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Mutational analysis of a function of xeroderma pigmentosum group A (XPA) protein in strand-specific DNA repair

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ABSTRACT

To analyze the function of the xeroderma pigmentosum group A (XPA) protein in strand-specific DNA repair, we examined repair of UV-induced cyclobutane pyrimidine dimer (CPD) in transcribed and non-transcribed strands of the dihydrofolate reductase gene of xeroderma pigmentosum group A (XP-A) cell line (XP12ROSV) which was transfected with various types of mutant XPA cDNA. The transfectant overexpressing mutant XPA with a defect in the interaction with either ERCC1, replication protein A (RPA), or general transcription factor TFIIH, showed more or less decreased repair of CPD in each strand in parallel, while in the transfectant overexpressing R207G (Arg^{207} to Gly) mutant XPA derived from XP129, a UV-resistant XP12ROSV revertant, the rate of CPD repair was almost normal in each strand. We also examined the dose responses of the XPA protein on CPD repair in each strand by the modulation of the expression levels of wild-type or R207G mutant XPA using an inducible expression system, LacSwitch™ promoter. There were good correlations between the rate of CPD repair in each strand and the amount of XPA protein produced in these Lac cells. Our results indicate that the XPA protein is equally important for the CPD repair in both transcribed and non-transcribed strands and that the R207G mutation found in XP129 may not be responsible for a selective defect in CPD repair in the non-transcribed strand in XP129.

INTRODUCTION

Ultraviolet (UV) irradiation induces two major forms of DNA damage, the cyclobutane pyrimidine dimer (CPD) and the 6–4 pyrimidine–pyrimidone photoproduct [(6–4) photoproduct]. These damages are removed mainly by nucleotide excision repair (NER) in mammalian cells. There are two strand-specific DNA repair subpathways in NER. One is designated as transcription-coupled repair (TCR) which preferentially occurs in the transcribed strand of transcriptionally active genes. The other is global genome repair (GGR) which occurs throughout the genome including the non-transcribed strand of active genes. Lesions not easily recognized by GGR, such as CPD, are repaired more efficiently by TCR, but damage such as (6–4) photoproducts is repaired quickly by GGR (1,2).

Xeroderma pigmentosum (XP) is an autosomal recessive human genetic disorder characterized by extreme sensitivity to sunlight, high incidence of skin cancer on sun-exposed skin and neurological complications. XP consists of eight different genetic complementation groups (XP-A–G and a variant). The primary defect in XP patients, with the exception of those with the variant, resides in an early stage of NER (3). Both NER subpathways are defective in groups A–G but not group C, in which only GGR is impaired (4–6). Another inherited disorder, Cockayne syndrome (CS), which shows photosensitivity, cachectic dwarfism and severe mental retardation but no predisposition to UV-induced skin cancer, also displays a defect in the NER. In CS cells, the TCR subpathway is selectively deficient, whereas the GGR subpathway is proficient (7,8). There are two different complementation groups in CS (CS-A and CS-B). To date, the genes responsible for XP-A–G, CS-A and CS-B have been cloned (XPA, XPB, XPC, XPD, XPF, XPG, CSA and CSB genes) (9–17). However, the exact mechanisms of strand-specific DNA repair are unknown.

The XPA gene encodes a C4 type zinc finger protein consisting of 273 amino acids (9). It binds preferentially to DNA damaged by UV irradiation or by chemical agents such as cisplatin, *N*-acetoxy-2-acetylaminofluorene and osmium tetroxide, suggesting that the XPA protein is involved in damage recognition of NER (18–20). It has also been shown that XPA protein associates with other proteins involved in NER such as ERCC1 (21–24), replication protein A (RPA) (25–28), general transcription factor TFIIH (29,30) and CSB (31). These interactions are thought to play important roles in the early stage of NER.

The majority of XP-A patients exhibit clinically severe forms of the disease, and cells derived from such patients are extremely hypersensitive to killing by UV irradiation, are completely defective in repair of CPD and (6–4) photoproducts, and show no retention of TCR (3,4,32). XP12ROSV is a SV40-transformed fibroblast cell line derived from an XP-A patient with severe XP phenotype who was homozygous for the nonsense mutation at

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codon Arg^{207} (33). XP129 is a UV-resistant revertant cell line created by mutagenizing XP12ROSV cells. In XP129 cells this stop codon has been mutated further and encodes glycine in one allele instead of the wild-type arginine [R207G (Arg^{207} to Gly)]. XP129 is proficient in genome overall repair of (6–4) photoproducts but deficient in genome overall repair of CPD while parental XP12ROSV is deficient in genome overall repair of both damages (34,35). Moreover, XP129 has been shown to be proficient in CPD repair in the transcribed strand but deficient in the non-transcribed strand (36). The reason why CPD repair in the non-transcribed strand is selectively absent in XP129 has not been elucidated. An analysis of the defect(s) in XP129 cells might provide insight into the strand-specific DNA repair mechanisms in mammalian cells.

On the other hand, some XP-A patients such as XP39OS and XP8LO manifest only mild skin symptoms and minimal neurological findings. Cells derived from these patients display mild or intermediate UV sensitivity compared with typical XP-A cells (33,37). In addition, the various cell lines derived from XP12ROSV cells into which mutant XPA cDNAs were transfected, also exhibited mild or intermediate UV sensitivity (38). Strand-specific DNA repair activity has not been examined in these cells.

To elucidate the function of the XPA protein in strand-specific DNA repair, we investigated CPD repair in transcribed and non-transcribed strands of the dihydrofolate reductase gene of XP12ROSV cell line expressing wild-type and various mutant type XPA cDNAs such as R207G found in XP129, ΔC46 (deletion of 46 C-terminal amino acid residues) in XP39OS, H244R (His^{244} to Arg) in XP8LO, ΔN36 (deletion of 36 N-terminal amino acid residues), ΔE-cluster (deletion of 78–84 amino acid residues) and C264S (Cys^{264} to Ser).

MATERIALS AND METHODS

Cells

XP12ROSV, kindly provided by Jan H. J. Hoeijmakers of Erasmus University Rotterdam, is an SV40-transformed fibroblast cell line derived from XP-A patient XP12RO who is homozygous for the nonsense mutation at codon Arg^{207} (33). XP12ROSV cells are hypersensitive to killing by UV. In XP12ROSV cells, no XPA protein was detected by western blot analysis and CPD repair in both strands is absent (Figs 5 and 7; our unpublished results). Thus, XP12ROSV is a null mutant of XP-A (25). WI38VA13 is an SV40-transformed normal fibroblast cell line obtained from Masayoshi Namba of Okayama University. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS). The XP12ROSV transfectants overexpressing mutant XPA cDNA, ΔC46, H244R or R207G, were established in this study, whereas those overexpressing Wt (wild-type), ΔN36, ΔE-cluster and C264S XPA cDNA had been established previously (38). All the transfectants were named according to their cDNAs. These transfectants were cultured in the presence of 200 μg/ml of G418.

Overexpression of normal and mutant XPA cDNAs

The *Eco*RI fragment containing the entire open reading frame of XPA was cut out from plasmid pGM-H19-WS (39), and cloned into pBluescript SK(–) plasmid from which single-stranded DNA was prepared. *In vitro* mutagenesis reactions were carried out using the Mutan-K kit according to the manufacturer's protocol (Takara, Tokyo, Japan). The oligonucleotides used in the mutagenesis reactions were as follows: ΔC46 (Δ228–273), 5′-AGAATTGCGGTGAGCAGTAAG-3′; H244R, 5′-GTTCA-TCAACGTGAGTATGGA-3′; R207G, 5′-AAAGGAAGTCG-GACAGGAAAA-3′. The corresponding double-stranded DNAs were prepared and the DNA sequencing excluded additional mutations introduced elsewhere in the mutant XPA cDNA produced by the site-directed mutagenesis. The *Eco*RI fragments were cloned into the expression vector pCAGGS containing cytomegalovirus enhancer, chicken β-actin promoter and neo cassette (32). XP12ROSV cells (1×10^7 cells) were transfected with 1 μg of wild- or mutant-type XPA cDNA expression plasmids by the electroporation method using Gene Pulser (Bio-Rad, Richmond, CA). Transfectants were selected in medium containing 400 μg/ml of G418. Permanent transfectants were named ΔC46, H244R and R207G cells according to their transfected cDNAs. The mutations of XPA cDNA expressed in ΔC46, H244R and R207G cells corresponded to those found in XP39OS (33), XP8LO (37) and XP129 (34) cells, respectively. The clone transfected with only pCAGGS vector was designated pCAGGS cells.

Inducible expression of normal and mutant XPA cDNAs

LacSwitch™ system (Stratagene, La Jolla, CA) was used for studying the NER efficiency in the presence of various amounts of XPA protein. This system consists of a Lac-repressor-expressing vector p3′SS and a lac-operator-containing vector pOPI3CAT, into which the gene of interest is inserted. Lac-repressor binds as a homotetramer to the lac-operator, blocking transcription of the inserted gene. Inducers such as isopropyl-β-D-thiogalactoside (IPTG) bind to the Lac-repressor which results in its conformational change and an effective decrease in the affinity of the repressor for the operator, leading to an increase in transcription of the cloned gene. The p3′SS vector was transfected into XP12ROSV cells by the electroporation method. Stable transfectants were selected in medium containing 200 μg/ml of hygromycin, and examined for expression of the Lac-repressor by indirect immunofluorescence. In this work, the wild-type and R207G mutant XPA cDNAs were cloned into pOPI3CAT vectors, and the resultant plasmids were transfected into the XP12ROSV cells containing the p3′SS plasmid. The selection of stable transfectants was carried out by adding 400 μg/ml of G418 to the media. For induction of XPA protein production, expanded cell lines were treated with 1 mM IPTG 24 h before experiments.

UV survival

Exponentially growing fibloblasts were plated at 2×10^3 cells per 100-mm Petri dish and exposed to UV at various dosages ~12 h after plating. The cells were then cultured for 10–14 days in adequate medium, fixed with 3% formaldehyde and stained with 0.1% crystalviolet, after which colonies were counted.

Strand-specific DNA repair analysis

The repair of CPD was examined in a 20 kb *Kpn*I fragment within the dihydrofolate reductase (DHFR) gene. The incidence of CPD was determined using a method described elsewhere (40,41) with some modifications. Cells were irradiated with 7.5 J/m^2 of UV (254 nm), lysed immediately for an initial sample or incubated in medium containing 10^{-5} M bromodeoxyuridine and 10^{-6} M fluorodeoxyuridine to incorporate density label into the newly replicated DNA. After incubation for 4, 8 and 24 h, high molecular

DNAs were extracted, restricted with *Kpn*I endonuclease and centrifuged in a neutral CsCl gradient to separate the unreplicated parental DNA from the replicated DNA. The parental DNA was dialyzed to remove CsCl. Two 10 µg aliquots of DNA were either treated or mock treated with 180 ng of T4 endonuclease V, which generates single-strand breaks specifically at CPD sites. The sample aliquots were mixed with alkaline loading dye, separated by electrophoresis through 0.6% alkaline agarose gel, transferred onto Hybond N^+ membranes (Amersham, Buckinghamshire, UK), and hybridized with strand-specific ^{32}P-labeled DNA probes. The membranes were washed under stringent conditions. The intensity of the bands was quantitated by scanning using a BAS2000 imaging analyzer (FUJIX, Tokyo, Japan). The number of CPD per fragment was calculated from the ratio of densities of bands in the enzyme- and mock-treated samples using the Poisson expression. The strand-specific probes were generated by linear PCR based on a method described by Ruven *et al.* (42) with modifications. A 690 bp *Eco*RI–*Hin*dIII DHFR gene fragment from PGEM0.69EH plasmid (43), which was kindly provided by Dr P. C. Hanawalt of Stanford University, was used as a template in a linear PCR, using a single primer recognizing each strand specifically. The primers are as follows: transcribed strand, 5′-CTGTTTCCAGTTTATTT-AGT-3′; non-transcribed strand, 5′-CTCCCATAGCCTTGTTTA-GA-3′. Linear PCR was carried out for four consecutive cycles at 94°C for 30 s, 53°C for 30 s and 72°C for 3 min in the presence of 165 nM [α-^{32}P]dCTP (3.0 kCi/mmol, Amersham) as the only dCTP source and 5 µM of the other nucleotides. Strand specificity of the probes was checked on dot blots by hybridization to single-stranded pBluescript vectors containing one of the strands of the probe.

Genome overall repair of (6–4) photoproducts and CPD

The rate of genome overall repair of CPD and (6–4) photoproducts was measured by ELISA using the monoclonal antibodies TDM-1 (specific for CPD) and 64M-2 [specific for (6–4) photoproducts] (44).

RESULTS

Establishment of XP12ROSV transfectants expressing mutant XPA cDNA

To examine the function of the XPA protein in strand-specific DNA repair, we established XP12ROSV transfectants overexpressing either ΔN36, ΔE-cluster, ΔC46, H244R, C264S or R207G mutant XPA cDNA. Figure 1 indicates the locations of these XPA mutations which were superimposed on the functional domains of the XPA protein. ΔN36 protein lacks 36 N-terminal amino acids including the RPA p34 binding region and a part of the nuclear localizing signal (28,38). ΔE-cluster protein has a deletion of amino acids 78–84 in the ERCC1 binding region (23). ΔC46 mutation corresponded to the nonsense mutation at codon 228 found in patient XP39OS. H244R mutation was detected in one of the alleles of patient XP8LO. XP39OS and XP8LO showed a mild phenotype for XP-A and cells from these patients showed less UV hypersensitivity for XP-A (33,37,38). It has been shown that ΔC46, H244R and 264CS mutations affect TFIIH binding to XPA (29,30; our unpublished data). The R207G mutation was detected in XP129 cells, which are UV-resistant XP-A revertant cells derived from mutagenized

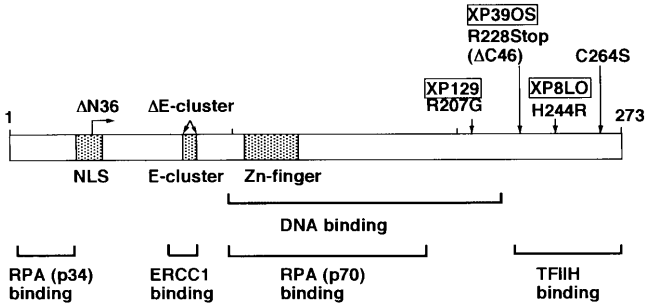

Figure 1. Locations of the XPA mutations superimposed on the functional domains of XPA. NLS, nuclear localization signal; E-cluster, seven consecutive glutamic acid residues in the 78–84 amino acid region; Zn-finger, C4-type Zn-finger motif. The regions of XPA for the bindings with UV-damaged DNA (98–219 amino acid residues), RPA p34 subunit (4–29 residues), ERCC1 (53–97 residues), RPA p70 subunit (98–187 residues) and TFIIH (226–273 residues) are shown by parentheses. Arrows indicate the positions of XPA mutations.

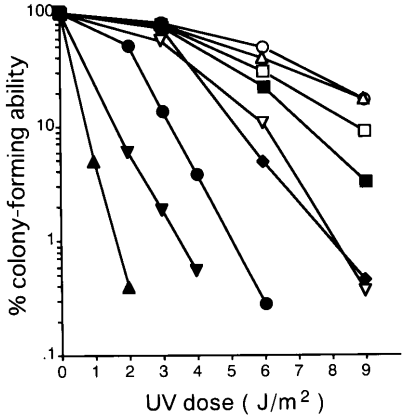

Figure 2. Ultraviolet sensitivity measured by colony-forming ability. Values are for WI38VA13 (○), Wt (□), R207G (Δ), ΔN36(■), 264CS (▽), H244R (◆), ΔC46 (●), ΔE-cluster (▼) and pCAGGS (▲) cells after various doses of UV irradiation. Each point represents the average value for duplicate dishes.

XP12ROSV (45) and exhibited proficient genome overall repair of (6–4) photoproducts and CPD repair in the transcribed strand of active genes, but deficient genome overall repair of CPD and CPD repair in the non-transcribed strand (34,36).

Immunoblotting using polyclonal anti-XPA antibody revealed that the expression levels of XPA proteins in the Wt, ΔN36, ΔE-cluster, 264CS, ΔC46, H244R and R207G cells were 5–10 times higher than in normal WI38VA13 and HeLa cells (data not shown).

Cell survival

Colony forming abilities of the transfectants and WI38VA13 cells after UV-irradiation were then assessed (Fig. 2). pCAGGS cells were extremely sensitive to UV irradiation, whereas Wt and R207G cells exhibited almost the same level of UV resistance as WI38VA13 cells. Other cell strains showed intermediate UV resistance between pCAGGS and Wt cells. ΔN36 cells were slightly more sensitive than normal cells. 264CS and H244R cells

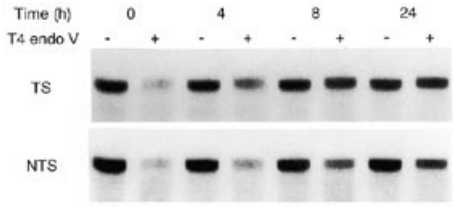

Figure 3. Autoradiograms showing removal of UV-induced CPD from the 20 kb DHFR *Kpn*I fragments in Wt cells irradiated with 7.5 J/m^2. The DHFR fragments were analyzed with strand-specific probes recognizing the transcribed (TS) or the non-transcribed (NTS) strand.

showed partially reduced UV resistance. ΔC46 and ΔE cells retained marginal UV resistance compared with pCAGGS cells.

Strand-specific removal of CPD in the DHFR gene

To examine the effects of XPA mutations on strand-specific repair of CPD in active genes, we measured the removal of CPD from each strand of the active DHFR gene in the XP12ROSV cells transfected with wild- or mutant-type XPA cDNA using strand-specific DNA probes. Autoradiograms showing strand-specific repair of CPD in Wt cells are represented in Figure 3. It is clearly shown that CPD was repaired more efficiently in the transcribed than in the non-transcribed strand, especially at early time points after UV irradiation. The intensity of each band was quantified. The percent repair of each strand at appropriate post-irradiation incubation times was determined and plotted (Fig. 4). Each point in Figure 4 shows the average value for more than two independent experiments. Approximately 50% of the CPD was removed in the transcribed strand in Wt cells within 4 h after UV irradiation, and 80% within 8 h, whereas only 60% of CPD was removed by 24 h in the non-transcribed strand. The pattern of repair for each strand of the DHFR gene in Wt cells was similar to the repair rate reported previously in primary and SV40-transformed normal human fibroblasts (8,46). pCAGGS cells exhibited no substantial repair of CPD in each strand. In R207G cells, the rate of CPD repair in the transcribed strand was almost the same as that in Wt cells, and repair in the non-transcribed strand was also proficient but a little less efficient than that in Wt cells. The other transfectants showed a significantly decreased rate of CPD repair in each strand. ΔE-cluster and ΔC46 cells had very little CPD repair in each strand. No significant difference in the repair rate between H244R and 264CS cells was observed. These two cell

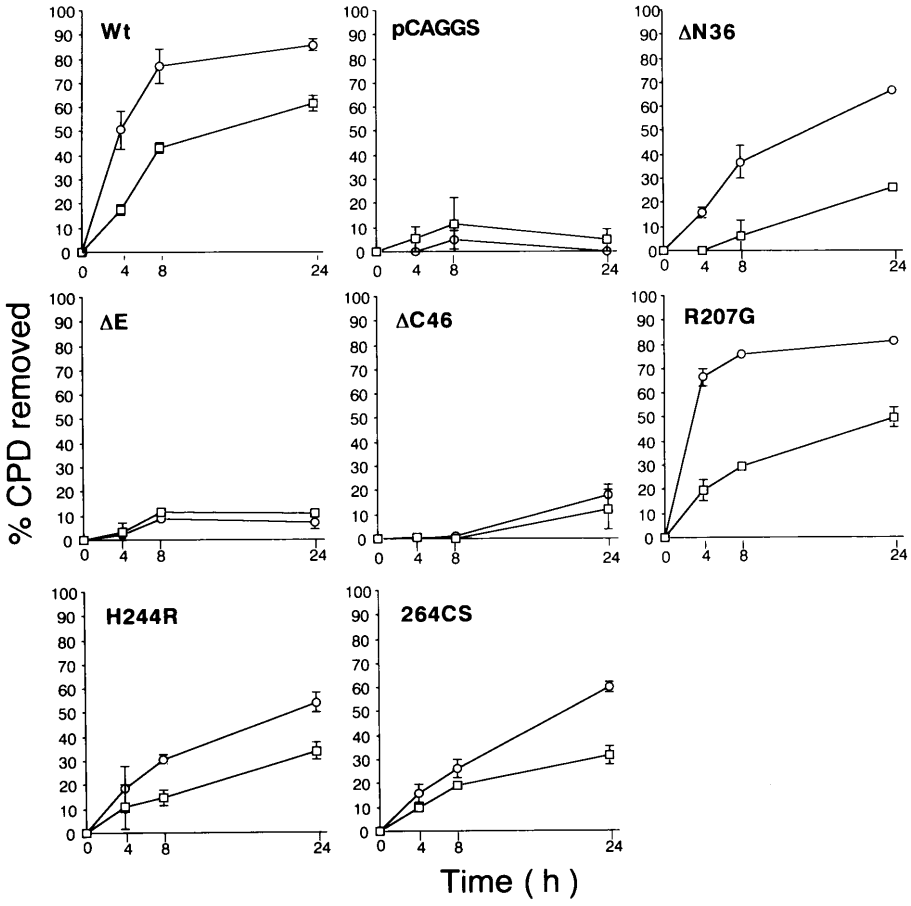

Figure 4. Strand-specific repair within the DHFR gene in Wt, pCAGGS, ΔN36, ΔE, ΔC46, R207G, H244R and 264CS cells. The frequency of CPD was measured as described in the text. The percentage of repair was calculated from the CPD frequency at each time point. (○), transcribed strand; (□), non-transcribed strand. The values are the averages of more than two independent experiments for each cell line. Bars represent standard errors. Bars are not drawn when the standard errors are too small for the symbols.

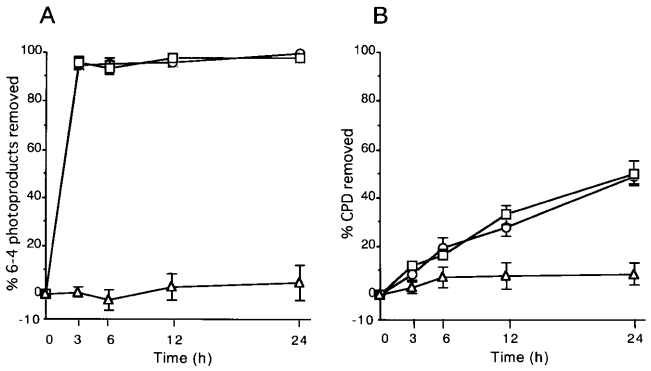

Figure 5. Repair kinetics of (6–4) photoproducts (**A**) and CPD (**B**) in Wt (○), R207G (□) and pCAGGS (Δ) cells after UV irradiation. Each point represents the average value for three separate experiments. Bars indicate standard errors.

lines exhibited moderately decreased rates of repair of CPD in each strand compared with Wt cells. In ΔN36 cells, CPD repair was decreased in each strand. However, the repair rate of ΔN36 cells was higher than that of H244R and C264S cells. Taken together, repair of CPD in transcribed and non-transcribed strands more or less decreased in parallel in each mutant cell line except R207G cells, and the strand-specific repair rate correlated well with colony forming ability of the cells after UV irradiation.

Genome overall repair of (6–4) photoproducts and CPD

It has been reported that in XP129 cells, the genome overall repair of (6–4) photoproducts occurred normally but that repair of CPD did not. We therefore examined genome overall repair of (6–4) photoproducts and CPD in R207G cells. Figure 5 shows the kinetics of genome overall repair of (6–4) photoproducts and CPD at different times post-UV in Wt, pCAGGS and R207G cells. In Wt and R207G cells, >90% of (6–4) photoproducts were repaired within 3 h (Fig. 5A). These repair kinetics of (6–4) photoproducts were consistent with previously reported properties of wild-type cells (47). There was no detectable repair of (6–4) photoproducts in pCAGGS cells. On the other hand, Figure 5B shows that 50% of CPD was repaired in Wt and R207G cells within 24 h whereas no repair of CPD was observed in pCAGGS cells. In our present experimental conditions, the rate of genome overall repair of both CPD and (6–4) photoproducts in R207G cells was indistinguishable from that in Wt cells.

Effect of the level of XPA protein on the removal of CPDs from each strand of the DHFR gene

To investigate the effect of the XPA protein level on strand-specific DNA repair, we established three cell lines designated LacW, LacM1 and LacM5, in which Wt (LacW) and R207G (LacM1 and LacM5) XPA protein are inducible by IPTG. In immunoblot analysis using anti-XPA protein antiserum (Fig. 6), XPA protein in LacW cells was undetectable in the absence of IPTG in our present experimental conditions, but expressed at the same level as in normal HeLa and WI38VA13 cells on IPTG induction. XPA protein was also practically undetectable without IPTG in LacM1 cells. After IPTG induction, the amount of XPA protein in LacM1 cells was about half the level of that in normal cells. The amount of R207G type XPA protein without IPTG in LacM5 cells was

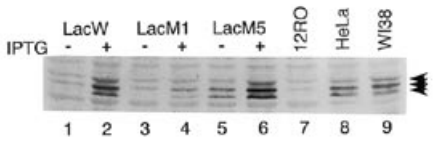

Figure 6. Induction of wild-type and R207G-type XPA protein with IPTG. Each transfectant clone was incubated with (+) or without (–) IPTG for 24 h (lanes 1–6). Ten micrograms of whole cell extract was loaded in each lane, separated on a 12% SDS–PAGE gel and analyzed by immunoblotting with anti-XPA polyclonal antibody. Detection of the XPA protein was performed using alkaline phosphatase and nitro blue tetrazolium (NBT)/5-bromo-4-chloro-3 indolyl phosphate (BCIP). As controls, untransfected XP12ROSV (lane 7), HeLa (lane 8) and WI38VA13 (lane 9) whole cell extracts were loaded. Positions of XPA proteins are indicated by arrows. Since the size of the truncated XPA protein produced in XP12ROSV, if any, is ~30 kDa in the SDS–PAGE gel (38), all the bands in lane 7 are non-specific.

~40% of that in HeLa cells. In the presence of IPTG, LacM5 cells produced about twice as much XPA protein as normal cells.

Strand-specific repair of CPD in these Lac cells with or without IPTG induction was then examined (Fig. 7). In the presence of IPTG, the repair rate of CPD in each strand of the LacW cells was similar to that in Wt cells. Even without IPTG induction, LacW cells were able to perform CPD repair to some extent: 35% of CPD repair within 4 h in the transcribed strand and 30% of CPD repair within 24 h in the non-transcribed strand. The LacM1 and LacM5 cells without IPTG induction retained some CPD repair in both transcribed and non-transcribed strands, while the LacM1 and LacM5 cells with IPTG induction showed more proficient CPD repair in each strand than the LacM1 and LacM5 cells without IPTG induction, respectively. Thus, there is also a good correlation between the rate of CPD repair in each strand and the amount of R207G mutant XPA protein in LacM1 and LacM5 cells. On the other hand, although the induced LacM5 cells had almost twice as much XPA protein as the induced LacW cells, the rate of CPD repair in each strand in the induced LacM5 cells was apparently lower than that in the induced LacW cells, indicating that the wild-type XPA protein was more efficient in repair of CPD in each strand than the R207G mutant XPA protein. The R207G XPA protein may have abnormal XPA function.

Figure 8 shows colony forming ability after UV irradiation in Lac cells with or without IPTG induction. Even the LacW cells without IPTG induction, which showed a subnormal rate of CPD repair in each strand, acquired a normal UV-resistance, indicating that a normal rate of CPD repair in each strand is not necessary for normal UV-resistance and that even an undetectable level of wild-type XPA protein is sufficient for normal UV-resistance. On the other hand, R207G protein conferred normal UV-resistance on XP12ROSV cells when the amount of R207G mutant XPA protein in the transfectant was >40% of that in HeLa cells.

DISCUSSION

In the present study, we examined the strand-specific DNA repair characteristics of XP12ROSV transfectants expressing either ΔN36, ΔE-cluster, ΔC46, H244R, C264S or R207G mutant XPA cDNA (Fig. 1). pCAGGS cells transfected with vector alone showed the same hypersensitivity and strand-specific DNA repair deficiency as parental XP12ROSV cells. Careful evaluation of UV survival revealed that ΔN36 cells are a little more UV sensitive than Wt and normal cells and obviously have a reduced ability for CPD repair in each strand. ΔN36 lacks a part of the nuclear localization signal as

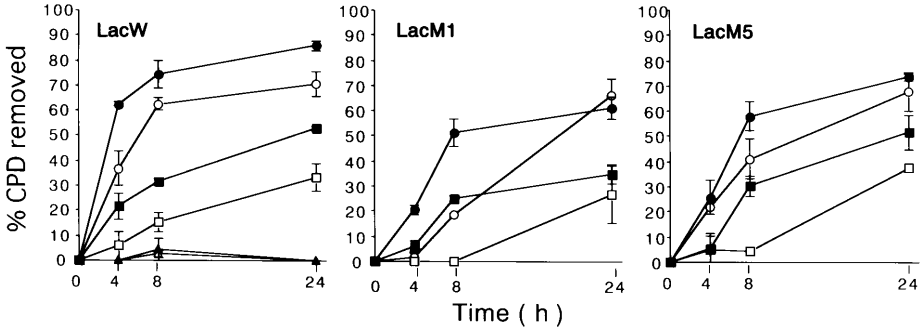

Figure 7. Strand-specific repair within the DHFR gene in LacW (XP12ROSV carrying the Wt XPA cDNA), LacM (XP12ROSV carrying the R207G-type XPA cDNA)1 and LacM5 cell strains with or without IPTG induction. Transcribed strand with (●) or without (○) IPTG. Non-transcribed strand with (■) or without (□) IPTG. As a negative control, CPD repair in the transcribed (▲) and non-transcribed (Δ) strands in parental XP12ROSV cells is included. The values are the average of two independent experiments for each cell strain. Bars represent standard errors.

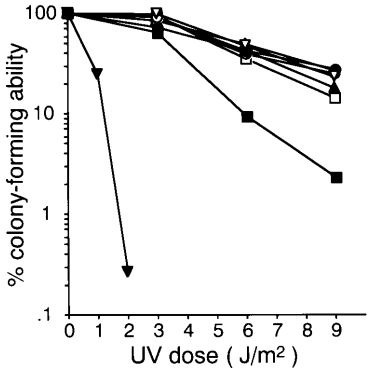

Figure 8. UV sensitivity measured by colony-forming ability. Values are for WI38VA13 (▽), XP12ROSV (▼), LacW with (○) or without (●) IPTG, LacM1 with (□) or without (■) IPTG and LacM5 with (Δ) or without (▲) IPTG. Each point represents the average value for duplicate dishes.

well as the RPA p34 subunit binding domain. We have shown that the overexpressed XPA mutant protein without nuclear localization signal localized almost evenly in the nucleus and cytoplasm due to passive diffusion across the nuclear membranes (38). Therefore, the reduced strand-specific repair of CPD in ΔN36 cells might be due to the absence of interaction between RPA p34 subunit and XPA protein rather than the partial deletion of the nuclear localization signal. XPA binds the p70 subunit as well as the p34 subunit of RPA (28). Efficient repair of CPD might be performed when XPA binds not only p70 subunit but also p34 subunit of RPA. ΔE-cluster cells showed only marginal UV-resistance and almost no strand-specific DNA repair activities, indicating that the interaction between XPA and ERCC1 is indispensable for CPD repair in both transcribed and non-transcribed strands. H244R and C264S cells with partially reduced UV-survival showed partially decreased strand-specific DNA repair in each strand. ΔC46 cells with only marginal UV resistance showed almost no strand-specific DNA repair. Thus, the TFIIH/XPA interaction is important for the CPD repair in each strand. All these results indicate that colony forming ability after UV irradiation correlated well with strand-specific DNA repair activities, that the rate of CPD repair in transcribed and non-transcribed strands varied in parallel in the ΔN36, ΔE-cluster, H244R, 264CS and ΔC46 cells, and that the XPA protein is important for the CPD repair in both transcribed and non-transcribed strands.

R207G cells with normal UV sensitivity showed almost normal CPD repair in transcribed and non-transcribed strands, whereas XP129 cells with normal UV sensitivity are proficient in CPD repair in the transcribed strand but deficient in the non-transcribed strand. Moreover, the genome overall repair of both CPD and (6–4) photoproducts was normal in R207G cells, whereas in XP129 cells, removal of (6–4) photoproducts was proficient but that of CPD was absent. It has been suggested that R207G mutation is responsible for the proficient CPD repair in the transcribed strand and deficient CPD repair in the non-transcribed strand in XP129 cells (36). The present results suggest that the reversion of the nonsense mutation at codon 207 to glycine in XP12ROSV cells is responsible for the partial restoration of the function of the XPA protein and subsequent recovery of CPD repair in the transcribed strand in XP129 cells, but it may not be the cause of selective deficiencies in CPD repair in the non-transcribed strand and genome overall repair of CPD in XP129 cells.

On the other hand, the amount of XPA protein produced in XP129 cells was ~30% of that in normal human cells (48), while in R207G cells, the XPA protein was overproduced (~5–10 times as much as normal). The discrepancy of strand-specific DNA repair activity in R207G and XP129 cells could be due to the difference in the amount of XPA protein produced in these cells. To examine whether the lower amounts of R207G protein may cause selective deficiency in CPD repair in the non-transcribed strand, we investigated the relationship between the amounts of XPA protein, strand-specific repair of CPD and cell survival after UV irradiation. We established LacW and LacM cells derived from XP12ROSV cells that had been transfected with wild-type and R207G XPA cDNA driven by LacSwitch promoter, respectively.

As shown in Figure 6, in LacW cells without IPTG induction, XPA protein was practically undetectable in our present experimental conditions. However, it is known that the LacSwitch™ system exhibits low basal expression of the inserted gene even without IPTG induction (Stratagene LacSwitch™ Instruction Manual). Therefore, it was plausible that the LacW cells without IPTG induction produced a little wild-type XPA protein. However, in spite of this, they showed normal levels of UV survival (Fig. 8) and slightly lower rates of CPD repair in each strand than normal cells (Fig. 7). These findings indicate that even a very low level of wild-type XPA protein results in efficient CPD removal in each strand and that a normal rate of CPD repair is not necessary for normal UV resistance. In addition, the parallel reduction of CPD repair in each strand without IPTG induction indicates that XPA protein is a rate limiting factor for CPD repair in each strand.

Production of R207G type XPA protein was also inducible by IPTG in LacM cells harboring R207G type XPA cDNA. In the

absence of IPTG, the amount of R207G type XPA protein in LacM5 cells is ~40% of that in HeLa cells, which is similar to that in XP129, but significant CPD repair in the non-transcribed strand was observed. Moreover, R207G type XPA protein was not practically detected in the non-induced LacM1 cells, but still some CPD repair in non-transcribed strand was observed. These results suggest that the discrepancy of strand-specific DNA repair activity in R207G and XP129 cells is not due to the difference in the amount of XPA protein produced in these cells, and that the R207G mutation is not the cause of the selective defect in the CPD repair in the non-transcribed strand and that there exist mutation(s) responsible for CPD repair deficiency in the non-transcribed strand of the XP129 cell line other than the R207G mutation.

On the other hand, although LacM5 cells with IPTG induction produced about twice as much XPA protein as LacW cells with IPTG induction, the CPD repair in each strand was less efficient in LacM5 cells than in LacW cells. These results indicate that R207G-type XPA protein has abnormal function in the repair of CPD. Since the R207G mutation resides in the damaged DNA binding domain of the XPA protein (49,50), the R207G XPA protein may have lower affinity for CPD than wild-type XPA protein. However, R207G protein is able to play an almost normal role in CPD repair in each strand when it is overproduced in the cells (5–10 times higher than in normal WI38VA13 and HeLa cells).

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