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# Lack of DNA enzymatic photoreactivation in HeLa cell-free extracts

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Using a DNA–protein binding assay, we have previously identified and characterized a UV-damaged DNA recognition protein (UVDRP) from HeLa cells [(1991) Nucleic Acids Res. 19, 6413–6418]. In this report, the photoreactivating activity of UVDRP from the yeast, *Saccharomyces cerevisiae*, and HeLa cells was investigated. Although yeast and human cells are evolutionarily different from each other, both UVDRPs were conserved in the sense of their biochemical characteristics except that the yeast UVDRP also exhibited an enzymatic photoreactivating activity. A mammalian expression vector plasmid DNA carrying the bacterial chloramphenicol acetyltransferase (CAT) gene was UV irradiated in vitro followed immediately by exposure to photoreactivating light, and its transient expression in repair-deficient xeroderma pigmentosum (XP) cells was investigated. More than 80% of the CAT activity was inhibited by UV irradiation, which was partially restored (>60%) by a partially purified yeast photolyase. In contrast, HeLa cell extracts did not express a photoreactivatable recovery from UV-induced inhibition of the CAT activity tested in the same system. This study has demonstrated the potential use of the DNA-mobility shift assay to investigate enzymatic photoreactivation, and has indicated the absence of the repair mechanism in human cells.

Damage-binding protein; DNA repair; Photoreactivation

## 1. INTRODUCTION

Ultraviolet (UV) radiation causes UV-DNA adducts, including cyclobutane-type pyrimidine dimers, (6-4)-photoproducts, as well as other minor DNA base adducts on cellular DNA. These UV-DNA adducts are normally repaired in mammalian cells through excision repair [1]. Since UV-type excision repair involves multiple steps, which require different enzymes and accessory proteins that presumably must have access to the damaged DNA for effective repair to occur [1,2], it is reasonable to think that some of these factors can be identified by a DNA-binding assay. Much of the knowledge about repair of damaged DNA in higher eukaryotic systems has been gained from bacterial and yeast studies. We and others have recently identified UV- and cisplatin damage-recognition proteins (DRP) in mammalian cells [3–5]. For some cases, nuclear proteins which recognize cisplatin- or UV-DNA adducts were over-expressed in resistant HeLa cells [6–7]. There is a rough correlation between the level of UVDRP and nucleotide excision repair [8]. Furthermore, it has been demonstrated that nuclear extracts isolated from some mammalian and yeast repair mutants fail to interact with damaged DNA [3,9,10]. These results strongly suggest the important role of cellular DRP in assisting in DNA repair. However, it is not clear whether there is evolutionary conservation of the UVDRP function in diverged species.

Studies in bacteria have shown proteins that interact with modified DNA (e.g. *E. coli* uvrAB) and target catalytic subunits to the lesion sites (e.g. uvrC) [1]. DRPs that might belong to this group have been identified and partially purified from mammalian cells and tissues. Examples include proteins that bind specifically to UV-damaged non-pyrimidine dimer-containing DNA [11–13], UV or *N*-acetoxy-*N*-acetyl-2-aminofluorene-damaged supercoiled DNA [14,15], or cisplatin-modified DNA [16,17]. A DRP, which can complement in vitro repair synthesis of extracts from xeroderma pigmentosum group A (XPA) cells, has been identified in calf thymus [18]. Recently, a photolyase-binding activity was also detected in yeast and *Drosophila* [9,19]. In this report, we identified a UVDRP from the budding yeast, *Saccharomyces cerevisiae*, which also exhibits a photolyase activity. The biochemical characteristics of the yeast UVDRP is similar to that of HeLa cells except for the photolyase activity. The results may provide an explanation for the loss of mammalian photoreactivation in cultured cells.

## 2. MATERIALS AND METHODS

### 2.1. *Cell lines*

Budding yeast BJ2168 (*MATa, prb1-1122, pep4-3, prc1-407, leu2, trp1, ura3-52, gal2*) (obtained from Dr. Soo-Chen Cheng, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan ROC) was cultured at 30°C in rich glucose-based YPD media (1% yeast extract, 2% Bacto-peptone, 2% glucose) as previously described [20]. HeLa S3 cells and xeroderma pigmentosum group A (XP) fibroblasts were main-

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*Abbreviations*: UVDRP, UV-damaged DNA recognition protein; PRL, photoreactivating light; UV, ultraviolet radiation; XP, xeroderma pigmentosum.

tained according to the supplier's specifications (American Type Culture Collection). Cells were grown in Dulbecco's modified Eagle's medium (Gibco, Gaithersburg, MD) containing 10% (v/v) fetal bovine serum, 100 μg/ml streptomycin and 100 U/ml penicillin, and incubated at 37°C in a humidified atmosphere of 5% (v/v) $CO_2$ in air.

2.2. *Cell extracts, DNA probes and DNA mobility shift assay*

Yeast cells that had grown to an $OD_{600}$ of 1.3–1.5 (~ $5 \times 10^9$ cells/ml) were used for preparation of cell extracts as previously described [20]. The cell pellet was resuspended in reducing buffer I (1 M sorbitol, 50 mM Tris-HCl, pH 7.5, 30 mM DTT) at a density of $1 \times 10^9$ cells/ml, and incubated at 30°C for 10 min. After being spun down, the pellet was resuspended in reducing buffer II (1 M sorbitol, 50 mM Tris-HCl, pH 7.5, 2 mM DTT) at a density of $1 \times 10^9$ cells/ml. Lyticase (Sigma) at 2,000 U/ml was added, and incubated with occasional swirling at 4°C for 30 min. Spheroplasts were monitored by phase-contrast microscopy, and collected by centrifugation at 2,200 rpm (4°C, 5 min), with two washes using 4 vols. of reducing buffer II to remove the lyticase. The final spheroplast pellet was resuspended in 50 mM Tris-HCl (pH 8.0), 2 mM DTT at $5 \times 10^9$ spheroplasts/ml. Lysis solution (1 mM EDTA, 0.2% Triton X-100, 0.2 M KCl) was added with gentle mixing. The lysate was centrifuged at 34,500 rpm (Beckman VTi65.1 rotor) at 4°C for 1 h. The crude nuclear and cytosolic extracts of yeast as well as HeLa cells were then prepared by the method of Dignam et al. [21]. The protein concentration was measured by a Bio-Rad kit using the Bradford method [22] and visualized by SDS-polyacrylamide gel electrophoresis [23]. Gel mobility shift assay was performed in a buffer containing 12% glycerol, 12 mM HEPES (pH 7.9), 100 mM KCl, 5 mM $MgCl_2$, 4 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 300 μg/ml BSA [24]. Unless otherwise specified, exactly 0.3 ng of the DNA probe was incubated with 4 μg of cell extracts, in 15 μl at 30°C for 30 min. The DNA probe for UVDRP binding and processing of the gels was prepared as described before [8]. The intensity of the shifted DNA bands was determined by the average of three scannings along the center and the two sides of each band on the X-ray film through a scanning densitometer (Hoefer GS 300).

2.3. *Heparin–agarose chromatography*

10 mg of crude nuclear extracts were loaded onto a 1-ml Affi-Gel heparin–agarose column (Bio-Rad Laboratories) equilibrated with low-salt phosphate buffer (0.01 M $KH_2PO_4$, 0.15 M NaCl, pH 7.4) at a flow rate of 12 ml/h as suggested by the supplier. Proteins were eluted by a FPLC (Pharmacia) at a flow rate of 2 ml/min from the column with a salt gradient from 0.1 to 2.15 M NaCl. Fractions were then dialysed against Tris buffer, and lyophilized. The fractions were stored at −70°C, or were used immediately.

2.4. *Photoreactivation assay*

Photoreactivation light (PRL) was adapted from the method previously described [25]. The UVDRP binding reaction or plasmid DNA was exposed to an ambient fluorescent lamp (Toshiba FL20) at a distance of 30 cm. The light was passed through 8-mm lead glass, which acted as a heat barrier and filter for removing wavelengths shorter than 350 nm. Under these conditions, the binding reaction or plasmid DNA remained at 25°C.

2.5. *DNA transfection and CAT assays*

20 μg of plasmid pRSVcat DNA was introduced into XP cells by the electroporation technique and analyzed for CAT activity as previously described [6]. Following 40 h incubation to allow transient expression of the CAT gene, the cell lysates were harvested and assayed for CAT enzymatic activity in vitro. The CAT assay reaction was incubated at 37°C for 1 h, followed by development on silica thin-layer chromatography (TLC) plates (Macherey-Nagel, Germany). After autoradiography, density on the X-ray film corresponding to the modified chloramphenicol was quantitated through a scanning densitometer (Hoefer GS300). CAT activity was calculated as % of chloramphenicol converted into acetylated derivatives. 20 μg of plasmid pSVβ (Clontech) was co-transfected with pRSVCAT, and its β-galactosidase activity was determined [26] as an internal control.

## 3. RESULTS

### 3.1. *Detection of yeast and human UVDRP by DNA-mobility shift assay*

UV damage-specific binding proteins were detected in yeast and HeLa nuclear extracts. Specificity of the yeast nuclear protein binding was analyzed by binding competition assay (Fig. 1). 4 μg of yeast (left panel) or 2 μg of HeLa (right panel) nuclear extracts was incubated in binding reactions containing 1-, 10- or 100-fold molar excess of the UV-irradiated competitor, f130-UV (Fig. 1A), or a 100-fold non-specific competitor (Fig. 1B): unirradiated f130 (lane 6), single-stranded f130-UV (lane 7) and single-stranded f130 (lane 8). Probe alone and binding reaction without competitor are shown in lanes 1 and 2, respectively. The binding of yeast and HeLa extracts was dramatically inhibited by 10-fold

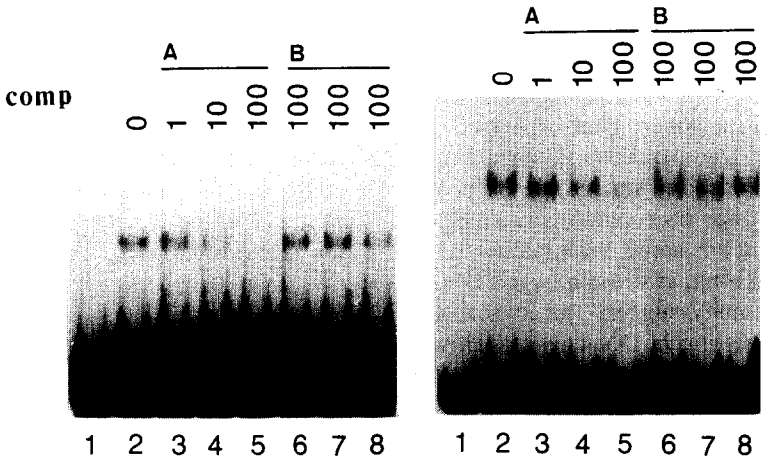


Fig. 1. Competition for the yeast (left panel) and HeLa (right panel) UVDRP binding activity by increasing amounts of competitors. 1-, 10- or 100-fold molar excess of competitors were used to compete against the binding of yeast (4 μg) or HeLa (2 μg) nuclear extracts. Competitors: specific f130-UV (A), non-specific (B): un-irradiated f130 (lane 6), single-stranded f130-UV (lane 7), and single-stranded f130 (lane 8). Controls without cell extract or without competitor are shown in lanes 1 and 2, respectively. Comp., competitor; arrowhead, bound probe; f, free probe.

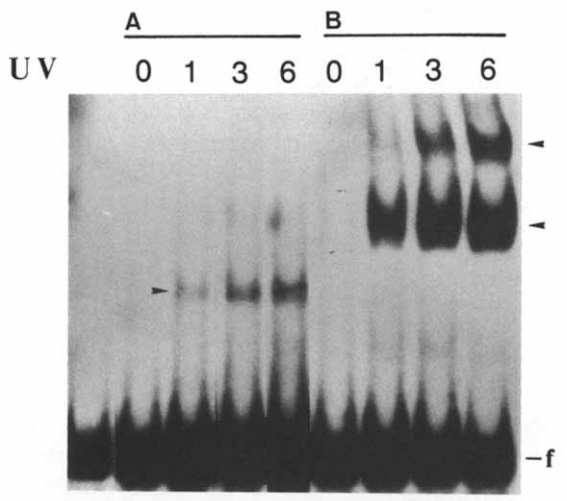


Fig. 2. UVDRP binding activities of yeast (A) and HeLa (B) nuclear extracts. 4 $\mu$g of nuclear extracts were incubated with 0, 1, 3, or 6 kJ/m$^2$ UV-irradiated f130. Control without cell extract is in the left lane. Symbols are as in Fig. 1.

specific f130-UV competitor and inhibited further by 100-fold. In contrast, 100-fold of non-specific competitors showed only a slight, if any, inhibition. The results indicate that UV-irradiated DNA is the target for yeast and HeLa UVDRP binding. Furthermore, both UVDRP binding activities were extremely sensitive to protease K, but were resistant to RNase (data not shown), suggesting that UVDRP is a protein or protein complex. To elucidate whether the UVDRP binding activity is dependent on the level of UV damage, f130 was irradiated with 0, 1, 3, or 6 kJ/m$^2$ UV and used as a probe for 4 $\mu$g of yeast (Fig. 2A) or HeLa (Fig. 2B) nuclear extract binding. Compared to the far left control lane (probe alone) of each panel, the binding activity (indicated with arrowheads) increased with the UV dose. It is interesting that at a high UVDRP binding, a second slower migrating band also appeared.

### 3.2. *Haparin–agarose (HA) chromatography of yeast and human UVDRP binding activities*

To analyze further the yeast and HeLa UVDRPs, both crude nuclear extracts were separated by HA chromatography (Fig. 3). The eluates were analyzed for UVDRP binding activity in the absence of EDTA. There are three yeast peak DNA binding activities eluted by 0.15–0.3 M, 0.35 M and 1.5 M NaCl (indicated with arrowheads, Fig. 3A). Competition analysis indicates that only the 0.35 M eluate of yeast nuclear extracts contains UVDRP binding activity, whereas the binding activities from 0.15 M and 1.5 M eluates were not specific for UV damage (data not shown). HeLa crude nuclear extracts were prepared under the same salt conditions. A nuclease activity was detected in HeLa extracts in association with UVDRP binding activity. Through HA chromatography of HeLa crude nuclear extracts, a peak UVDRP binding activity was also detected in the 0.35 M eluate (indicated with an arrowhead in Fig. 3B). The HeLa nuclease activity, reflected by the appearance of DNA degradation (indicated with an asterisk), was separated from the UVDRP binding activity by prolonged elution with 0.35 M salt. A non-specific binding activity was also detected in the 0.55 M eluate of HeLa nuclear extracts.

### 3.3. *Yeast UVDRP also exhibits photolyase activity*

Two recent studies suggest that yeast [9] and *Drosophila* [19] photolyase constitute the majority of UVDRP binding activities. Here, we show that the yeast UVDRP binding activity can be reduced by exposure immediately after the binding reaction to PRL (Fig. 4). The binding reactions using crude nuclear extracts from yeast (Fig. 4A) or HeLa (Fig. 4B) were treated with PRL for 0 (lanes 3 and 6), 20 (lanes 4 and 7), or 60 min (lanes 5 and 8). The yeast UVDRP binding activity (indicated with arrowheads pointing towards the right) decreased with the PRL exposure. Approximately 60% of UVDRP binding activity was inhibited by 60 min of PRL exposure. In contrast, HeLa UVDRP binding ac-

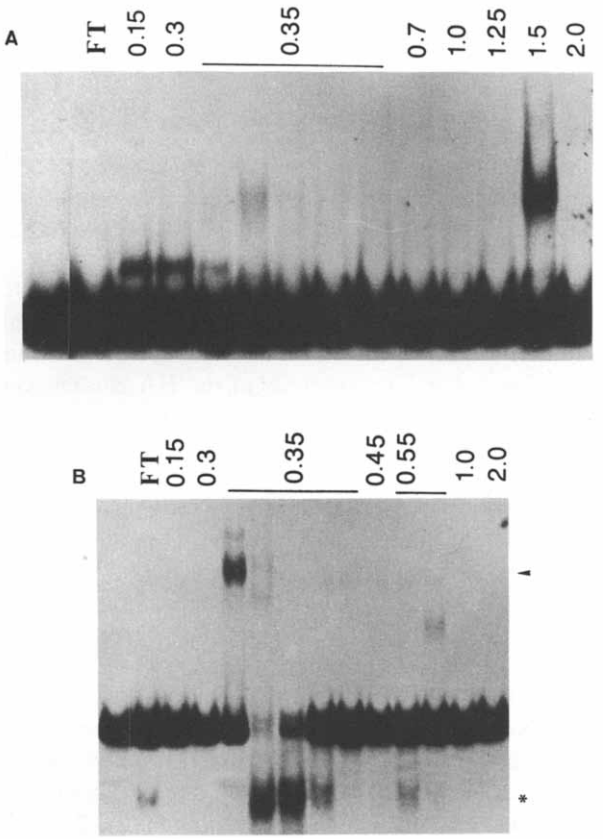


Fig. 3. UVDRP binding activities of 0.15–2 M NaCl-eluted yeast (A) and HeLa (B) HA nuclear extracts. Exactly 2 $\mu$l of HA eluates from 5 mg of nuclear extracts by indicated NaCl concentrations (M) were incubated with the f130-UV probe. The 0.35 M and 0.55 M eluates include more than one fraction with 8 ml each by extended elution at 2 ml/min. Control without cell extract is in the left lane. Nuclease-degraded DNA fragments are indicated with a asterisk. FT, flow through. Other symbols are as in Fig. 1.

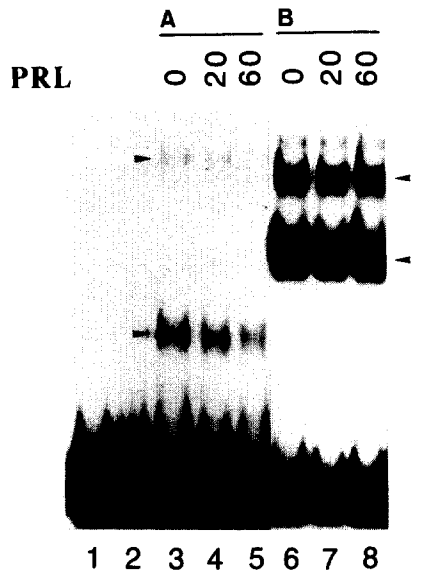


Fig. 4. Photoreactivation of UVDRP binding activity by crude nuclear extracts. The UVDRP binding activities of crude nuclear extracts (4 μg) from yeast (A) or HeLa cells (B) were detected by exposure immediately after the binding reaction to photoreactivating light (PRL) for 0, 20 or 60 min. Probe alone and 60 min PRL-exposed probe are shown in lanes 1 and 2, respectively. Symbols are as in Fig. 1.

tivity (indicated with arrowheads pointing towards the left) was not affected by the same PRL treatment. Probe alone (lane 1) or probe with 60 min PRL alone (lane 2) were conducted in parallel as controls. The results indicate that yeast UVDRP is associated with a photoreactivating activity.

Partially purified HA fractions were also assayed for photoreactivating activity (Fig. 5). Since there were only limited amounts of the HA eluates, exact quantitation was not possible. The amounts of the HA eluates were adjusted according to the DNA binding activity. Equal aliquots of cell extracts eluated by 0.35 M NaCl (HA0.35) or 1.5 M NaCl (HA1.5) (see Fig. 3) were mixed with DNA probe as for the standard binding reaction for 30 min, and then incubated in the dark for an additional 0 min (dk0) or 60 min (dk60) (lanes 2,6, and lanes 3,7) or in PRL for 20 min (pr20) and 60 min (pr60) (lanes 4,8, and lanes 5,9). Lane 1 shows probe alone. The reaction was conducted at 25°C (Fig. 5A) or at 4°C (Fig. 5B). The probe bound by 0.35 M and 1.5 M NaCl eluates was indicated by b0.35 and b1.5, respectively. For both HA fractions, further incubation of the binding reaction at 25°C in the dark caused a further increase of the binding (compare lanes 2 with 3, and lanes 6 with 7 in Fig. 5A). In the presence of PRL, 30–50% of the binding by HA0.35 was inhibited within 20 min (lane 4). 60 min PRL resulted in a similar extent of inhibition of the binding (compare lane 5 with lane 3). In contrast, more than 90% of the HA1.5 binding was abolished by PRL within 20 min (lane 8), and remained the same or even a greater inhibition for 60 min (compare lane 9 with lane 7). However, when the same experiments were conducted in parallel at 4°C, the photoreactivation was not detected (Fig. 5B). The data indicate that both HA eluates contain enzymatic photoreacting activity. It should be noted that only the 0.35 M, but not the 1.5 M eluate, showed UVDRP characteristics, i.e. a binding activity specific for UV-damaged DNA according to binding competition assays (see Table I). Thus, HA partial purification by 0.35 M NaCl generated a photoreactivable UVDRP.

### 3.4. *Photoreactivation in vitro increases cellular reactivation of damaged plasmid DNA*

Although biochemical analysis in vitro suggested that partially purified HA0.35 fraction contains enzymatic photoreactivating activity, it is not clear whether the finding is reproduced in vivo. This was tested by an adapted CAT assay system (see section 2 for details). 20 μg of pRSVcat plasmid DNA was irradiated with 250 J/m$^2$ UV with or without photoreactivation treat-

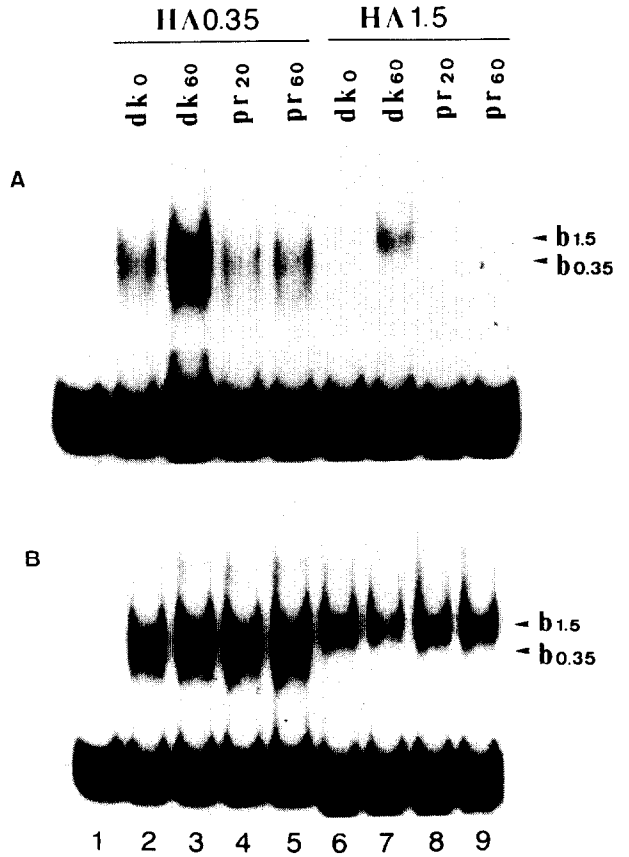


Fig. 5. Photoreactivation of UVDRP binding activity by partially purified yeast nuclear extracts. The UVDRP binding activities of HA 0.35 M eluate (HA0.35) or 1.5 M eluate (HA1.5) were detected at 25°C (A) or at 4°C (B). The binding reaction was continued for 30 min and further incubated in the dark for 0 min (lanes 2 and 6) or 60 min (lanes 3 and 7), or in the presence of PRL for 20 min (lanes 4 and 8) or 60 min (lanes 5 and 9). Probe alone is shown in lanes 1. dk0 and dk60, incubation in dark for 0 and 60 min, respectively; pr20 and pr60, incubation in PRL for 20 and 60 min, respectively. b0.35, bound probe by HA0.35; b1.5, bound probe by HA1.5.

ment, and transient expression in XP cells allowed (Fig. 6). CAT activity expressed in XP cells from un-irradiated plasmid was set as 100% (lane 1). More than 80% CAT activity was inhibited by UV irradiation (lane 2). UV- irradiated plasmids followed by PRL alone or additionally including HeLa HA0.35 extracts did not alter much of the inhibition of the CAT activity (lanes 3 and 4, respectively). However, when yeast HA0.35 was used, more than 60% CAT activity was restored (lane 5). Since the expression of CAT activity depends on the intactness of the plasmid, restoration of UV-inhibited CAT activity by yeast extract-dependent photoreactivation indicates a repair mechanism which is demonstrable in a cell-free system. In contrast, this biochemical characteristic was not detected in HeLa extracts.

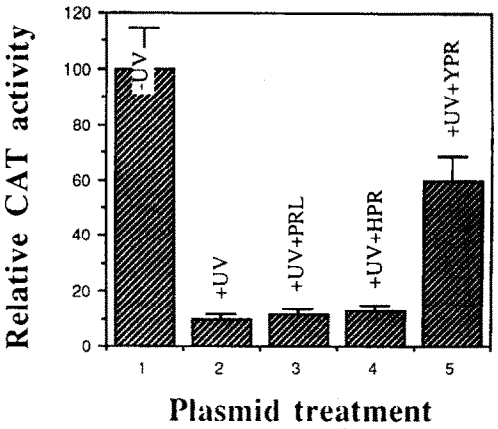


Fig. 6. Relative CAT activity expressed from transfected pRSVcat in XP cells. 20 $\mu$g of pRSVcat was transiently expressed in XP cells for 40 h and assayed for CAT activity in vitro. Being normalized to the internal control $\beta$-galactosidase activity (see section 2 for details), relative CAT activity was calculated by setting un-irradiated plasmid as 100%. Lanes 1, (−UV), un-irradiated pRSVcat; 2 (+UV), 250 J/m$^2$ UV-irradiated pRSVcat; 3 (+ UV + PRL), UV-irradiated pRSVcat followed by 60 min PRL; 4 (+ UV + HPR), UV-irradiated pRSVcat followed by 60 min PRL in HeLa HA0.35 extracts; 5 (+ UV + YPR), UV-irradiated pRSVcat followed by 60 min PRL in yeast HA0.35 extracts. The standard deviation bars are also indicated ($n$ = 3).

## 4. DISCUSSION

In this study we have identified yeast nuclear proteins that preferentially bind to UV-damaged DNA. Biochemical characteristics of the yeast and HeLa UVDRP are essentially the same (Table I). The amount of UVDRP from yeast and HeLa crude nuclear extracts is 0.55 and 0.077 molecules per megabase of genomic DNA, respectively. The human UVDRP binding increased by at least 30-fold using nuclear extracts partially purified from the HA column (data not shown), which resulted in an estimated value of more than 2.2 molecules per megabase reported by Patterson and Chu [9]. The yeast UVDRP from crude nuclear extracts is about 0.55 molecules per megabase of genomic DNA. The peak UVDRP binding from HA eluate increased 2- to 3-fold (data not shown), i.e. 1.1–1.65 molecules per megabase. This value is similar to the 1.8 molecules per megabase estimated by others [9]. Therefore, the yeast and human cells have similar UVDRP quantities with respect to genome size. In addition, a protein complex, which consists of more than one polypeptide, has been identified from calf thymus that can complement in vitro the XPA defect in repair synthesis and bind to damaged DNA [18]. It has also been shown that mammalian UVDRP consists of multiple forms of polypeptides [27]. Further, DRP for cisplatin-induced DNA damage is composed of multiple polypeptides, including high-mobility group (HMG) proteins or HMG box-containing proteins [17]. It is likely to be the case in UVDRP since the denaturing protein gel electrophoresis of the UVDRP complex isolated from the gel shift assay indicates proteins with $M_r \sim 25$ kDa (C.C.-K.C., unpublished data), which is comparable to the HMG proteins. Therefore, there is not only conservation of biochemical characteristics between yeast and human UVDRPs, but possibly also between UVDRP and cisplatin-specific DRP.

Table I

Comparison of the UVDRP binding activities from yeast and HeLa cells

| | Yeast | HeLa |
|---|---|---|
| Major location | nucleus | nucleus |
| Protein conformation modifier resistance | yes | yes |
| Abundance[a] (molecules per megabase) | 0.55 | 0.077 |
| Heparin salt elution[b] | 0.35 M | 0.35 M |
| Photoreactivation activity | yes[c] | no |

[a] Estimated from three determinations using 6,000 J/m$^2$ UV-irradiated f130 probe and 4 $\mu$g of nuclear extracts. The variations are 15–20%.
[b] The NaCl concentration at which the peak binding activity was eluted.
[c] Greater than 60% binding released by 60 min of PRL exposure.

Unexpectedly, the yeast nuclear extracts also displayed photoreactivating activity, which was absent in HeLa extracts. Although the yeast 0.35 M and 1.5 M eluates showed photoreactivating activity, only the 0.35 M eluate was associated with the UVDRP binding. It is not clear whether HA0.35 is structurally related to HA1.5. If so, the HA0.35 binding activity is probably part of the HA1.5 because, according to the DNA mobility shift assay, the 1.5 M eluate contains a binding activity which migrates slightly slower than that of the 0.35 M eluate. In addition, the HA1.5 binding activity is preferential for un-damaged DNA. It may be that the damage-specific domain of the UVDRP presented in HA0.35 is shielded by a factor, resulting in a protein complex that appears in the HA1.5 eluate. Enzymatic photoreactivation is present in a wide variety of organisms [1]. However, the absence of photolyase in placen-

tal mammals, including humans, is puzzling from an evolutionary point of view. We have found that UVDRP binding can be reduced by exposure of binding reaction to a photoreactivating light in the majority of the yeast extracts, but not in HeLa extracts. This is consistent with the finding that the UVDRP binding activity disappeared in a *phr* mutant yeast [9]. Since HeLa extract exhibits only UVDRP binding, the absence of photolyase activity in placental mammals may be elucidated by UVDRP binding assay. There is evidence indicating that a majority of the UVDRP from a variety of cells, including HeLa, interact with (6-4)-photoproduct, a major UV adduct besides pyrimidine dimers [19]. Since (6-4)-photoproducts are important for the cellular response to UV damage [28,29], the UVDRP binding activity may be biologically relevant. Recently, a new *Drosophila* photolyase has been purified, which interacts mainly with the (6-4)-photoproduct [19]. These findings raise an immediate question as to whether yeast photolyase also binds to (6-4)-photoproducts. In any case, this study did not prove the yeast UVDRP to be photoreactivable, rather it could be that two activities are partially co-purified. However, it is certain that the in vitro photoreactivation of UVDRP detected in yeast is absent in HeLa cells. The physiological significance of this biochemical charactistic is supported by the host cell reactivation of damaged plasmid DNA (Fig. 6). The level of UVDRP binding activity is inversely proportional to the extent of CAT activity (with ~ 20% variation) in our assay system. Since XP cells are deficient in excision repair and lack photolyase [1], variations detected in this study should essentially be caused by the transfection of the plasmid DNA and the in vitro CAT assay. Recently, Sancar and co-workers [30] have also found evidence for lack of DNA enzymatic photoreactivation in human cells. In conclusion, this study, together with others, strongly suggests a lack of enzymatic photoreactivation in human cells, and the potential use of DNA-mobility shift assay to study this repair mechanism.

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## REFERENCES

[1] Friedberg, E.C. (1985) DNA repair, Freeman, New York.
[2] Hanawalt, P.C. (1986) in: Mechanisms of DNA Damage and Repair (Grossman, L. and Upton, A.C. eds.) pp. 489–498, Plenum, New York.
[3] Chu, G. and Chang, E. (1988) Science 242, 564–567.
[4] Hirschfeld, S., Levine, A.S., Ozato, K. and Protic, M. (1990) Mol. Cell. Biol. 10, 2041–2048.
[5] Chao, C.C.-K., Huang, S.-L., Lee, L.-Y. and Lin-Chao, S. (1991) Biochem. J. 277, 875–878.
[6] Chao, C.C.-K., Huang, S.-L., Huang, H. and Lin-Chao, S. (1991) Mol. Cell. Biol. 11, 2075–2080.
[7] Chu, G. and Chang, E. (1990) Proc. Natl. Acad. Sci. USA 87, 3324–3327.
[8] Chao, C.C.-K. (1992) Biochem. J. 282, 203–207.
[9] Patterson, M. and Chu, G. (1989) Mol. Cell. Biol. 9, 5105–5112.
[10] Kataoka, H. and Fujiwara, Y. (1991) Biochem. Biophys. Res. Commun. 175, 1139–1143.
[11] Feldberg, R.S. and Grossman, L. (1976) Biochemistry 15, 2402–2408.
[12] Feldberg, R.S. (1980) Nucleic Acids Res. 8, 1133–1143.
[13] Feldberg, R.S., Lucas, J.L. and Dannenberg, A. (1982) J. Biol. Chem. 257, 6394–6401.
[14] Tsang, S.S. and Kuhnlein, U. (1982) Biochim. Biophys. Acta 697, 202–212.
[15] Abramic, M., Levine, A.S. and Protic, M. (1991) J. Biol. Chem. 266, 22493–22500.
[16] Donahue, B.A., Augot, M., Bellon, S.F., Treiber, D.K., Toney, J.H., Lippard, S.J. and Essigmann, J.M. (1990) Biochemistry 29, 5872–5880.
[17] Hughes, E.N., Engelsberg, B.N. and Billings, P.C. (1992) J. Biol. Chem. 267, 13520–13527.
[18] Robins, P., Jones, C.J., Biggerstaff, M., Lindahl, T. and Wood, R.D. (1991) EMBO J. 10, 3913–3921.
[19] Todo, T., Takemori, H., Ryo, H., Ihara, M., Matsunaga, T., Nikaido, O., Sato, K. and Nomura, T. (1993) Nature 361, 371–374.
[20] Jazninski, S.M. (1990) Methods Enzymol. 182, 154–174.
[21] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475–1489.
[22] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[23] Laemmli, U.K. (1970) Nature 227, 680–685.
[24] Carthew, R.W., Chodosh, L. and Sharp, P. (1985) Cell 43, 439–448.
[25] Chao, C.C.-K., Rosenstein, R.B. and Rosenstein, B.S. (1985) Mutat. Res. 149, 443–450.
[26] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[27] Abramic, M., Levine, A.S. and Protic, M. (1991) J. Biol. Chem. 266, 22493–22500.
[28] Brash, D.E. (1988) Photochem. Photobiol. 48, 59–66.
[29] Mitchell, D.L. and Nairn, R.S. (1989) Photochem. Photobiol. 49, 805–819.
[30] Li, Y.F., Kim, S.-T. and Sancar, A. (1993) Proc. Natl. Acad. Sci. USA 90, 4389–4393.