Reduced extractability of the XPA DNA repair protein in ultraviolet light-irradiated mammalian cells

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Received 11 October 1999; received in revised form 9 November 1999

Edited by Vladimir Skulachev

Abstract The XPA protein is essential for both of the known modes of nucleotide excision repair (NER) in human cells: transcription-coupled repair (TCR) and global genome repair (GGR). In TCR, this protein is thought to be recruited to lesion sites in DNA at which RNA polymerase II is blocked and in GGR, by direct recognition of damages by repair protein complex containing XPC/HR23B or DNA damage-binding protein. However, details of the recruitment of the XPA protein in vivo are unknown. It was shown earlier that a portion of another NER protein, PCNA, which is completely extractable from non-S-phase mammalian nuclei, becomes insoluble after ultraviolet (UV) light irradiation and cannot be extracted by methanol or buffer containing Triton X-100. In the present study, we have found that UV light irradiation of human or Chinese hamster cells leads to decrease of extractability of the XPA protein by Triton X-100. Maximal insolubilization of the XPA protein is observed 1–4 h after irradiation but it is not detectable by 22 h. This effect is dose-dependent for UV light from 2.5 to 15 J/m^2 and is unaffected by the pre-treatment of cells with sodium butyrate, an inhibitor of histone deacetylation. The UV light-induced insolubilization of the XPA protein was also observed in two lines of Cockayne syndrome complementation group A cells, indicating that the effect is not dependent upon TCR. The results are discussed in relation to possible mechanisms of NER.

Key words: Nucleotide excision repair; Xeroderma pigmentosum complementation group A; Transcription-coupled repair; Global genome repair; Ultraviolet irradiation

1. Introduction

Nucleotide excision DNA repair (NER), which is important for cell survival after ultraviolet (UV) irradiation or after treatment with some chemical carcinogens, is a complex biochemical pathway involving many proteins, most of which have been well studied in vitro [1]. However, the temporal and spatial organization of the NER process in the mammalian nucleus in vivo is poorly understood. There are two major modes of NER: transcription-coupled repair (TCR) and global genome repair (GGR). TCR is thought to be initiated by stalled RNA polymerase II transcription complex at a lesion in the transcribed DNA strand [2,3]. GGR can be initiated by the XPC/human homolog B of the yeast RAD23 protein protein complex [4] and by p120/p48 (DNA damage-binding protein) complex in a p53-dependent fashion [5]. Both NER modes require the XPA protein [2,3] that is defective or deficient in cells of xeroderma pigmentosum patients of complementation group A [6]. XPA deficient cells are unable to repair cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (6-4PPs) produced by UV light [7]. Although the complete reaction of the GGR pathway has been reconstituted in vitro, this has not yet been possible for TCR. Furthermore, the cell-free NER systems are generally inefficient, in part perhaps because they lack the cellular compartmentalization and attachment to structures that may be important for the repair pathways in vivo.

After UV irradiation of mammalian non-S-phase cells, a portion of the DNA replication 'sliding clamp' protein proliferating cell nuclear antigen (PCNA) becomes insoluble in methanol or in a buffer containing a low concentration (0.1–0.5%) of the non-ionic detergent Triton X-100 [8,9]. This phenomenon has been confirmed [10–17] and evidence was obtained that PCNA is required for NER [18]. It was also shown that a fraction of the NER protein XPB becomes insoluble in Triton X-100 in UV-irradiated cells, with evidence for partial colocalization in the nucleus with insoluble PCNA [17]. These results suggested that during NER, large protein complexes may be assembled at the nuclear matrix. Very recently, it has been found [19], using a novel variant of the photobleaching assay, that UV irradiation limits mobility (diffusion in nucleus) of the NER nuclease complex XPF/ERCC1.

In this study, using an immunoblotting assay, we analyzed solubility in Triton X-100 of the mammalian XPA protein. It is found that like PCNA, the XPA protein may be extracted from unirradiated human or Chinese hamster cells with a buffer containing indicated detergent but after irradiation with a sufficient UV dose, a large fraction of the protein (40–50%) becomes insoluble in Triton. The insolubilization is not affected by inhibition of histone deacetylation and may be observed in the absence of TCR.

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Abbreviations: NER, nucleotide excision repair; TCR, transcription-coupled repair; GGR, global genome repair; UV, ultraviolet light; XPA, xeroderma pigmentosum complementation group A; XPB, xeroderma pigmentosum complementation group B; XPC, xeroderma pigmentosum complementation group C; XPF, xeroderma pigmentosum complementation group F; ERCC1, excision repair complementing protein 1; PCNA, proliferating cell nuclear antigen; CPD, cyclobutane pyrimidine dimer; 6-4PP, (6-4) pyrimidine-pyrimidone photoproduct; PBS, phosphate-buffered saline

0014-5793/99/$20.00
PII: S0014-5793(99)01592-6

2. Materials and methods

2.1. Cell cultures

Primary human fibroblasts line VH-10 were obtained from Dr. A. Kolman, Chinese hamster ovary (CHO) line CHO K1 from the Cell culture collection of the Institute of Cytology RAS, SV3-transformed complementation group A Cockayne syndrome cells CS3BE (CSA line 1) from the collection of the Department of Biological Sciences of Stanford University, complementation group A Cockayne syndrome cells CS1SP (CSA line 2) from Dr. V. Mikheslon. Cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 100 μg/ml penicillin.

2.2. Immunoblotting

Cells grown in plastic plates were irradiated in the absence of growth medium under a germicidal lamp (254 nm) and then incubated for different times in growth medium. The UV dose was measured using a commercial dosimeter. After washing with phosphate-buffered saline (PBS) (2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 8.1 mM Na_2HPO_4, pH 7.1), cells were treated with 0.1% Triton X-100 in PBS for 2 min at room temperature (RT) and then again washed twice with PBS. After detachment using rubber policeman cells were suspended in the Laemmli buffer (50 mM Tris-HCl, pH 6.8, 0.8 mM EDTA, 4% sodium dodecyl sulfate (SDS), 0.25% bromophenol and 20% glycerol), proteins separated in a 12% SDS-polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane (Hybond) in 12 mM Tris-HCl, pH 8, 95 mM glycine, 20% methanol. The membrane was blocked overnight at 4°C in PBS containing 5% nonfat dry milk or in 5% blocking solution from Boehringer. All antibodies were diluted in 0.5% blocking solution containing 0.02% Tween 20, incubations were at RT and all washes were in PBS with 0.1% Tween 20. Rabbit polyclonal antibodies against human XPA protein were obtained from Dr. R. Wood. Membranes were sequentially incubated with anti-XPA antibodies (1:2000, 1 h) and goat anti-rabbit IgG antibodies conjugated with peroxidase (Sigma, 1:2500, 1 h). Peroxidase was detected using chemiluminescence (ECL) and exposure to X-ray film. The relative intensity of bands on X-ray films was estimated after their scanning using computer program Quantiscan (Biosoft).

3. Results

Fig. 1 shows typical Western blots of the XPA protein in non-damaged and UV-irradiated human fibroblasts (line VH-10) and CHO cells. It is seen that the major fraction of the protein may be extracted from unirradiated cells by short treatment with PBS containing 0.1% Triton X-100 (Fig. 1, lanes 2), but 1–4 h after UV irradiation (30 J/m^2), XPA protein remains tightly bound (lanes 3 and 4). Immunofluorescent analysis of fixed cells showed that after UV irradiation, XPA protein resistant to extraction with Triton X-100 does not change its nuclear localization typical for unextracted non-damaged cells (not shown). In control experiments, it was found that another abundant nuclear protein, transcription factor Yin Yang 1 which is not involved in NER, does not become resistant to extraction with Triton X-100, indicating that UV-dependent XPA insolubilization is not a consequence of an unspecific direct photochemical linking of nuclear proteins to nuclear matrix.

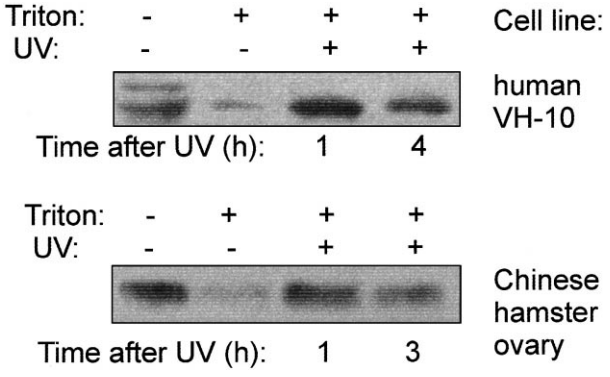

Fig. 1. Immunoblots showing XPA protein bands in human and Chinese hamster cells under different experimental conditions. Each track contained total proteins from 4×10^4 cells and in the Triton (+) variants, plastic-attached cells were treated for 2 min at RT with PBS containing 0.1% Triton X-100. The UV dose was 30 J/m^2 and after irradiation, cells were incubated for indicated times at 37°C in growth medium.

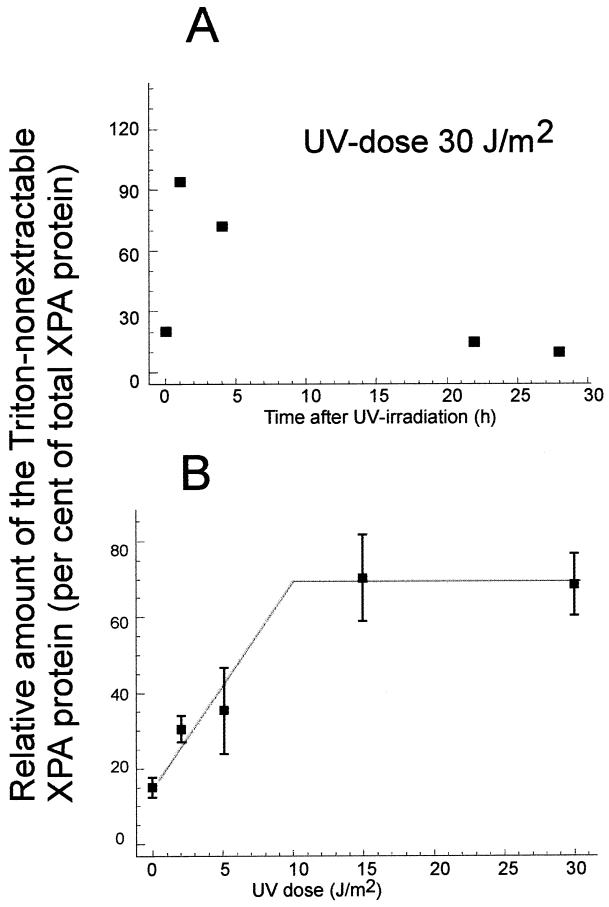

Fig. 2. Time-course (A) and UV dose-dependence (B) of the appearance of the Triton-non-extractable XPA protein in human fibroblasts VH-10. Total XPA protein in unirradiated cells not treated with Triton (see left lanes in Fig. 1) measured in each experiment was taken as 100%. Bars in (B) show S.E.M.s of three independent experiments.

UV-induced insolubilization of the XPA protein is maximal at 1–4 h after irradiation and is not observed at 22–28 h after damage (Fig. 2A). The effect of the XPA insolubilization is also dose-dependent (Fig. 2B) within UV doses 2.5–15 J/m^2 and upon higher doses, no further increase of insoluble XPA protein is observed (Fig. 2B). Quantitation of the immunoblots indicates that maximal increase of the Triton-insoluble XPA protein in VH-10 cells observed 1 h after UV corresponds to about 50% of the total XPA protein (Fig. 2B).

Since histone hyperacetylation induced by sodium butyrate facilitates NER in chromatin [20], we examined whether the drug affects UV-induced insolubilization of the XPA protein.

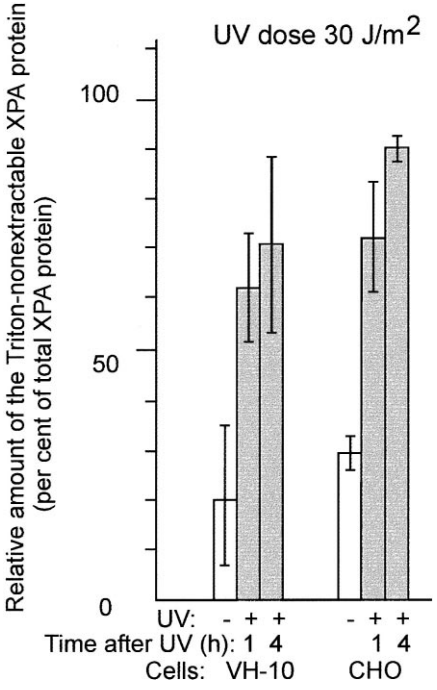

Fig. 3. Triton X-100-insoluble XPA protein in mammalian cells pretreated for 24 h before UV irradiation with 10 mM sodium butyrate. Results of two independent experiments are shown. Butyrate was also present in growth medium after UV.

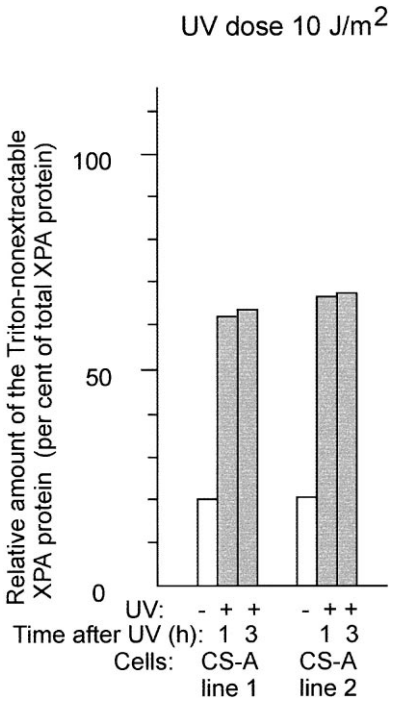

Fig. 4. UV-dependent induction of the Triton-insoluble XPA protein in human cells of patients with Cockayne syndrome (CS-A). For details about cell lines, see Section 2.

No effect of pre-treatment of cells with 10 mM sodium butyrate on XPA protein was detected in experiments with human or Chinese hamster cells (Fig. 3), suggesting that the UV-induced XPA insolubilization is not a consequence of changes of chromatin acetylation during NER.

It is known that TCR is defective in cells from patients with Cockayne syndrome [2,3]. We examined UV-induced XPA insolubilization in two lines of Cockayne cells (both complementation group A). The results (Fig. 4) indicate that XPA insolubilization may be observed in CSA cells after irradiation (10 J/m^2). The results indicate that UV-induced insolubilization of the XPA protein takes place in cells with defective TCR.

4. Discussion

Results obtained in this study indicate that not only NER/replication protein PCNA becomes Triton-insoluble in mammalian nuclei after UV irradiation, but similar behavior also shows another NER protein XPA. Insoluble forms of both proteins are clearly detectable at 1–4 h after irradiation and UV dose-dependence of induction of the tightly bound PCNA reaching plateau at 10 J/m^2 [12] is similar to those for XPA protein (Fig. 2B). Dose-dependent immobilization of the ERCC1/XPF complex is also observed at UV fluences up to 8 J/m^2 [19]. Interestingly, maximal immobile fraction of the ERCC1/XPF complex is 30–40% [19], which corresponds to the maximal fraction (50%) of insolubilized XPA protein (Fig. 2B), suggesting that a photobleaching assay and Triton X-100 extractability assay may reflect the same events. UV-induced insolubilization of the PCNA is detectable at 24 h after irradiation [9,15] while at this time, most of the XPA protein becomes soluble (Fig. 2A). This may suggest that XPA insolubilization is preferentially associated with fast repair of 6-4PPs and PCNA is also required for slow repair of CPDs but other explanations are possible.

Recent experimental evidence indicates that chromatin rearrangements take place during NER [21,22]. Well known chromatin modification effecting DNA repair is acetylation of histones which may be stimulated by an inhibitor of histone deacetylases, sodium butyrate [20]. In principle, the extent of chromatin acetylation may influence the solubility of NER proteins but it is found in this study that the pre-treatment of cells with 10 mM sodium butyrate does not effect UV-induced extractability of XPA protein by Triton X-100 (Fig. 3). It appears, therefore, that either other chromatin modifications may be important for the decreased solubility of XPA protein in UV-irradiated mammalian cells or XPA insolubilization takes place on non-chromatin nuclear structures, e.g. nuclear matrix. It is shown that damaged DNA and some NER proteins are recruited to the nuclear matrix in hamster cells following UV irradiation [23,24], but sites of DNA repair synthesis in UV-damaged permeabilized human cells do not show association with the nucleoskeleton [25]. Further studies of potential chromatin and non-chromatin targets for the UV-induced insolubilization of the XPA protein may be helpful in resolving this problem.

It should be noted that the biochemical method used in this study allows only for global analysis of the solubility of NER proteins in a large number of cells. In contrast, the recently developed modified photobleaching method [19] allows for estimation of the time of UV-induced spatial immobilization of proteins, which for the ERCC1/XPF complexes was found to be about 4 min [19]. These results suggest that NER operates by assembly of individual NER factors at sites of DNA damage rather than by pre-assembly of large complexes and that ERCC1/XPF participates in repair of DNA damage in a distributive fashion rather than by processive scanning of

large genome segments [19]. However, global dynamics of the ERCC1/XPF immobilization indicates that the immobile fraction of the complexes (which is more than 30% immediately after irradiation) drops to less than 10% at 4 h after UV [19]. At this time point, a major fraction of XPA protein still remains Triton-insoluble (Figs. 1, 2A and 3) and tightly bound PCNA clearly persists at 4–24 h after irradiation [9,15]. This indicates that the global behavior of XPA and PCNA is different from that of ERCC1/XPF complex. It is possible, therefore, that only some repair proteins (e.g. ERCC1/XPF) are involved in NER in a distributive fashion while the main holocomplex is working processively. Our recent results on visualization of focal sites of DNA repair synthesis in UV-irradiated human cells [26] are more consistent with a processive scanning of large chromatin domains during NER.

Acknowledgements: We would like to thank Dr. Rick Wood for antibodies against XPA protein. This work was supported by the Civilian Research and Development Foundation Grant RN2-408.

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