The Journal of Microbiology (2012) Vol. 50, No. 1, pp. 112–118
DOI 10.1007/s12275-012-1597-4

Saccharomyces cerevisiae Cmr1 Protein Preferentially Binds to UV-Damaged DNA *In Vitro*

Do-Hee Choi, Sung-Hun Kwon, Joon-Ho Kim, and Sung-Ho Bae*

Department of Biological Sciences, College of Natural Science, Inha University, Incheon 402-751, Republic of Korea

(Received November 12, 2011 / Accepted December 20, 2011)

DNA metabolic processes such as DNA replication, recombination, and repair are fundamentally important for the maintenance of genome integrity and cell viability. Although a large number of proteins involved in these pathways have been extensively studied, many proteins still remain to be identified. In this study, we isolated DNA-binding proteins from *Saccharomyces cerevisiae* using DNA-cellulose columns. By analyzing the proteins using mass spectrometry, an uncharacterized protein, Cmr1/YDL156W, was identified. Cmr1 showed sequence homology to human Damaged-DNA binding protein 2 in its C-terminal WD40 repeats. Consistent with this finding, the purified recombinant Cmr1 protein was found to be intrinsically associated with DNA-binding activity and exhibited higher affinity to UV-damaged DNA substrates. Chromatin isolation experiments revealed that Cmr1 localized in both the chromatin and supernatant fractions, and the level of Cmr1 in the chromatin fraction increased when yeast cells were irradiated with UV. These results suggest that Cmr1 may be involved in DNA-damage responses in yeast.

Keywords: Cmr1, DNA-binding protein, damaged DNA binding, WD40 repeats, UV-irradiation, *Saccharomyces cerevisiae*

Introduction

DNA-binding proteins play important roles in DNA metabolism such as DNA replication, recombination, and repair. For example, many DNA-binding proteins involved in recognition of DNA lesions are required for proper function of nucleotide excision repair (NER) (Prakash and Prakash, 2000). NER is the most important repair system in the removal of a large variety of DNA lesions. The xeroderma pigmentosum C (XPC) protein in human and its yeast ortholog Rad4 protein initially bind to a site that has a DNA helix distortion, and thus play a central role in initiating global-genomic NER (GG-NER) by recruiting downstream factors (Sugasawa *et al.*, 2001).

Damaged-DNA binding protein 2 (DDB2), which is encoded by the XPE gene in humans, is another example of a DNA-binding protein that functions in GG-NER. DDB2 forms a heterodimeric complex with DDB1 (UV-DDB) and specifically recognizes the UV-induced DNA lesion (Tang and Chu, 2002; Wittschieben *et al.*, 2005). Extensive biochemical studies have suggested that UV-DDB cooperates with XPC to initiate GG-NER. UV-DDB is also part of a cullin family ubiquitin ligase. It associates with cullin4 (CUL4) and forms the CUL4-DDB1 ubiquitin E3 ligase complex *in vivo* (Groisman *et al.*, 2003). This finding may hold some clues to the role of UV-DDB in GG-NER. Biochemical studies have revealed that DDB2 itself, XPC, and histone are directly ubiquitylated by this E3 ligase complex upon UV-irradiation. Polyubiquitylation was shown to alter the DNA binding properties of UV-DDB and XPC, which may result in transfer of the UV-induced lesion from UV-DDB to XPC (Matsuda *et al.*, 2005; Sugasawa *et al.*, 2005). The UV-induced histone ubiquitylation appeared to affect nucleosome stability, which may facilitate the recruitment of repair proteins to damaged DNA (Wang *et al.*, 2006).

CMR1 (changed mutation rate) is a reserved gene name in the *Saccharomyces* Genome Database (Offman, personal communication), whose systematic gene name is YDL156W. Its product, Cmr1, is an uncharacterized protein of unknown function that contains WD40 repeats in its C-terminal region. It is a 60 kDa protein consisting of 522 amino acid residues, and shows sequence homology to human DDB2 (see Fig. 3). Genome-wide scale analyses revealed that it is localized to both the cytoplasm and nucleus (Huh *et al.*, 2003), and deletion of the YDL156W gene was shown to enhance resistance to tirapazamine, an anticancer drug that targets topoisomerase II (Hellauer *et al.*, 2005). Sequence analysis of the YDL156W promoter region predicted the presence of a potential regulatory target of Mbp1 (Brohée *et al.*, 2011). Mbp1 is a sequence-specific transcription factor that regulates gene expression during the G1/S transition of the cell cycle, and many of its target genes are involved in DNA synthesis and DNA repair (Bean *et al.*, 2005).

In this study, we isolated Cmr1 protein from the crude extract of *Saccharomyces cerevisiae* using DNA-cellulose columns. Here we show that the purified Cmr1 protein preferentially binds to UV-damaged DNA *in vitro*. We also provide evidence suggesting that Cmr1 localizes in the chromatin fraction and its levels in this fraction increase in response to UV-irradiation.

*For correspondence. E-mail: sbae@inha.ac.kr; Tel.: +82-32-860-7712; Fax: +82-32-874-6737

Materials and Methods

Oligonucleotides and DNA substrate preparation

All oligonucleotides utilized for the construction of DNA substrates were commercially synthesized (Bioneer, Korea) and gel-purified prior to use. The sequences of the oligonucleotides were derived from either ΦX174 virion DNA or the yeast *RNR3* gene and listed in Table 1. The oligonucleotides were labeled by the incorporation of [γ-^{32}P]ATP (GE Healthcare, USA) using T4 polynucleotide kinase, and the substrates were prepared as described previously (Lee *et al*., 2010). To prepare the UV-damaged DNA substrate, the labeled DNA substrate was irradiated with UV light (245 nm) on ice before use.

DNA binding and gel mobility shift assays

Standard assays to measure DNA-binding activity were conducted in a reaction mixture (20 μl) containing 50 mM Tris-HCl (pH 7.8), 0.5 mM DTT, 0.5 mg/ml bovine serum albumin, 0.02% NP-40, 5 fmol of 5'-^{32}P-labeled DNA substrate. After 5 min of incubation at 37°C, glycerol and bromophenol blue were added at concentrations of 5% and 0.05%, respectively, and the products were separated on 6% polyacrylamide gel in 0.5× TBE. The gels were dried on DEAE-cellulose paper and subjected to autoradiography. The quantity of complexes formed was then determined using a PhosphorImager (Fuji).

Isolation of DNA-binding proteins from *S. cerevisiae*

S. cerevisiae strain BJ2168 (MAT*a*, *pep4-3*, *prb1-1122*, *pre1-451*, *ura3-52*, *leu2*, *trp1*) was grown (4 L) at 30°C, and the crude extracts (19 mg/ml, 70 ml) were prepared in buffer T (25 mM Tris-HCl; pH 7.5, 10% glycerol, 1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 0.15 μg/ml leupeptin, and pepstatin A) containing 250 mM NaCl. After 1 h of centrifugation at 45,000 rpm in a Beckman 70 Ti rotor, the supernatant was loaded onto an single-stranded (ss) or double-stranded (ds) DNA-cellulose (Sigma-Aldrich, USA) column (0.79 cm^2× 2.5 cm, 2 ml) equilibrated with the same buffer. The column was then washed with 40 ml of buffer T_{300} [hereafter, the number indicates the concentration of NaCl (mM) in buffer T], and subsequently eluted with buffer T_{1000}. The peak fractions were pooled and analyzed by mass spectrometry (Genomine, Korea).

Cloning and purification of Cmr1

The open reading frame of the *CMR1* gene from *S. cerevisiae* was amplified by PCR and cloned into the *Bam*HI-*Xho*I sites of pFastBacHTb plasmid (Invitrogen, USA). A recombinant baculovirus was constructed to produce the Cmr1 protein with an N-terminal His tag fusion as recommended by the manufacturer (Invitrogen). Sf9 insect cells infected with the recombinant baculovirus (1×10^6 cells/ml, 500 ml) were harvested, resuspended in 50 ml of buffer T_{50}, and disrupted by sonication (five cycles of a 30 sec pulse and 1 min cooling interval). The extract was cleared by centrifugation at 45,000 rpm for 1 h, and the supernatant was directly applied to a Q-Sepharose (GE Healthcare) column (1.77 cm^2×5.6 cm, 10 ml) equilibrated with buffer T_{50}. The column was washed with 10 volumes of the same buffer containing neither EDTA nor DTT, and eluted with buffer T_{500} (-EDTA, -DTT) plus 5 mM imidazole. The peak protein was pooled and loaded onto a Ni^{2+}-NTA-agarose (QIAGEN, USA) column (1.77 cm^2×2.5 cm, 4.5 ml) equilibrated with buffer T_{500} (-EDTA, -DTT) plus 5 mM imidazole. The column was consecutively washed with 5 column volumes of buffer T_{500} (-EDTA, -DTT) plus 50 and 200 mM imidazole, respectively. The column was then eluted with 500 mM imidazole in the same buffer. The peak fractions containing Cmr1 were pooled, concentrated, and then loaded onto a glycerol gradient (5 ml, 15–35% glycerol in buffer T_{500}). The gradient was subjected to centrifugation for 24 h at 45,000 rpm in a Beckman SW55 Ti rotor. Fractions (200 μl) were collected from the bottom of the gradient and assayed for DNA-binding activity.

Chromatin purification

To examine chromatin binding of Cmr1, yeast strain YPH499 (Choi *et al*., 2008) was grown in YPD media and extracts were prepared from zymolase-treated spheroplasts as described previously (Donovan *et al*., 1997). After spheroplasting, cells were washed three times with lysis buffer (0.4 M sorbitol, 150 mM potassium acetate, 2 mM magnesium acetate, 20 mM Hepes-KOH; pH 6.8, 0.1 mM PMSF, 0.15 μg/ml leupeptin, and pepstatin A). Cells were resuspended in lysis buffer at 10^9 cells/ml and lysed by the addition of Triton X-100 to a final concentration of 1%, followed by centrifugation at 15,000 rpm for 15 min. For DNase I treatment, extracts were incubated with 200 units of DNase I (TaKaRa, Japan) for 2 h at 16°C before centrifugation. After the supernatant was carefully removed, the chromatin-enriched fraction (i.e. pellet) was resuspended with the same volume of lysis buffer. Aliquots (15 μl) of whole cell extract, supernatant, and chromatin fraction were subjected to SDS-PAGE, followed by Western blot analysis of Cmr1 protein using

Table 1. Oligonucleotides used for substrate preparation

No.	Nucleotide sequences (length in nt)
1	5'-CGG ACG CTC GAC GCC ATT AAT AAT GTT TTC-3' (30)
2	5'-GAA AAC ATT ATT AAT GGC GTC GAG CGT CCG-3' (30)
3	5'-GGA AAA CAT TAT TAA TGG CGT CGA GCT AGG CAC AAG GCG AAC TGC TAA CG-3' (50)
4	5'-CGT TAG CAG TTC GCC TTG TGC CTA GCT CGA CGC CAT TAA TAA TGT TTT CC-3' (50)
5	5'-GCC TCC GCT GCT ATT CAA TTT ACC ATT GAT CAA GAG GTT GCC GAT CAA GCC GCT ACA CAT ATT GCT TCC GTC TCA GAA TTG GAT CGT CCA GTT TAT GTT CCA AAG GGT ACA AAA TTC TCT GAA CAA AAG GCG GCA TCT G-3' (139)
6	5'-CAG ATG CCG CCT TTT GTT CAG AGA ATT TTG TAC CCT TTG GAA CAT AAA CTG GAC GAT CCA ATT CTG AGA CGG AAG CAA TAT GTG TAG CGG CTT GAT CGG CAA CCT CTT GAT CAA TGG TAA ATT GAA TAG CAG CGG AGG C-3' (139)

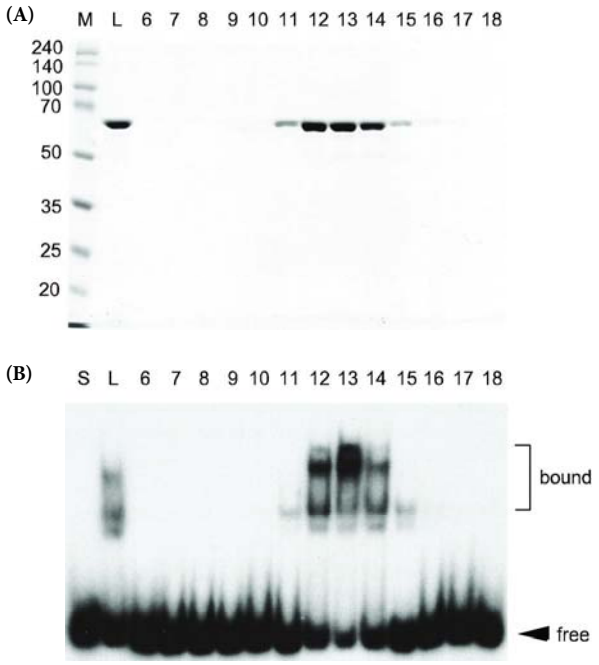

Fig. 1. Co-migration of DNA-binding activity with the purified Cmr1. Glycerol gradient fractions (15 μl) of Cmr1 were subjected to SDS-PAGE (10%), followed by Coomassie staining (A). DNA binding reactions were performed by incubation of each fraction (0.1 μl) with 5 fmol of ^{32}P-labeled dsDNA substrate constructed with oligonucleotides 3 and 4, and the resulting autoradiogram is shown (B). The load (L) and the fractions analyzed are indicated. M denotes molecular size markers. S denotes substrate alone control.

anti-Cmr1 antibody. To produce anti-Cmr1 antibody, full-length Cmr1 protein expressed in bacteria with pET28a vector was purified under a denaturing condition (8 M urea) using a Ni^{2+}-NTA-agarose (QIAGEN) column. The antibody was produced in rabbits and affinity purified.

Results

Identification of Cmr1

To identify novel DNA-binding proteins, we isolated proteins from the crude extract of *S. cerevisiae* that bound to an ss- or dsDNA-cellulose column as described in the 'Materials and Methods'. The proteins eluted at high salt concentration were analyzed by mass spectrometry. Using this analysis, many well-characterized DNA-binding or chromatin-associated proteins as well as some abundant metabolic enzymes were identified (data not shown). Along with these proteins, an uncharacterized protein, Cmr1, was isolated from both the ss- and dsDNA columns.

To determine if the DNA-binding activity is intrinsically associated with Cmr1, we purified a recombinant Cmr1 protein containing an N-terminal His tag that was expressed in insect Sf9 cells using baculovirus. The protein was initially purified with Q and Ni-columns, followed by additional purification via 15–35% glycerol gradient centrifugation as described in the 'Materials and Methods'. SDS-PAGE analysis and DNA binding assay of the glycerol gradient fractions revealed co-purification of Cmr1 with the DNA-binding activity (Fig. 1). In the gel mobility shift assay, stable Cmr1-DNA complexes, which migrated slower than the DNA substrate alone, were detected and the amount of these complexes was proportional to the concentration of Cmr1 protein in each fraction, indicating that the DNA-binding activity is intrinsic to Cmr1.

Cmr1 binds to both single- and double-stranded DNA

Since Cmr1 was isolated from both ss- and dsDNA-cellulose columns, we examined the binding affinity of Cmr1 to ss- and dsDNA substrates. DNA-protein complexes were hardly observed when a 30-nt ssDNA substrate was used (Fig. 2A, lanes 1–4). In contrast, Cmr1 readily formed a stable complex with dsDNA of the same length (lanes 5–8), suggesting that the binding affinity to dsDNA is higher than that to ssDNA. However, the amount of complex formed in the presence of a longer ssDNA substrate (50-nt) was comparable to that with dsDNA (compare lanes 9–12 with lanes 13–16), indicating that the binding affinity is depended on the length of the DNA substrate.

Since two or more bands of distinct mobility were observed in the gel mobility shift assay as shown in Figs. 1 and 2A, we examined whether all of these bands contained Cmr1 protein using a supershift assay with antibodies that bind to the N-terminal His tag of the recombinant Cmr1 protein. When an anti-HA antibody was added to the reaction mixture as a negative control antibody, no further mobility

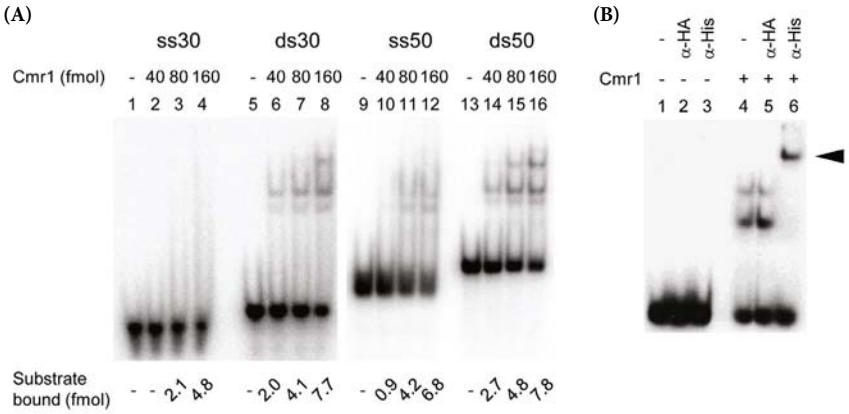

Fig. 2. Comparison of Cmr1 binding to ss- and dsDNA. (A) Ss30 and ss50 denote 5'-^{32}P-labeled oligonucleotides 1 and 3, respectively. Ds30 and ds50 denote the corresponding dsDNA substrates constructed by annealing with the complementary oligonucleotides 2 and 4, respectively. The indicated amounts of Cmr1 were incubated with 15 fmol of ^{32}P-labeled DNA substrates and the resulting DNA-protein complexes were separated by PAGE. The amounts of products are indicated at the bottom of the figure. (B) DNA binding reactions were performed with dsDNA substrate, ds50. The reaction mixtures were incubated in the presence (+) or absence (-) of 80 fmol of Cmr1 for 5 min, followed by the addition of antibodies, α-HA or α-His. An arrowhead indicates the migration position of the DNA-Cmr1-antibody complex.

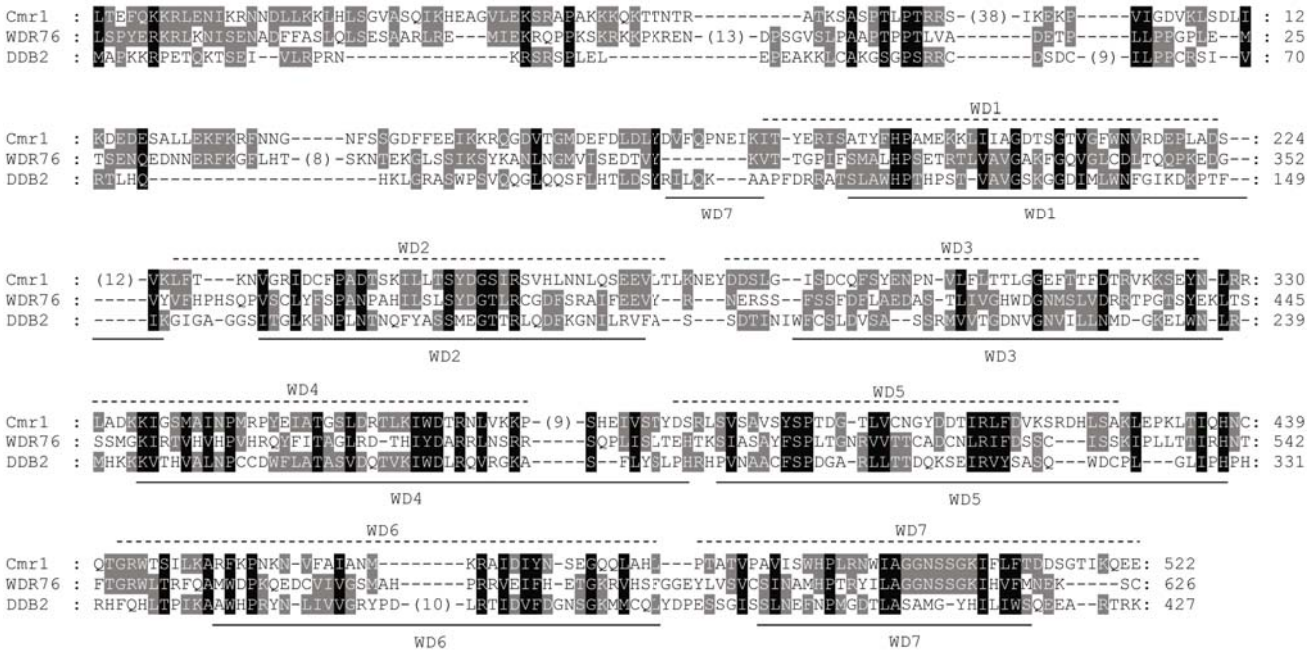

Fig. 3. Alignment of amino acid sequences of Cmr1, human WDR76, and human DDB2. The seven WD40 repeats in DDB2 (solid lines) and those predicted in Cmr1 (dashed lines) are indicated.

shift was observed (Fig. 2B, lane 5). In contrast, addition of anti-His antibody resulted in the formation of a larger slow-migrating band (lane 6), suggesting that each protein-DNA complex contained Cmr1 protein.

Cmr1 binds preferentially to the UV-damaged DNA

When the Cmr1 sequence was analyzed, it was shown to be homologous to human WD40 repeat-containing protein 76 (WDR76) and DDB2. Comparison of amino acid sequences revealed overall sequence identities of 24% and 21%, and similarities of 42% and 38%, respectively. The homologies were higher at the C-terminal 340 amino acid residues of Cmr1, where the WD40 repeats were located (Fig. 3). X-ray crystallographic studies revealed that DDB2 has a WD40 β-propeller domain composed of seven WD40 repeats (Scrima *et al.*, 2008). Sequence analysis of Cmr1 pre-

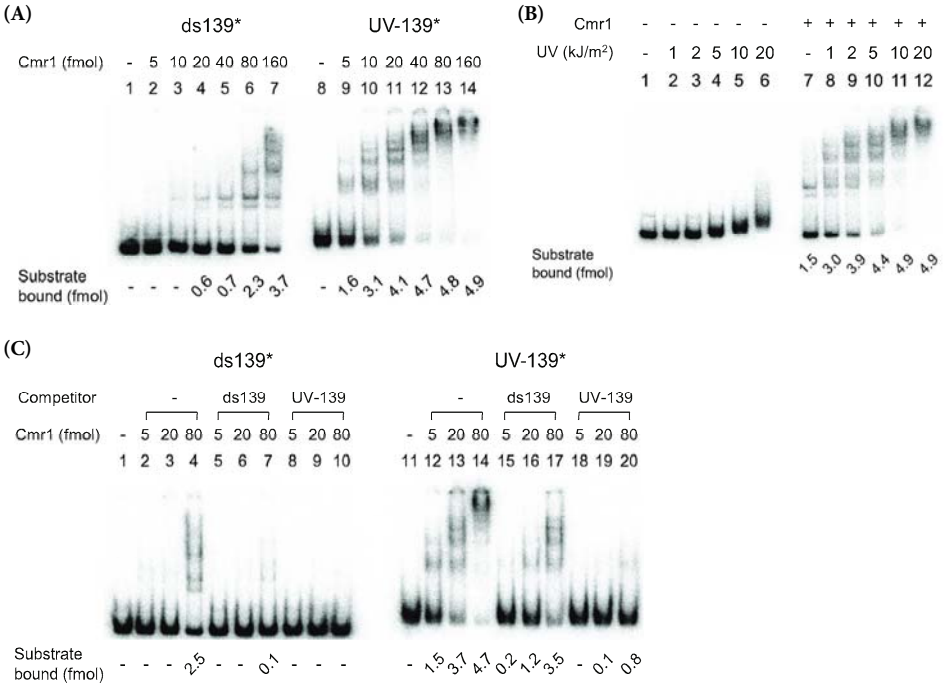

Fig. 4. Gel mobility shift analysis of Cmr1 binding to UV-damaged DNA. (A) The indicated amounts of Cmr1 were incubated with 5 fmol of either unirradiated (ds139*) or UV-irradiated (10 kJ/m^2, UV-139*) ^{32}P- labeled DNA substrates (139-bp in length), and the resulting DNA-protein complexes were separated by PAGE. The amounts of products are indicated at the bottom of the figure. (B) Effect of UV-irradiation on DNA binding of Cmr1. Reaction mixtures containing ^{32}P-labeled 139-bp dsDNA irradiated with indicated dose of UV light were incubated in the presence (+) or absence (-) of 40 fmol of Cmr1. The resulting DNA-protein complexes were separated by PAGE and the amounts of products are indicated at the bottom of the figure. (C) Competition experiment using 5 fmol of ^{32}P-labeled substrates (ds139* or UV-139*) in the presence of 2.5 pmol of unlabeled DNA (ds139 or UV-139). Reaction mixtures were incubated with the indicated amount of Cmr1 and the amounts of products are indicated at the bottom of the figure.

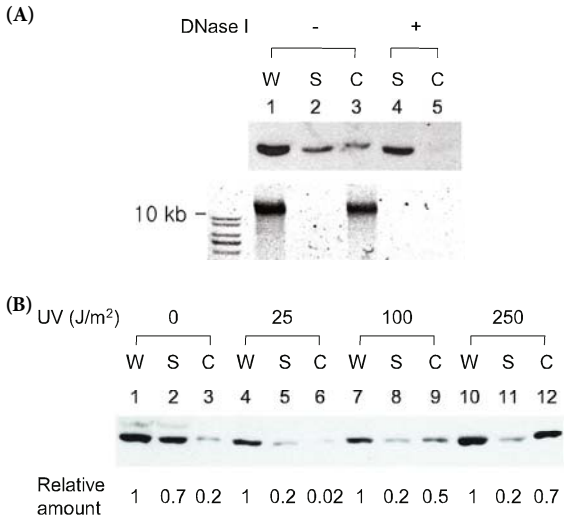

Fig. 5. Chromatin binding of Cmr1. (A) Whole-cell extracts (W), supernatants (S), and chromatin fractions (C) were prepared from the extracts as described in the 'Materials and Methods'. Each fraction (15 μl) was analyzed by immunoblotting using anti-Cmr1 antibodies (A). DNA was purified by phenol extraction and ethanol precipitation from the same fractions, and subjected to 0.7% agarose gel electrophoresis (B). The agarose gel was stained with ethidium bromide. (B) Yeast cells were irradiated with the indicated UV dose before isolation of chromatin fractions. Each fraction (15 μl) was subjected to SDS-PAGE, followed by immunoblotting analysis of Cmr1. The relative amounts of Cmr1 protein were determined using Image Quant LAS4000 (GE Healthcare) and indicated at the bottom of the figure.

dicted the presence of seven WD40 repeats (Fig. 3), implying that it probably folds into a stable β-propeller structure similar to that of DDB2. Based on this finding, we examined the possibility that Cmr1 might bind preferentially to UV- damaged DNA.

To examine the UV-damaged DNA binding properties of Cmr1, a 139-bp DNA fragment containing 39 target sites (TT or TC) for UV photoproduct formation was irradiated with UV light. The unirradiated DNA and UV-damaged DNA were incubated with increasing amounts of the purified Cmr1 protein, followed by electrophoretic analysis. As shown in Fig. 4A, incubation of Cmr1 with the UV-damaged DNA resulted in the formation of more DNA-protein complexes than with the unirradiated DNA. The efficiency of UV-damaged substrate binding was estimated to be about 7-fold higher than that of undamaged substrate binding (compare lanes 4 and 5 with 11 and 12). When the unirradiated substrate was used, the reaction was not saturated even at the highest Cmr1 concentration (160 fmol, lane 7), whereas complex formation with the UV-irradiated substrate reached a saturation level in the presence of much lower concentrations of Cmr1 (40 fmol, lane 12). The DNA-protein complex formation was proportional to the UV dose, and most of the substrates were converted into DNA-protein complexes at 10 kJ/m^2 (Fig. 4B). In addition, the level of slower migrating complexes were more prevalent at higher UV doses, suggesting that these complexes contained multiple Cmr1 proteins bound to separate UV lesions in the same DNA molecule.

We next performed a gel mobility shift assay in the presence of excess amounts (500-fold) of unlabeled competitors to confirm that Cmr1 is associated with a UV-damage-specific DNA-binding activity. Both the unirradiated and irradiated competitor DNA effectively titrated out Cmr1 binding to the labeled unirradiated substrate (Fig. 4C, lanes 1-10). In contrast, Cmr1 binding to the irradiated substrate was not significantly inhibited by the unirradiated competitor DNA (compare lanes 12-14 with 15-17), indicating that Cmr1 had a higher affinity for the UV-damaged DNA than the unirradiated DNA.

Cmr1 is found in the chromatin fraction

We next examined the possibility that Cmr1 might be in the chromatin-enriched fraction. In these experiments, yeast spheroplasts prepared by treatment with zymolase were lysed by the addition of non-ionic detergent, and chromatin was collected by centrifugation using sorbitiol-containing buffer. As shown in Fig. 5A, Cmr1 protein was found both in the chromatin and the supernatant fractions (upper, lanes 2 and 3). When the protein content in the fractions was analyzed by SDS-PAGE, less than 5% of the total protein was found in the chromatin fraction (data not shown), whereas nearly all of the genomic DNA was found in this fraction (Fig. 5A, lower), indicating that Cmr1 protein was selectively enriched by precipitation of chromatin. To provide further evidence that the presence of Cmr1 in the chromatin fraction was dependent on intact chromatin DNA, the whole cell extract was treated with DNase I before centrifugation. As shown in Fig. 5A, Cmr1 was not precipitated at all by treatment with DNase I, but a higher level of Cmr1 was found in the supernatant fraction (lanes 4 and 5), indicating that intact chromatin DNA was required for the sedimentation of Cmr1. This result suggests that Cmr1 is indeed associated with chromatin *in vivo*. Through multiple independent experiments, we estimated that approximately one third of the Cmr1 was associated with chromatin.

To examine whether UV-irradiation increases the amount of Cmr1 associated with chromatin, yeast cells were irradiated at different UV doses before spheroplasting, and the levels of Cmr1 in the chromatin and supernatant fractions were compared by western analysis. At the lower dose of UV irradiation (25 J/m^2), the amount of chromatin-bound Cmr1 did not increase (Fig. 5B, lane 6). However, at the higher dose (100 J/m^2), more than half of the total Cmr1 protein was found in the chromatin fraction (lane 9), and at the highest dose (250 J/m^2), most Cmr1 proteins was associated with chromatin (lane 12).

Since the Cmr1 was shown to be enriched in the chromatin fraction upon UV-irradiation, we constructed a *cmr1Δ* strain and assessed its susceptibility to UV-irradiation as well as DNA-damaging agents. In these experiments, the deletion of the *CMR1* gene did not significantly affect the growth of the mutant cells after either UV-irradiation or exposure to a variety of chemicals such as methyl methanesulfonate (MMS), mycophenolic acid, 4-nitroquinoline 1-oxide, 6-azauracil, and hydroxyurea (data not shown). Therefore, Cmr1 is not likely to be directly involved in the major DNA repair pathways. However, we cannot exclude the possibility that Cmr1 may play a minor role during the UV-damage response in yeast.

Discussion

In this study, we found that a WD40 repeat-containing protein Cmr1 possesses DNA-binding activity. In addition, its binding affinity to UV-irradiated DNA was much higher than that to unirradiated DNA, which is consistent with the finding that Cmr1 is homologous to human DDB2. We also found that part of the cellular Cmr1 protein pool localized in the chromatin fraction, and the Cmr1 levels found in this fraction increased in response to UV-irradiation.

Despite these biochemical data, the deletion of *CMR1* gene did not affect the growth of yeast cells after UV-irradiation (data not shown). In contrast, mutations in DDB2 gene have been shown to be responsible for the human disorder XP group E, which shows similar clinical and cellular characteristics to those of other XP complementation groups but are generally milder (Tang and Chu, 2002). DNA lesions in mammalian cells are recognized by the XPC/hHR23 complex in concert with CUL4-DDB1 ubiquitin ligase complex to initiate GG-NER. In yeast, the primary recognition of UV-damage is dependent on Rad4, the yeast ortholog of human XPC (Dantuma *et al*., 2009). Although orthologs of DDB1 and DDB2 have not been found in yeast (Tang and Chu, 2002), a similar cullin-containing ubiquitin ligase, Cul3-Rad16-Rad7-Elc1 complex has been implicated in the initial recognition of DNA lesions, based on the observation that the purified Rad16-Rad7 complex binds to UV- damaged DNA with high specificity (Dantuma *et al*., 2009). Mutations in Rad4, Rad7, or Rad16 were shown to cause yeast cells to be sensitive to UV-irradiation; however, no phenotypic change in the *cmr1Δ* mutant was observed, which indicates that Cmr1 is unlikely to be involved in the primary recognition of UV-damage in yeast.

The WD40 domain is one of the most abundant domains in eukaryotic genomes, and its common function is to serve as a platform for protein-protein or protein-DNA interaction (Xu and Min, 2011). DDB1 contains three WD40 propeller domains, which provide attachment sites for both CUL4 and DDB2, whereas DDB2 contains a single domain, which exclusively acts as the DNA lesion binding site (Scrima *et al*., 2008, 2011). Cmr1 is also likely to possess a WD40 β-propeller structure since the seven possible WD40 repeats in Cmr1 matched well with those of DDB2 in terms of their sequences and arrangements (Fig. 3). However, the striking difference between Cmr1 and DDB2 is that DDB2 requires DDB1 for its DNA-binding activity (Wittschieben *et al*., 2005). DNA-binding activity was observed only with the purified DDB1-DDB2 complex but not with DDB2 alone. In contrast, purified Cmr1 protein alone exhibited DNA-binding activity with higher affinity to UV-damaged DNA (Figs. 1 and 4). To examine whether Cmr1 forms a complex with other proteins, we constructed a yeast strain expressing tandem affinity purification (TAP)-tagged Cmr1. After TAP purification of Cmr1, the peak fractions were analyzed by SDS-PAGE to identify co-purifying proteins. However, in this analysis, no co-purifying proteins were isolated in amounts comparable to that of Cmr1 (data not shown).

Cullin family ubiquitin ligases use different substrate-specific adaptor proteins to target distinct substrates. In the case of CUL4-DDB1 ubiquitin ligase complex, CUL4 acts as scaffold protein for the assembly of ubiqutin ligase complex and DDB1 serves as a key linker to bridge adaptor proteins to the CUL4 E3 ligase (Lee and Zhou, 2007; Scrima *et al*., 2011). DDB2 is one of these adaptors and forms a complex with DDB1 in response to UV irradiation, resulting in the recruitment of CUL4-DDB1 ubiquitin ligase to the DNA lesion. Many WD40-repeat proteins (WDRs), including DDB2, were coimmunoprecipitated with DDB1 and acted as substrate-specific adaptor proteins (Higa *et al*., 2006; Lee and Zhou, 2007). WDR76, which is the most homologous protein to Cmr1, was one of these possible adaptor proteins. This observation implies that Cmr1 may function as an adaptor molecule for yeast cullin-containing ubiquitin E3 ligase.

S. cerevisiae encodes three cullin proteins, Cdc53, Cul3, and Rtt101. Although Cdc53 and Cul3 are orthologs of human CUL1 and CUL3, respectively, Rtt101 lacks homology to any known cullins. However, a recent study demonstrated that Mms1 is distantly conserved with human DDB1 and associates with Rtt101 to form a CUL4-DDB1-like ubiquitin E3 ligase (Zaidi *et al*., 2008). Although the overall sequence identity between Mms1 and DDB1 was less than 10%, close inspection of the conserved localized regions predicted that Mms1 probably adopts DDB1-like WD40 β-propeller domains, which mediate its interactions with cullin and adaptor proteins. Moreover, Mms22 and Crt10 were found to interact with Rtt101 via Mms1 and act as adaptor proteins (Zaidi *et al*., 2008). The interaction between Mms22 and Mms1 was stimulated by MMS treatment. On the basis of these observations, we hypothesize Cmr1 can associate with Mms1 and act as an adaptor protein that allows the Rtt101-Mms1 complex to recognize UV-damaged DNA. Although no co-purifying proteins were detected by TAP purification of Cmr1 from yeast cells (see above), we cannot exclude the possibility that Cmr1 may associate with a cullin E3 ligase under certain conditions, such as UV-irradiation. To understand the function of the DNA-binding activity of Cmr1, it will be essential to isolate proteins that interact with Cmr1.

Acknowledgements

This work was supported by the National Research Foundation of Korea (313-2008-2-C00666) and Inha University.

References

Bean, J.M., Siggia, E.D., and Cross, F.R. 2005. High functional overlap between MluI cell-cycle box binding factor and Swi4/6 cell-cycle box binding factor in the G1/S transcriptional program in *Saccharomyces cerevisiae*. *Genetics* **171**, 49–61.

Brohée, S., Janky, R., Abdel-Sater, F., Vanderstocken, G., André, B., and van Helden, J. 2011. Unraveling networks of co-regulated genes on the sole basis of genome sequences. *Nucleic Acids Res*. **39**, 6340–6358.

Choi, D.H., Oh, Y.M., Kwon, S.H., and Bae, S.H. 2008. The mutation of a novel *Saccharomyces cerevisiae* SRL4 gene rescues the lethality of *rad53* and *lcd1* mutations by modulating dNTP levels. *J. Microbiol*. **46**, 75–80.

Dantuma, N.P., Heinen, C., and Hoogstraten, D. 2009. The ubiquitin receptor Rad23: at the crossroads of nucleotide excision repair and proteasomal degradation. *DNA Repair* **8**, 449–460.

Donovan, S., Harwood, J., Drury, L.S., and Diffley, J.F. 1997. Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. *Proc. Natl. Acad. Sci. USA* **94**, 5611–5616.

Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A.F., Tanaka, K., and Nakatani, Y. 2003. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* **113**, 357–367.

Hellauer, K., Lesage, G., Sdicu, A.M., and Turcotte, B. 2005. Large-scale analysis of genes that alter sensitivity to the anticancer drug tirapazamine in *Saccharomyces cerevisiae*. *Mol. Pharmacol.* **68**, 1365–1375.

Higa, L.A., Wu, M., Ye, T., Kobayashi, R., Sun, H., and Zhang, H. 2006. CUL4-DDB1 ubiquitin ligase interacts with multiple WD40-repeat proteins and regulates histone methylation. *Nat. Cell Biol.* **8**, 1277–1283.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. 2003. Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691.

Lee, C.H., Shin, Y.K., Phung, T.T., Bae, J.S., Kang, Y.H., Nguyen, T.A., Kim, J.H., Kim, D.H., Kang, M.J., Bae, S.H., and *et al.* 2010. Involvement of Vts1, a structure-specific RNA-binding protein, in Okazaki fragment processing in yeast. *Nucleic Acids Res.* **38**, 1583–1595.

Lee, J. and Zhou, P. 2007. DCAFs, the missing link of the CUL4-DDB1 ubiquitin ligase. *Mol. Cell* **26**, 775–780.

Matsuda, N., Azuma, K., Saijo, M., Iemura, S., Hioki, Y., Natsume, T., Chiba, T., Tanaka, K., and Tanaka, K. 2005. DDB2, the xeroderma pigmentosum group E gene product, is directly ubiquitylated by Cullin 4A-based ubiquitin ligase complex. *DNA Repair* **4**, 537–545.

Prakash, S. and Prakash, L. 2000. Nucleotide excision repair in yeast. *Mutat. Res.* **451**, 13–24.

Scrima, A., Fischer, E.S., Lingaraju, G.M., Böhm, K., Cavadini, S., and Thomä, N.H. 2011. Detecting UV-lesions in the genome: The modular CRL4 ubiquitin ligase does it best! *FEBS Lett.* **585**, 2818–2825.

Scrima, A., Konícková, R., Czyzewski, B.K., Kawasaki, Y., Jeffrey, P.D., Groisman, R., Nakatani, Y., Iwai, S., Pavletich, N.P., and Thomä, N.H. 2008. Structural basis of UV DNA-damage recognition by the DDB1-DDB2 complex. *Cell* **135**, 1213–1223.

Sugasawa, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S., and Hanaoka, F. 2001. A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev.* **15**, 507–521.

Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K., and *et al.* 2005. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* **121**, 387–400.

Tang, J. and Chu, G. 2002. Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein. *DNA Repair* **1**, 601–616.

Wang, H., Zhai, L., Xu, J., Joo, H.Y., Jackson, S., Erdjument-Bromage, H., Tempst, P., Xiong, Y., and Zhang, Y. 2006. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. *Mol. Cell* **22**, 383–394.

Wittschieben, B.Ø., Iwai, S., and Wood, R.D. 2005. DDB1-DDB2 (xeroderma pigmentosum group E) protein complex recognizes a cyclobutane pyrimidine dimer, mismatches, apurinic/apyrimidinic sites, and compound lesions in DNA. *J. Biol. Chem.* **280**, 39982–39989.

Xu, C. and Min, J. 2011. Structure and function of WD40 domain proteins. *Protein Cell* **2**, 202–214.

Zaidi, I.W., Rabut, G., Poveda, A., Scheel, H., Malmström, J., Ulrich, H., Hofmann, K., Pasero, P., Peter, M., and Luke, B. 2008. Rtt101 and Mms1 in budding yeast form a CUL4(DDB1)-like ubiquitin ligase that promotes replication through damaged DNA. *EMBO Rep.* **9**, 1034–1040.