shown (left panel).

defect in *fos−/−* cells is different from the repair defect reported for p53-deficient MEFs (5,12).

CPD removal is accomplished by NER, which can be divided into four steps: recognition, incision/excision, re-synthesis and ligation. Which one of these steps is defective in *fos−/−* cells? To examine the incision/excision step, we analysed the formation and repair of DNA SSBs that are formed during NER by dual incision at defined positions flanking the DNA damage (33,34). Although the formation of SSBs upon UV-C occurs in both cell lines equally, their sealing during NER clearly did not occur in *fos−/−* cells. As c-Fos is part of the dimeric transcription factor AP-1, it was pertinent to conclude that c-Fos regulates genes involved in the repair of UV-C induced DNA lesions. To verify this hypothesis, microarray analysis was performed using a self-constructed array containing the presently known DNA repair genes (5). As to the basal level of expression, there was no significant difference in the expression of *NER* genes between wt and c-Fos lacking cells. However, upon UV-C irradiation a remarkable and highly reproducible change was observed in the expression pattern of the endonucleases *xpf* and *xpg*. This was substantiated by semi-quantitative PCR and real-time RT–PCR. The study revealed that *xpg* and, even more pronounced, *xpf* mRNA is more reduced in *fos−/−* cells than in the wt upon irradiation. With high doses of UV-C (20 J/m$^2$) the expression of *xpf* and *xpg* was also slightly attenuated in wt cells. However, it recovered quickly to basal level, which was not the case in *fos−/−* cells. Therefore, we conclude that c-Fos regulates the re-synthesis of *xpf* and *xpg* genes upon DNA damage.

As demonstrated in experiments using the transcription inhibitor actinomycin D (and α-amanitin), the *xpf* and *xpg* mRNAs are quite unstable, being degraded already after 2 h of treatment with the inhibitor. Therefore, even a transient block of transcription of *xpf* and *xpg* will result in reduced transcript levels. Interestingly, the overall inhibition of transcription induced by UV-C was similar in wt and c-Fos-deficient cells. Therefore, it appears that c-Fos/AP-1 is a key component involved in the abolition of the block of transcription of a subset of genes, notably the repair genes *xpf* and *xpg*.

Interestingly, the impaired re-synthesis of *xpf* and *xpg* mRNA resulted in a significant reduced level of XPF but not XPG protein in *fos−/−* cells. This indicates that either the XPG protein is much more stable or the remaining mRNA is sufficient for maintaining the XPG protein expression. Since the XPG protein was found to be present in *fos−/−* cells, we conclude that the blocked re-synthesis of XPF protein is responsible for the NER defect observed in c-Fos-deficient cells. This was strongly supported by the finding that transfection of XPF provoked CPD removal in *fos−/−* cells.

The important role of c-Fos/AP-1 for triggering the re-synthesis of *xpf* was verified using a specific inhibitor of AP-1 and ChIP analysis. Inhibition of Jun kinase and, thereby, AP-1 transactivation abolished the re-synthesis of *xpf* in wt cells. We also show by ChIP experiments that c-Fos/AP-1 binds *in vivo* to the *xpf* promoter.

The lack of XPF but not XPG protein in c-Fos-deficient cells upon UV-C treatment is compatible with the data
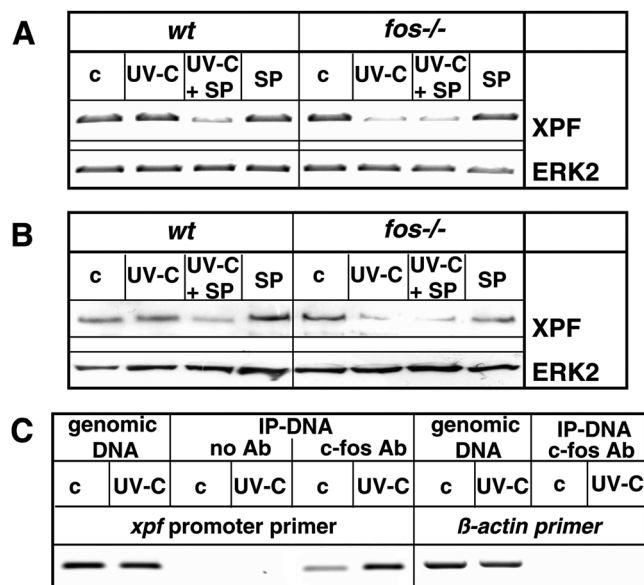
**Figure 7.** Effect of JNK inhibition on XPF level and Chip analysis. (**A** and **B**) Exponentially growing wt and *fos−/−* cells were not exposed (c, control), exposed to 20 J/m² UV-C (UV-C), pre-incubated with 10 µM SP600125 and thereafter exposed to 20 J/m² UV-C (UV-C + SP) or incubated with 10 µM SP600125 only (SP). Total RNA was isolated 6 h later and protein extracts were prepared 12 h after exposure. Expression of *xpf* mRNA (analysed by RT–PCR) (**A**) and XPF protein (determined by western blot analysis) (**B**) upon treatment with the JNK inhibitor. (**C**) Chip analysis: exponentially growing wt cells were not exposed (c, control) or exposed to 20 J/m² UV-C (UV-C). Six hours after exposure, cells were harvested and dealt with as described in Materials and Methods. Immunoprecipitation was performed using a c-Fos-specific antibody. PCR was performed using specific primers for the AP-1-binding site of the *xpf* promoter and, as negative control, for the β-actin 5′-untranslated region.

obtained by measuring the level of DNA SSBs. Here we found incision but not restitution of SSBs to occur in *c-fos−/−* cells. During NER, the 3′ incision is performed by XPG (35,36) whereas the 5′ incision is accomplished by the XPF–ERCC1 complex (37). The 3′ incision is a pre-requisite for the 5′ incision (34). Obviously, *fos−/−* cells are able to perform the 3′ incision (by XPG) but not the subsequent 5′ incision step (executed by XPF-ERCC1). This explains why SSBs occurred and CPDs were not removed from DNA of c-Fos-deficient cells. Thus, they resemble the phenotype of XPF defective cells that are able to generate 3′ but not 5′ incision next to the lesion (34). Since mouse cells remove CPDs only from transcriptional active genes (38), we suppose the repair defect of *fos−/−* cells is restricted to transcription coupled repair.

In summary, we provide evidence that c-Fos-deficient cells display a defect in the repair of CPDs due to impaired re-synthesis of XPF. *c-fos* is a paradigmatic example of a gene that is promptly induced upon DNA damage, notably UV light. How relevant is the finding as to the natural UV exposure? A dose of 10 J/m² induces ∼0.3 CPDs per kbp DNA. This dose is comparable to ∼80 kJ/m² simulated sun light or 1 kJ/m² UV-B (39). It has further been reported that 0.062 CPDs per kbp are induced during a 30 min sunbath in August (in Paris). This would correspond to a sun exposure period of 2.5 h (40), yielding a similar amount of CPDs than 10 J/m² UV-C light. Therefore, our finding as to the role of c-Fos/AP-1 in regulating the re-synthesis of XPF might be

highly relevant for the recovery of skin cells from DNA damage after sun exposure. We should also consider the possibility that individuals might be variable in the regulation of the expression of c-Fos/AP-1 triggered genes and, therefore, might be impaired in DNA repair and susceptibility to UV light without showing mutations in NER genes. The data provide a firm basis for future studies in this direction.

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