1002–1008 *Nucleic Acids Research, 1997, Vol. 25, No. 5*

The fission yeast UVDR DNA repair pathway is inducible

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Received November 19, 1996; Revised and Accepted January 10, 1997

DDBJ/EMBL/GenBank accession no. U78487

ABSTRACT

In addition to nucleotide excision repair (NER), the fission yeast *Schizosaccharomyces pombe* possesses a UV damage endonuclease (UVDE) for the excision of cyclobutane pyrimidine dimers and 6–4 pyrimidine pyrimidones. We have previously described UVDE as part of an alternative excision repair pathway, UVDR, for UV damage repair. The existence of two excision repair processes has long been postulated to exist in *S.pombe*, as NER-deficient mutants are still proficient in the excision of UV photoproducts. UVDE recognizes the phosphodiester bond immediately 5′ of the UV photoproducts as the initiating event in this process. We show here that UVDE activity is inducible at both the level of *uve1*$^+$ mRNA and UVDE enzyme activity. Further, we show that UVDE activity is regulated by the product of the *rad12* gene.

INTRODUCTION

Multiple pathways exist for the repair of the major cytotoxic and carcinogenic UV photoproducts, cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine pyrimidones (6–4 PPs). These include nucleotide excision repair (NER), photoreactivation, recombination and UV damage repair (UVDR). NER is the classical DNA excision repair system, which is the major repair pathway in nearly all organisms responsible for the repair of not only UV photoproducts but a variety of DNA damage types, including bulky lesions and cross-linked DNA (1–3). Photoreactivation, often referred to as light-dependent repair, had until recently thought to only be involved in reactivating CPDs (4), however, recently a 6–4 PP photoreactivating enzyme was described (5). In photoreactivation, a photolyase recognizes the bonds joining adjacent pyrimidines and in the presence of near UV light resolves the bases back to monomers. Recombination is a less well-characterized mechanism of DNA repair, where DNA damage is removed by recombination with its sister chromatid (6,7). UVDR is the most recently described DNA repair pathway. While the exact mechanism of this process is not known, repair of UV photoproducts is initiated by cleavage of the phosphodiester bond immediately 5′ of either CPDs or 6–4 PPs. The enzyme which catalyzes this reaction, first described in *Schizosaccharomyces pombe*, was named SPDE, for *S.pombe* DNA endonuclease (8), or UVDE, for UV damage endonuclease (9). A similar endonuclease has been identified in *Neurospora crassa* (10) and a homolog of the gene exists in *Bacillus subtilis* (9). To maintain continuity of nomenclature, we will refer to the *S.pombe* endonuclease as UVDE, for UV damage endonuclease, as defined by Takao *et al.* (9). Further, we will refer to the gene encoding UVDE as *uve1*$^+$ and the UVDE-dependent repair pathway UVDR, for UV damage repair.

Our studies have previously shown that UVDR is distinct from NER, based on both genetic and biochemical evidence (11). Yeast double mutant strains which carry mutations in genes involved in both NER (*rad13-A*) and UVDR (*rad12-502*) are hypersensitive to UV light (11). Further, the site of 5′ incision in UVDR is at the phosphodiester bond immediately adjacent to the site of damage (8), while both 5′ and 3′ incisions in NER occur at a distance from the site of damage (1). Following 5′ incision, DNA repair synthesis can be demonstrated *in vitro* (11). DNA repair synthesis is deficient in cell extracts prepared from *rad12-502* mutants and this deficiency can be complemented by the addition of partially purified UVPE (11). This demonstrates that the reason UVDR is defective in *rad12* extracts is because of limiting UVDE activity. The existence of a second DNA excision repair pathway for the removal of UV photoproducts was clearly demonstrated genetically using antibodies directed against CPDs and 6–4 PPs (12). It has been proposed that UVDE may act to initiate a recombinational repair process, which involves the *rad2*, *rad18* and *rph51* gene products (13). However, studies using our UVDR *in vitro* repair system (14) indicate that extracts prepared from both *rad2-44* and *rad18-X* cells have normal levels of repair synthesis

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(K.Sidik, unpublished data). Further studies will be necessary to resolve the steps following UVDE incision in this repair process.

Some enzymes involved in various DNA repair processes are induced in response to DNA damage. In *Escherichia coli* transcription activation is controlled by the RecA protein (15). In *Saccharomyces cerevisiae* there are a number of genes which are activated in response to DNA damage. Some of these gene products are involved directly in DNA repair, such as RAD2, RAD7, RAD18, RAD51, RAD54 and SNM1, while others are involved in DNA metabolism, such as RNR1, RNR2 and RNR3 (16–22). In *S.pombe* subtraction cloning was used to clone four cDNAs whose mRNAs were induced in response to UV damage (23). Recently, it was reported that the *S.pombe* recombination repair gene *rhp51* is transcriptionally induced in response to DNA damage (24). Analysis of the *rhp51* promoter region revealed that it contained damage-responsive elements (DRE) homologous to sequences identified in *S.cerevisiae* as being involved in regulating induction by DNA damage. Interestingly, *rhp51* encodes a protein with amino acid similarity to the *E.coli* RecA protein. In this paper we show that UVDE is inducible at the level of transcription and that UVDR, as measured by *in vitro* excision repair, is similarly induced. Further, we show that the product of the *rad12*$^+$ gene does not code for UVDE, but rather is a regulator of UVDE activity.

MATERIALS AND METHODS

Schizosaccharomyces pombe genetic manipulations

Schizosaccharomyces pombe was cultured by standard techniques (25). Complete genotypes of the strains used in this study are summarized in Table 1. Sp272, h^{-S} *rad12-502*, was constructed by outcrossing Sp264 (h^{+N} *rad12-502*) twice with 972 (h^{-S}).

Table 1. Strains used in this study

Strain	Genotype	Reference
972	h^{-S}	25
Sp18	h^{-S} *cdc25-22*	26
Sp264	h^{+N} *rad12-502*	11
Sp269	h^{-S} *rad12-502 rad13-A*	11
Sp272	h^{-S} *rad12-502*	This study
Sp273	h^{-S} *leu1-32 rad13-A*	11

Preparation of *S.pombe* whole cell extracts

Whole cell extracts were prepared from 10^{10}–10^{11} *S.pombe* cells. These cells were grown to late log phase in 1.5× YEA (7.5 g/l yeast extract, 45 g/l dextrose, 100 mg/l adenine). Cells were collected by centrifugation, washed in water, resuspended in an equal volume of extraction buffer [20 mM Tris–HCl, pH 7.9, 10% glycerol, 1 mM EDTA, 10 mM $MgCl_2$, 0.3 M $(NH_4)_2SO_4$, 1 mM PMSF, 1 mM DTT] and frozen at –80°C. Frozen cells were thawed and lysis was performed using a 50 ml bead beater (BioSpec Products Inc). After separating the beads, the cellular debris was removed by centrifugation for 1 h at 100 000 *g*. The supernatant was dialyzed for 5 h to overnight against 100 vol dialysis buffer (20 mM HEPES–KOH, pH 7.6, 10 mM $MgSO_4$, 10 mM EGTA, 5 mM DTT, 20% v/v glycerol, 1 mM PMSF). Protein concentrations of the extracts, determined by Bradford assay (BioRad), were between 30 and 60 mg/ml.

Cells used to produce UV-induced extracts were grown in YEA to late log phase, collected by centrifugation, washed with water and resuspended in 1 vol water. Cells were placed in a large Petri dish or a glass tray and irradiated with constant mixing. Inductions were performed with 254 nm light, using a total dose of 200 or 400 J/m^2 (as indicated) and a dose rate of 2.68 J/m^2/s. The cells were transferred to fresh YEA and incubated with shaking at 30°C for the appropriate times. Cells were then collected by centrifugation and mixed with an equal volume of extraction buffer prior to freezing at –70°C. Extracts were prepared as described above. Viability experiments on cells irradiated by this protocol yielded 90–75% viability, indicating the actual dose absorbed by the yeast was 50–100 J/m^2 for the 200 or 400 J/m^2 total doses given respectively.

Preparation of the damaged oligonucleotide and plasmid substrates

The 6–4 PP 49mer and CPD 49mer were synthesized as described (27). 3′-End-labeling was carried out by incubating 1 pmol of either oligonucleotide with [α-^{32}P]dGTP (50 µCi, 3000 Ci/mmol), 0.2 mM dATP and 5 U T4 DNA polymerase for 40 min at 14°C. This created the 3′-end-labeled 6–4 PP or CPD 51mer. UV-damaged plasmid DNA was prepared by spotting 10 µl droplets of supercoiled pUC18 DNA in TE at 0.1 µg/µl onto a sheet of parafilm. The DNA was exposed to 100 J/m^2 254 nm light.

UVDE assays

UVDE assays were carried out essentially as described (11). Whole cell extract (100 µg) was incubated with 0.02 pmol 3′-end-labeled 6–4 PP 51mer at 37°C for 5–15 min in 45 mM HEPES–KOH, pH 7.8, 70 mM KCl and 7 mM $MgCl_2$ in a 20 µl reaction. The samples were treated with proteinase K, extracted with phenol/chloroform and the DNA analyzed on denaturing 15% polyacrylamide–urea gels. The gel was dried, exposed to X-ray film and the results were quantified on an Image Analysis System (Fuji).

In vitro repair assay

Extract preparation and reaction conditions were as described (14). Following incubation at 30°C for 2 h plasmid DNA was repurified (14). The DNA was separated on a 0.8% agarose gel. The gel was dried, exposed to X-ray film and the results were quantified on an Image Analysis System (Fuji).

Radioimmunoassay (RIA) to determine *in vivo* excision of UV photoproducts

Details of the RIA have been published (28,29). Briefly, *S.pombe* was grown to late log phase in YEA, collected, resuspended in an equal volume of dH_2O and irradiated with 200 J/m^2 254 nm UV light. The cells were returned to YEA and grown for the indicated times prior to harvest of total DNA. Next, poly(dA)·poly(dT) (Boehringer-Mannheim) was nick translated with [α-^{32}P]dTTP (Amersham) to a specific activity of 5–10 × 10^8 c.p.m./µg. The labeled DNA was irradiated in water at a fluence rate of 14 J/m^2/s, measured at 254 nm, for a total dose of 30 kJ. About 5–10 pg

UV-irradiated, radiolabeled ligand competed with 7.5 µg heat-denatured sample DNA for binding to antiserum. Rabbit polyclonal antisera that bind 6–4 PPs or CPDs were added to TES (10 mM Tris, pH 7.8, 150 mM NaCl, 1 mM EDTA) containing 0.15% gelatin (Type III; Sigma) at a concentration that yielded 30–50% binding. The absolute specificities of these antisera for the 6–4 PP and *cis,syn* cyclobutane dimer have been demonstrated using mobility shift immunoassays of damage-specific oligonucleotides (30). After overnight incubation at 4°C, goat anti-rabbit IgG (Calbiochem) and carrier γ-globulin (Calbiochem) were added and incubated for 2–3 days at 4°C to form a precipitable immune complex. The immune pellet was collected by centrifugation, dissolved in tissue solubilizer (Amersham) and counted in a Packard liquid scintillation counter. For DNA repair curves, percentage inhibition of sample DNA harvested at increasing times post-irradiation was extrapolated through a linear regression of the unrepaired sample harvested immediately after irradiation, to give the percentage remaining photoproduct.

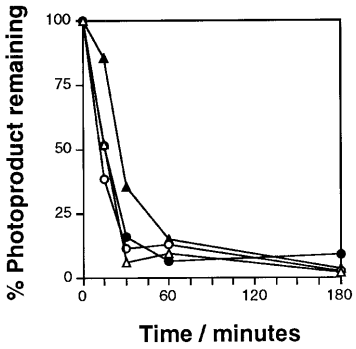

Figure 1. *rad12-502 rad13-A* double mutants are capable of *in vivo* excision of UV photoproducts. *Schizosaccharomyces pombe* wild-type (972, Δ), *rad12-502* (Sp272, ○), *rad13-A* (Sp273, ▲) or *rad12-502 rad13-A* (Sp269, ●) strains were subjected to radioimmunoassay to detect UV photoproducts at various times after UV irradiation. The data show removal of UV photoproducts *in vivo* in all of these strains.

Quantitation of mRNAs

Schizosaccharomyces pombe 972 cells were grown in YEA to a density of 2×10^7 cells/ml at 32°C. Cells were collected and resuspended in 2 vol dH_2O. The cells were irradiated with 400 J/m^2 254 nm UV light. Cells were then transferred to fresh YEA prewarmed to 32°C and grown with shaking for the indicated times. Cells were collected by centrifugation at 3000 *g* for 2 min and rapidly frozen. Because of the rapid induction times unirradiated cells were collected and frozen and used to measure basal transcription. In addition, aliquots of irradiated and unirradiated cells were plated and counted for survival.

Total RNA was isolated by lysing the cells with glass beads. The frozen cell pellets (0.4 ml) were resuspended in 4 ml Trizol (Gibco BRL) in 50 ml conical tubes and enough glass beads (0.5 mm) added so that no liquid remained. The cells were vortexed for 2 × 40 s. Then, 4 ml Trizol were added and mixed followed by the addition of 1.6 ml $CHCl_3$. The samples were again mixed and the aqueous layer was separated by centrifugation. The aqueous layer was extracted with an equal volume of phenol/chloroform and isopropanol precipitated. Total RNA was collected by centrifugation and the pellets briefly air dried. The RNA was suspended in 400 µl DEPC-treated dH_2O. Based on absorbance at 260 nm, between 1 and 1.5 mg total RNA were recovered. Poly(A)$^+$ mRNA was isolated on Oligotex (Qiagen). Between 20 and 35 µg poly(A)$^+$ mRNA were recovered from each sample.

Northern blot analysis (31) was done by resuspending 5 µg poly(A)$^+$ mRNA from each sample in 10 µl loading buffer (50% formamide, 20% formaldehyde, 20 mM MOPS, pH 7.0, 1 mM sodium acetate, 1 mM Na_2EDTA and 400 µg ethidium bromide). The samples were heated to 65°C for 10 min and loaded onto a 1.2% agarose gel containing 2.2% formaldehyde, 20 mM MOPS, pH 7.0, 1 mM sodium acetate and 1 mM Na_2EDTA. The RNAs were separated by electrophoresis for 3 h at 80 V. The gel was washed for 5 min in dH_2O and blotted onto Zetablot (BioRad). Blots were probed with ^{32}P-labeled PCR product produced from UVDE DNA using *Pfu*I polymerase (Stratagene). Quantitation of mRNA levels from the *leu1* gene were carried out by probing with a ^{32}P-labeled *leu1* PCR product. Following washing the blots were either autoradiographed or analyzed on a Molecular Dynamics phosphorimager.

Gene isolation and sequencing

A wild-type genomic library, made by a partial *Hin*dIII digest cloned into pWH5, was screened for UVDE sequences. The probe was made by PCR amplifying a region of the UVDE cDNA using [α-^{32}P]dCTP and [α-^{32}P]dGTP in the reaction. The clone (pUVDE12) contained several *Hin*dIII fragments. One of these fragments of ~5.4 kb was shown to contain the entire sequence for UVDE. This fragment was subcloned into pUC18 and named pgUV2. The sequencing of *uve1*$^+$ and its promoter region was accomplished by both conventional dideoxy sequencing using Sequenase (US Biochemicals) and by automated sequencing (ABI).

RESULTS

UVDE activity is induced by UV light

Previous data had shown that extracts prepared from *rad12-502* cells were deficient in UVPE activity based on an *in vitro* excision repair system (11). Furthermore, when *rad12-502* cells were crossed with the NER-deficient mutant strain *rad13-A* the resulting double mutant was hypersensitive to UV light. Based on these data the *rad12-502 rad13-A* double mutant cells were tested for excision of UV photoproducts in an *in vivo* assay. In this assay cells were grown to late log phase, exposed to UV light and their DNA isolated at various times following irradiation. UV adducts remaining in the DNA were measured by an immunoassay using antibodies specific to CPDs or 6–4 PPs. Our results demonstrated that this double mutant was still proficient in the excision of both CPDs and 6–4 PPs (Fig. 1). These data contrast with similar experiments carried out in *S.cerevisiae*, where elimination of NER function alone is sufficient to prevent excision of UV photoproducts *in vivo* (12).

This result could be explained either by the existence of a third mechanism for the excision of UV photoproducts in *S.pombe* or by the *rad12-502* mutation being leaky. However, a third explanation was proven to be the case; UVDE activity is inducible in both wild-type and *rad12-502* mutant cells. Both UVDE endonuclease activity and UVDE-dependent repair are present at elevated levels in extracts prepared from cells that have

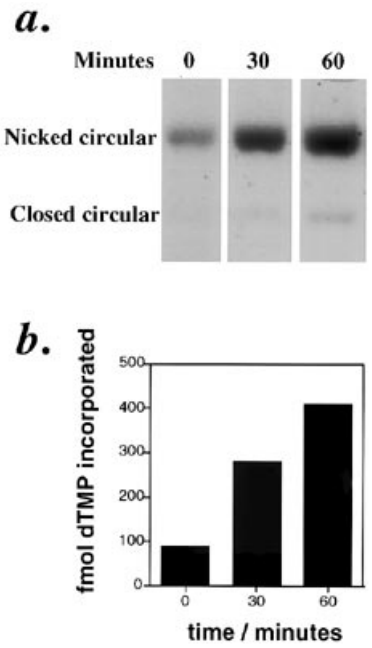

Figure 2. *In vitro* DNA repair is inducible by UV light. Wild-type *S.pombe* (972) was treated with UV light and harvested at the indicated times after irradiation. (**a**) *In vitro* DNA repair using a plasmid-based assay. (**b**) Quantitation of the amount of DNA repair synthesis in (a).

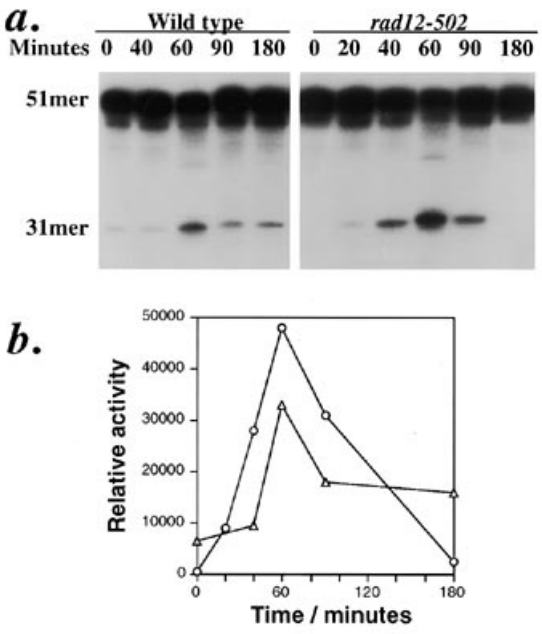

Figure 3. UVDE activity is inducible by UV light. *Schizosaccharomyces pombe* wild-type (972) or *rad12-502* (Sp272) strains were treated with UV light and harvested at the indicated times after irradiation. (**a**) UVDE assays of whole cell extracts show UVDE activity induction in both wild-type and *rad12-502* mutants, peaking 60 min after treatment. (**b**) Quantitation of the UVDE assays presented in (a) using a Fuji Image Analysis System. Strains: wild-type, Δ; *rad12-502*, ○.

been exposed to UV light 60–90 min prior to harvest. UV induction was done in the following manner. Cells were grown to late log phase, collected by centrifugation and resuspended in an equal volume of water. In suspension, the cells are largely shielded from the effects of UV light and of the total dose of 200 J/m^2 given, <50 J/m^2 was absorbed by the cells, as determined by 90% viability of wild-type cells. Following irradiation, cells were resuspended in fresh YEA and incubated at 30°C. Whole cell extracts were prepared at various times after irradiation and UVDE activity was assayed in these extracts. The extracts were then tested for elevated DNA repair activity using an *in vitro* DNA repair assay (Fig. 2). The peak of induction occurs ~60 min after UV exposure and represents a 5-fold induction of *in vitro* DNA repair activity.

We went on to show that this inducible activity is the UVDE-dependent repair pathway, using a specific assay to measure UVDE activity. UVDE activity is measured using a 51mer oligonucleotide containing a single internal UV photoproduct (either a CPD or a 6–4 PP), as previously described (11). Cleavage by UVDE converts a 3′-end-labeled 51mer to a labeled 31mer and an unlabeled 20mer. The products of the reaction are separated by denaturing polyacrylamide gel electrophoresis and the level of UVDE activity is determined by comparing the relative levels of 31mer and 51mer. When wild-type cells were exposed to UV light and harvested 60–90 min later, whole cell extracts exhibited 4- to 6-fold higher levels of UVDE activity compared with cells at 0 min following irradiation (Fig. 3). Cells isolated at 0 min following irradiation show the same levels of activity as unirradiated cells (data not shown). The induction of UVDE activity in extracts prepared from *rad12-502* mutants was much more striking, because of the low basal levels of UVDE activity in this background. However, the absolute level of UVDE activity in UV-induced *rad12-502* extracts is comparable with the levels seen in induced wild-type extracts (Fig. 3). We have shown previously that UVDE is limiting in our *in vitro* excision repair assay (11) and this result is consistent with that data and with UVDE activity being rate limiting in UV-induced extracts as well.

UVDE activity is constant during progression through the cell cycle

To show that induction of UVDE activity was DNA damage dependent and not due to different levels at specific points in the cell cycle, UVDE activity was measured as cells exited from a *cdc25-22*-dependent G_2 arrest. The *cdc25-22* mutant arrests the cell cycle at a point concomitant with the radiation-induced G_2 checkpoint (32,33). Cells were synchronized at the G_2/M transition by culturing at 36°C, the restrictive temperature for *cdc25-22*. Following release at 25°C, the permissive temperature for *cdc25-22*, there were no significant changes in UVDE activity as cells progressed through the cell cycle (Fig. 4). The time course presented includes two mitoses, with H1 kinase activity peaks at 20 and 140 min. These results indicate that the UVDE activity increase in response to UV light depends on UV-induced damage and not cell cycle phase changes after UV light exposure.

uve1$^+$ mRNA levels are increased following treatment with UV light

To determine if the induced levels of UVDE were due to increased transcription of the *uve1* gene, wild-type cells were grown to late log phase and irradiated with 400 J/m^2 254 nm UV light. Total RNA was isolated and poly(A)$^+$ mRNA selected.

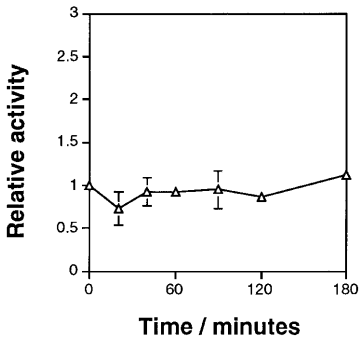

Figure 4. UVDE activity is constant across the cell cycle. Cells were synchronized at the G_2/M boundary by culture at the restrictive temperature for *cdc25-22*, 36°C. After 3 h at the restrictive temperature, cells were released to the permissive temperature of 25°C. Mitotic peaks of histone H1 kinase activity occur at 20 and 140 min after release from the *cdc25-22* block. UVDE assays were performed and quantified. Data were normalized to the UVDE activity level immediately prior to release from the *cdc25-22* block. There is no change in the UVDE activity levels as cells progress through this synchronous cell division. Data represent the average of three experiments and errors reflect the standard deviation of these data.

Preliminary studies had shown that the *uve1*$^+$ mRNA was not detected in total RNA by Northern blot analysis. Based on absorbance at 260 nm, very similar recoveries of mRNA for each sample were obtained. This was borne out by the results of probing with a *leu1* probe, which showed very similar levels of *leu1* mRNA in each sample. Because these cells were irradiated in suspension we wanted to check survival in order to determine how this dose related to that of cells irradiated on plates. Cells before and after UV irradiation were plated and counted for survival. In two separate experiments this dose of UV light gave 74 and 75% survival, which is an equivalent dose of plated cells of 80 J/m^2.

Poly(A)$^+$ mRNA (5 µg) was separated on 1.2% agarose–formaldehyde gels, blotted and hybridized to a fragment of *uve1*$^+$ generated by PCR. The blot was visualized and quantitated using a phosphorimager (Fig. 5). The UVDE mRNA band migrates at ~2.3 kb. A second slower migrating band is visible which we have determined to be cross-hybridization with rRNA. The data indicate that the mRNA levels elevate very quickly, increasing to 2.5-fold higher than unirradiated within 10 min of irradiation, then rapidly returning to normal levels. Induction was so rapid that cells collected by centrifugation and quick frozen immediately following irradiation showed significant induction (data not shown). For this reason induction was compared with unirradiated cells.

The UVDE promoter region has elements conserved in DNA damage-inducible genes

Since induction of *uve1* following DNA damage is transcriptionally regulated we compared its promoter sequence with that of the *S.pombe rhp51*, gene whose induction following DNA damage is also transcriptionally regulated. The promoter region of *uve1* (Fig. 6) appears to contain two DREs which share homology with regulatory sequences in *S.cerevisiae*. The two sequences, labeled DRE1 (CATGGCCTTC) and DRE2 (CTGGGAATGA), share reasonable homology with the DRE sequences of *rph51* and those of *S.cerevisiae* (C[T/G][T/A]GG[T/A]NT[T/C][A/C]). In addition,

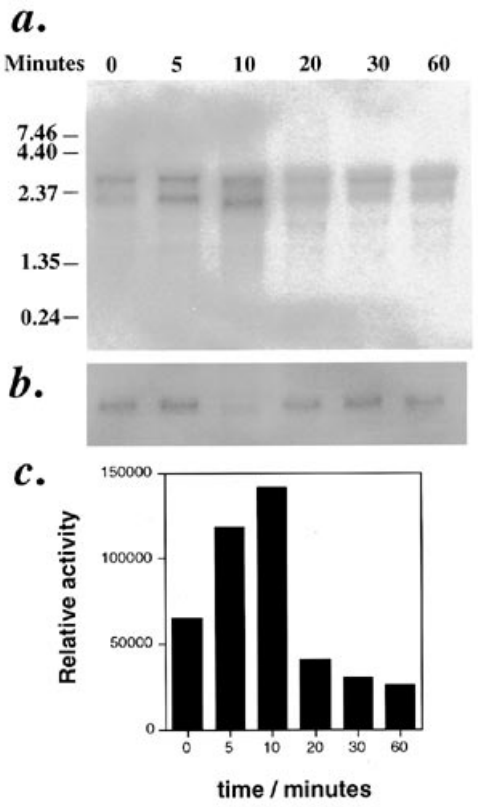

Figure 5. *uve1*$^+$ mRNA levels increase following UV irradiation. Wild-type *S.pombe* (972) was treated with UV light and harvested at the indicated times after irradiation. (**a**) Northern analysis of *uve1*$^+$ message level. (**b**) Control for loading using a PCR probe of the *leu1* gene. (**c**) Quantitation of the *uve1*$^+$ message level in (a).

a search of the sequence shows an exact 9 nt match with the c-Jun binding site (TGACGTAAC) at position –220.

DISCUSSION

Genetic data and *in vivo* studies had previously shown that *S.pombe* possesses an excision repair pathway independent of NER for the removal of UV photoproducts (12). Recent studies by our laboratories have shown that this second DNA excision repair pathway, which we have named UVDR, for UV damage repair, relies on the enzyme UVDE for the removal of both CPDs and 6–4 PPs (11). In that study we showed that extracts prepared from *rad12-502* cells were deficient in UVDE activity and that repair activity could be restored by adding back UVDE. This data demonstrated that UVDE is required in this reaction. The fact that partially purified UVDE and purified mus-18 protein, the *Neurospora crassa* homolog of UVDE, can recognize and cleave at CPDs and 6–4 PPs (8,10) suggests that this endonuclease alone is the damage recognition and repair initiating event in this process. In this study we have provided data demonstrating that UVDR is inducible and that the induction includes increased UVDE activity. While other unidentified proteins involved in this reaction may also be elevated in response to damage, our earlier studies indicated that UVDE was limiting in this reaction (11). Interestingly, while the *rad12* gene product is required for

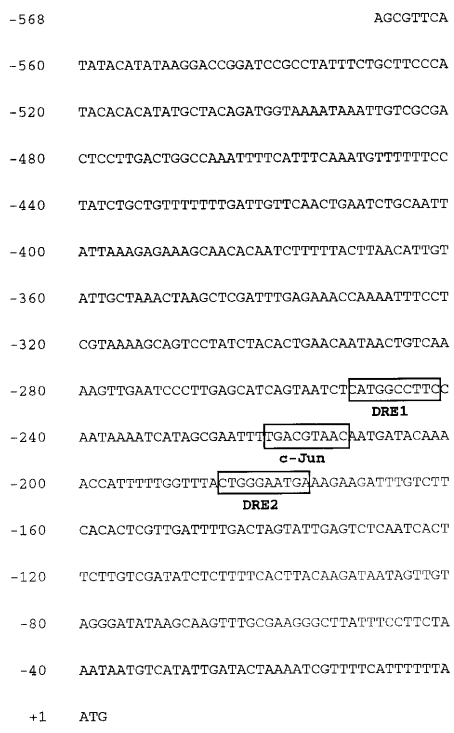

Figure 6. DNA sequence of the promoter region of *uve1*$^+$. The region covering the promoter of *uve1*$^+$, 568 nt upstream of the *uve1* translation start site, are shown (GenBank accession no. U78487). Putative DRE and c-Jun sequences are labeled and indicated by a box.

maintaining normal basal levels of UVDE, *rad12-502* cells induce normally, suggesting that induction of UVDE is independent of *rad12*. The mechanism of *rad12* regulation is currently under study. It is also unclear from these results why *rad12-502 rad13-A* double mutants are hypersensitive to UV damage, which we previously reported, while they have normal induction of UVDE activity. There are two possible explanations: the lack of basal levels of UVDE make the cells more sensitive or that the *rad12* gene product plays a broader regulatory role beyond UVDE regulation.

Increased levels of UVDE activity could be accounted for in a number of ways. A trivial explanation for increased UVDE activity could be that *uve1*$^+$ mRNA is synthesized only during the G_2 part of the cell cycle and that increased levels of UVDE following DNA damage is simply due to the fact that the cells are arrested in G_2. To test this possibility we studied UVDE levels through the cell cycle using a synchronized cell population. Cells containing a temperature-sensitive *cdc25* mutation were blocked in G_2 by incubation at the non-permissive temperature for 3 h followed by resumption of growth by shifting to the permissive temperature. UVDE activity was measured and shown to be at essentially constant levels throughout the cell cycle (Fig. 4). We next measured levels of the *uve1*$^+$ transcript following UV irradiation. Northern blot analysis demonstrated that UVDE mRNA levels increased (within 5 min) following exposure to UV light and that these elevated levels rapidly return to normal (within 20 min). It is interesting that while UVDE mRNA levels rise rapidly, peaking at 10 min, the levels of UVDE activity peak at 60 min. Whether this signals another level of regulation of UVDE activity or represents normal expression time is not clear from these studies. Future studies are planned to study post-transcriptional regulation of UVDE.

Cell-free extracts prepared from *S.pombe* cells following UV irradiation were previously reported to have elevated base excision repair activity (34). However, we believe that in fact they were measuring UVDR activity. Induction of DNA repair genes by transcriptional activation has been reported previously in *S.pombe*. Four transcripts were shown to be elevated following exposure to 254 nm UV light, named *uvi15*$^+$, *uvi18*$^+$, *uvi22*$^+$ and *uvi31*$^+$ (23). Two of these genes, *uvi18*$^+$ and *uvi31*$^+$, were induced only by UV light. It will be interesting to see if either of these genes are involved in UVDR. This same group has recently reported that *rhp51*$^+$, the fission yeast homolog of the *E.coli recA* and *S.cerevisiae RAD51* genes, is transcriptionally regulated (24). They further showed that the promoter region of *rhp51* contains sequences sharing homology with regulatory elements described in *S.cerevisiae*. Deletion analysis showed that a region containing two DREs, DRE1 and DRE2, was necessary for both basal levels of transcription and the inducible response. The *S.pombe* DRE sequences, which act as positive regulators of *rhp51*, share sequence homology with upstream repressor sequences, negative regulatory elements identified in *S.cerevisiae*. Promoter sequences in *rhp51* homologous with upstream activating sequences appear to be required for maintaining basal levels of transcription. Finally, deletion of a region very near the promoter led to loss of repression of *rhp51* expression, as transcription in these mutants is at the induced level. All of this suggests a relatively complex mechanism of regulation of this gene. Analysis of the sequences in the UVDE promoter (Fig. 6) shows the presence of DRE-like sequences. In addition there is homology with a number of other promoter elements, most notably the TGACGTAAC c-Jun binding sequence. The presence of a c-Jun binding site is interesting in the light of the fact that c-Jun, which is part of the AP-1 transcription complex, is activated in response to UV damage in mammalian cells and that AP-1 binding has been implicated in the regulation DNA damage-induced genes (35,36). As with *rhp51*, *uve1* transcriptional control would appear to be complex, with regulation of both its basal transcription levels and its induction. Ultimately, a detailed analysis of the *uve1*$^+$ promoter region will be required to determine all the elements involved in *uve1*$^+$ regulation.

ACKNOWLEDGEMENTS

S.D. is a Career Scientist of the Ontario Cancer Treatment and Research Foundation and this work was supported in part by OCTRF Start-up funds to S.D. G.A.F. was supported by NIH grant CA72647. D.L.M. was supported by NIH grant ES05914.

REFERENCES

1 Sancar,A. and Tang,M.S. (1993) *Photochem. Photobiol.*, **57**, 905–921.
2 Sancar,A. and Sancar,G.B. (1988) *Annu. Rev. Biochem.*, **57**, 29–67.
3 Friedberg,E.C. (1988) *Microbiol. Rev.*, **52**, 70–102.
4 Heelis,P.F., Kim,S.T., Okamura,T. and Sancar,A. (1993) *J. Photochem. Photobiol. B*, **17**, 219–228.
5 Todo,T., Ryo,H., Yamamoto,K., Toh,H., Inui,T., Ayaki,H., Nomura,T. and Ikenaga,M. (1996) *Science*, **272**, 109–112.
6 Jeggo,P.A., Taccioli,G.E. and Jackson,S.P. (1995) *Bioessays*, **17**, 949–957.
7 Keith,C.T. and Schreiber,S.L. (1995) *Science*, **270**, 50–51.
8 Bowman,K.K., Sidik,K., Smith,C.A., Taylor,J.S., Doetsch,P.W. and Freyer,G.A. (1994) *Nucleic Acids Res.*, **22**, 3026–3032.

9 Takao,M., Yonemasu,R., Yamamoto,K. and Yasui,A. (1996) *Nucleic Acids Res.*, **24**, 1267–1271.
10 Yajima,H., Takao,M., Yasuhira,S., Zhao,J.H., Ishii,C., Inoue,H. and Yasui,A. (1995) *EMBO J.*, **14**, 2393–2399.
11 Freyer,G.A., Davey,S., Ferrer,J.V., Martin,A.M., Beach,D. and Doetsch,P.W. (1995) *Mol. Cell. Biol.*, **15**, 4572–4577.
12 McCready,S., Carr,A.M. and Lehmann,A.R. (1993) *Mol. Microbiol.*, **10**, 885–890.
13 Lehmann,A.R., Walicka,M., Griffiths,D.J., Murray,J.M., Watts,F.Z., McCready,S. and Carr,A.M. (1995) *Mol. Cell. Biol.*, **15**, 7067–7080.
14 Sidik,K., Lieberman,H.B. and Freyer,G.A. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 12112–12116.
15 Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
16 Robinson,G.W., Nicolet,C.M., Kalainov,D. and Friedberg,E.C. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 1842–1846.
17 Perozzi,G. and Prakash,S. (1986) *Mol. Cell. Biol.*, **6**, 1497–1507.
18 Jones,J.S. and Prakash,L. (1991) *Nucleic Acids Res.*, **19**, 893–898.
19 Basile,G., Aker,M. and Mortimer,R.K. (1992) *Mol. Cell. Biol.*, **12**, 3235–3246.
20 Cole,G.M., Schild,D., Lovett,S.T. and Mortimer,R.K. (1987) *Mol. Cell. Biol.*, **7**, 1078–1084.
21 Elledge,S.J., Zhou,Z. and Allen,J.B. (1992) *Trends Biochem. Sci.*, **17**, 119–123.
22 Wolter,R., Siede,W. and Brendel,M. (1996) *Mol. Gen. Genet.*, **250**, 162–168.
23 Lee,J.K., Park,E.J., Chung,H.K., Hong,S.H., Joe,C.O. and Park,S.D. (1994) *Biochem. Biophys. Res. Commun.*, **202**, 1113–1119.
24 Jang,Y.K., Jin,Y.H., Shim,Y.S., Kim,M.J., Yoo,E.J., Choi,I.S., Lee,J.S., Seong,R.H., Hong,S.H. and Park,S.D. (1996) *Mol. Gen. Genet.*, **251**, 167–175.
25 Leupold,U. (1970) *Methods Cell Physiol.*, **4**, 169–177.
26 Nurse,P., Thuriaux,P. and Nasmyth,K. (1976) *Mol. Gen. Genet.*, **146**, 167–178.
27 Smith,C.A. and Taylor,J.S. (1993) *J. Biol. Chem.*, **268**, 11143–11151.
28 Mitchell,D.L. and Clarkson,J.M. (1981) *Biochim. Biophys. Acta*, **655**, 54–60.
29 Mitchell,D.L. (1996) In Pfeifer,G. (ed.), *Technologies for Detection of DNA Damage and Mutations*. Plenum Publishing Corp., New York, NY, pp. 73–85.
30 Ghosh,R., Johnston,D. and Mitchell,D.L. (1995) *Nucleic Acids Res.*, **23**, 2934–2937.
31 Rosen,K.M., Lamperti,E.D. and Villa-Komaroff,L. (1990) *BioTechniques*, **8**, 398–402.
32 Rowley,R., Subramani,S. and Young,P.G. (1992) *EMBO J.*, **11**, 1335–1342.
33 al-Khodairy,F. and Carr,A.M. (1992) *EMBO J.*, **11**, 1343–1350.
34 Jaeg,J.P., Bouayadi,K., Calsou,P. and Salles,B. (1994) *Biochem. Biophys. Res. Commun.*, **198**, 770–779.
35 Herrlich,P., Sachsenmaier,C., Radler-Pohl,A., Gebel,S., Blattner,C. and Rahmsdorf,H.J. (1994) *Adv. Enzyme Regulat.*, **34**, 381–395.
36 Devary,Y., Gottlieb,R.A., Lau,L.F. and Karin,M. (1991) *Mol. Cell. Biol.*, **11**, 2804–2811.