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 G1/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...
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% CO2 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/m2 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; Neomarker) 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 5 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 [3H]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 [5,6-3H]uridine was added to the medium. Thereafter, cells were washed twice with PBS and 6% trichloroacetic acid (TCA) to remove unincorporated [3H]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 ml phenylmethylsulfonyl fluoride (PMSF). Genomic DNA was fragmented by sonification 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 NaHCO₃) 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/m². 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/m². 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/m² 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/m²). (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/m²) 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/m²) 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 [14C]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/m² 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.
is due to impaired regulation of DNA repair gene(s), the
eexpression of genes involved in DNA repair and translesion
synthesis was studied by microarray analysis followed by
confirmative RT–PCR. Comparing wt and fos−/− cells trea-
ted with UV-C, we observed no differential expression of
most of the genes involved in NER (xpa, xph, xpc, xpd,
ddb1, ddb2, ercc1, csa, csb and lig1) or translesion
synthesis (polH, polF, polK, polL and polM) (data not
shown). Interestingly, however, microarray analysis revealed
that the xpf mRNA level was slightly enhanced 6 h after UV-C expo-
sure 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 per-
formed 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 sig-
nificantly reduced in the wt, whereas in fos−/− cells it
decreased 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 dra-
matically 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 demon-
strate 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−/−
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 \textit{xpf} and \textit{xpg} mRNA are responsible for the observed decrease in the mRNA levels observed in \textit{c-Fos}-deficient cells after UV-C exposure. It might be speculated that mRNA synthesis is generally blocked in \textit{fos−/−} cells compared with the wt. This however was not the case. As shown in Figure 5C, treatment with 20 J/m² UV-C reduced the overall transcription up to 40% control level both in wt and \textit{fos−/−} cells within a 12 h post-incubation period. Therefore, the stimulatory effect of \textit{c-Fos} on mRNA re-synthesis upon UV-C appears to be related to a specific subset of blocked genes, notably the repair genes \textit{xpf} and \textit{xpg}.

**Figure 4.** RNA expression of \textit{xpf}, \textit{xpg} and \textit{ercc1}. (A) Exponentially growing wt and \textit{fos−/−} MEFs were exposed to 7.5 or 20 J/m² UV-C for 6 h. Total RNA was isolated and real-time RT–PCR was performed using \textit{xpf}, \textit{xpg} or, as positive control, \textit{gapdh} specific primers. For quantification, the expression was normalized with \textit{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 \textit{fos−/−} cells were exposed to 20 J/m² UV-C for different time points, total RNA was isolated and semi-quantitative RT–PCR was performed using \textit{xpf}, \textit{xpg}, \textit{ercc1} or, as positive control, \textit{gapdh} specific primers (c, non-exposed control).
is in line with the observed decline and lack of re-synthesis in the XPF level in wt cells. It led, however, to a strong reduc-
tion of [3H]uridine. Cells were exposed or not exposed to 20 J/m² 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 prove 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 cells removed up to 40% of the CPDs 12–16 h after UV-C, whereas in mock transfected cells no repair was observed.
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 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.

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.

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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...
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 a pre-requisite for the 5' incision step (executed by XPF-ERCC1). This explains why SSBs occurred in c-fos 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.

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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