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cDNA Cloning of the Chicken *DDB1* Gene Encoding the p127 Subunit of Damaged DNA-binding Protein

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DDB (damaged DNA-binding protein) is a heterodimer, comprised of p48 (DDB2) and p127 (DDB1) subunits, which has a high affinity for a variety of DNA lesions including UV-photoproducts. The mutations in *DDB2* gene have been found in a subset of xeroderma pigmentosum complementation group E patients. However, no natural mutation has been identified so far in the cDNA of human *DDB1* and the precise roles of DDB1 are still unknown. We have cloned the *DDB1* cDNA from the chicken B lymphocyte line DT40 and revealed an open reading frame of 3420 bp encoding a polypeptide of 1140 amino acids, which is identical in size to the orthologs of human, monkey, mouse, rat and *Drosophila melanogaster* in databases. The amino acid sequence deduced from the chicken *DDB1* cDNA shows a high homology to the mammalian DDB1 orthologs (96–97% identity). Northern blot analysis using 5' portion of the chicken *DDB1* cDNA as a probe detected a single transcript of ~ 4.3 kb in chicken DT40 cells as well as in human HeLa cells and mouse embryonic fibroblasts. Furthermore, the chicken DDB1 (tagged with enhanced GFP) transiently expressed in human cells mainly localized in the cytoplasm, and coexpression of human DDB2 dramatically changed the localization from the cytoplasm to nucleus. These results suggest that DDB1 is evolutionarily conserved in the primary structure and function, and may play a fundamental role in higher eukaryotes.

Key words: damaged DNA-binding protein, *DDB1*, DT40, xeroderma pigmentosum

INTRODUCTION

Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by extreme sun sensitivity, pigmentation abnormalities, and predisposition to skin cancer. On the basis of cell fusion studies, XP patients have been divided into eight complementation groups: A–G and a variant form (Cleaver and Kraemer, 1989). The cultured cells derived from XP-A through XP-G patients manifest a defect in nucleotide excision repair (NER), which is the major pathway for removing UV-induced DNA lesions.

Among the repair-deficient XP patients, XP-E is the mildest form in the NER deficiency as well as clinical features. Some, but not all, XP-E patients have been shown to carry mutations in *DDB2* gene encoding p48 subunit of damaged DNA-binding protein (DDB) (Nichols et al., 1996). DDB is a stable heterodimer of p48 (DDB2) and p127 (DDB1) subunits and recognizes a variety of DNA lesions including UV-induced (6–4) photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs) (Reardon et al., 1993; Keeney et al., 1993; Fujiwara et al., 1999; Wakasugi et al., 2001). DDB is dispensable for the *in vitro* reconstituted reaction of NER (Mu et al., 1995; Aboussekhra et al., 1995), but it has been suggested to participate in global genomic repair of CPDs *in vivo* (Hwang et al., 1999; Tang et al., 2000). Recently, we have found that DDB stimulates the excision of CPDs in an *in vitro* system with cell-free extracts as well as in a defined system with purified proteins, indicating the accessory role of DDB in damage recognition step of NER (Wakasugi et al., 2001, 2002).

A number of protein-protein interaction studies have identified various kinds of physical and functional partners for DDB. DDB2 protein has been reported to interact with E2F1 and to enhance its transcriptional

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activation in conjunction with DDB1 (Hayes et al., 1998; Shiyanov et al., 1999a). In addition, DDB2 interacts with CBP/p300, a transcriptional coactivator with histone acetyltransferase activity (Datta et al., 2001), and also interacts with cullin 4A which is believed to be an ubiquitin-protein isopeptide ligase (Shiyanov et al., 1999b; Nag et al., 2001a; Chen et al., 2001). On the other hand, DDB1 has been shown to associate with the hepatitis B virus X protein (HBx) (Lee et al., 1995; Becker et al., 1998; Nag et al., 2001b) and V proteins from several viruses (Lin et al., 1998). Recent paper further suggested that HBx forms a complex with DDB1 in the cell nucleus and induces cell death (Bontron et al., 2002). Moreover, DDB1 has been found to interact with c-Abl tyrosine kinase using the yeast two-hybrid system (Cong et al., 2002). Taken together, DDB might play multiple roles in not only NER but also other cellular mechanisms.

In an attempt to further explore the molecular function of DDB1, we have cloned the *DDB1* cDNA from chicken DT40 cells. The amino acid sequence deduced from the cDNA shares an extremely high homology to mammalian DDB1 orthologs. We have also found that the chicken DDB1 protein transiently expressed in human cells makes a complex with human DDB2, consistent with the high sequence conservation between the two species.

MATERIALS AND METHODS

Cell Culture. The chicken B lymphocyte line, DT40, was cultured in RPMI1640 medium (Invitrogen) supplemented with 10 μM β-mercaptoethanol, 10% fetal bovine serum (FBS, ATLANTA biologicals) and 1% chicken serum (JRH) at 37°C in a 5% CO_2 atmosphere. HeLa cells and human lymphoblastoid cell line, GM01953, were grown in RPMI1640 medium supplemented with 10% FBS. Mouse embryonic fibroblasts were cultured in Dulbecco's modified Eagle's medium (Sigma) containing 10% FBS. GM01953 and DT40 cells were purchased from the Coriell Institute for Medical Research (NJ, USA) and the Health Science Research Resources Bank (Osaka, Japan), respectively.

In Vivo Repair Assay. Chicken DT40 and human GM01953 cells were collected by centrifugation, washed with PBS twice and suspended in 10 mL of phosphate-buffered saline (PBS). The cell suspensions were added to 100-mm dishes and exposed to 20 J/m^2 of UV light (254 nm) from a germicidal lamp (Toshiba, GL-10). After centrifugation, cells were incubated with fresh medium for various periods or directly processed for genomic DNA purification using the DNeasy kit (Qiagen). The amounts of CPD and 6-4PP were determined by an enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies, TDM-2 and 64M-2, respectively, as described previously (Mori et al., 1991).

Eletrophoretic Mobility Shift Assay (EMSA). Two fmol of ^{32}P-labeled 56-bp substrates containing a single 6-4PP were incubated with nuclear extracts (10 μg) or human recombinant DDB proteins (13.2 ng) at 30°C for 20 min. The nuclear extracts were prepared according to the method of Andrews and Faller (1991) and the recombinant DDB was purified from a baculovirus overexpression system as described previously (Wakasugi et al., 2001). The protein-DNA complex was separated by electrophoresis on 5% non-denaturing polyacrylamide gels at

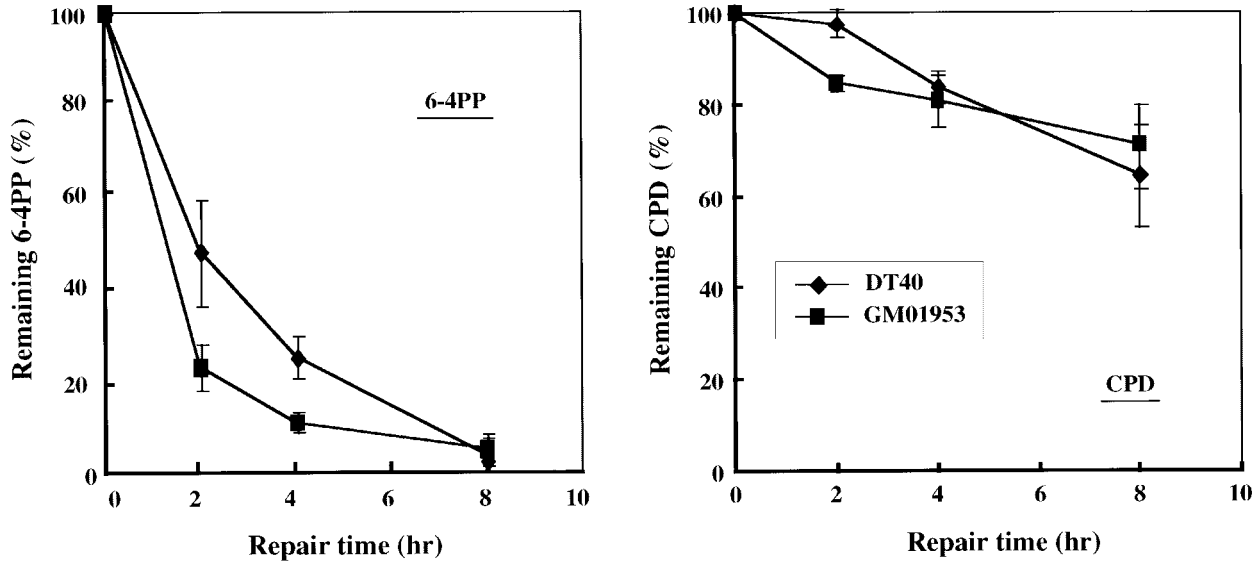


Fig. 1. Repair ability of 6-4PP and CPD in chicken DT40 cells and human GM01953 lymphoblastoid cells. The amounts of 6–4PP and CPD were determined using an ELISA at different times after receiving 20 J/m^2 of UV. Each point represents the mean of three independent experiments and *bars* show the S.D. values.

25 mA for 1.5 h and analyzed by autoradiography.

Cloning of Chicken *DDB1* cDNA. Total RNA was isolated from chicken DT40 cells using RNeasy Mini kit (Qiagen) and the first-strand cDNA was synthesized by M-MLV reverse transcriptase (Invitrogen). A pair of primers, 5'-ATGTCGTACAATTACGTC-3' (sense, 1–18) and 5'-TCAGGATAAAGAGCAGAT-3' (antisense, 224–241) was designed from the chicken expressed sequence tag (EST) sequence (udelptr1cpk0002g14) which shows 80% homology to 5' portion (1–272) of the human *DDB1* cDNA. The PCR product was confirmed to have the same sequence as the EST clone. We designed the other primer pair from the chicken EST sequence, 5'-GTCAAGGAGGTGGGCATGTA-3' (sense, 154–173), and human *DDB1* cDNA sequence (GenBank U18299), 5'-TGCAGCTTCTGGATCTCATC-3' (antisense, 2113–2132). The RT-PCR product was subcloned to pGEM-T easy vector and sequenced.

For the rapid amplification of cDNA ends (RACE), 5'-full RACE and 3'-full RACE core sets (Takara) were used according to the kit instructions. For the 5' RACE, five primers were used: 5'-(p)AAAGAGCAGATCC-3' (222–234); S1, 5'-CTATGTGGTGACAGCTGAGG-3' (sense, 123–142); S2, 5'-AAGGAGGTGGGCATGTATGG-3' (sense, 157–176); A1, 5'-CTCTAGGCGTGTGTTCTTGG-3' (antisense, 101–120); A2, 5'-AGGTTCAGGTCCTCTGCTGA-3' (antisense, 73–92). For the 3' RACE, a forward primer (2058–2079) containing three restriction sites (underlined) was designed: 5'-CTGATCTAGAGGTACCGGATCCGTATCCTGACAGCTTAGCATTG-3'. The RACE products were subcloned into pGEM-T easy vector and sequenced to find start and stop codons for an open reading frame (ORF).

Northern Blotting. Total RNAs were isolated from each cell lines as described above and 20 μg of each RNA was subjected to electrophoresis on a 1.0% agarose gel containing 6.6% formaldehyde and transferred to a positively charged nylon membrane. DNA probe was synthesized by PCR with a primer pair (sense 1–20 and antisense 1131–1150) and labeled with digoxigenin (DIG) using DIG High Prime DNA Labeling kit (Roche). After hybridization in DIG Easy Hyb buffer, the membrane was washed under a high stringency condition. The DNA probe retained on the membrane was detected with anti-DIG antibody conjugated with alkaline phosphatase, and visualized by the LAS-1000 Image Reader (Fuji Film) after incubating with a chemiluminescence substrate (CSPD). The size of the mRNA species was estimated from electrophoretic mobility of ribosomal RNA (18S and 28S).

Expression Plasmid Constructs. The full-length cDNA of chicken *DDB1* containing the *Not*I and *Sal*I restriction sites at 5' and 3' portions (underlined) , respectively, was generated by RT-PCR using synthetic primers: 5'-TGGCGGCCGCATGTCGTACAATTACGTCGTG-3' (sense) and 5'-CCAAGTCGACCTAGTGGATGCGGGTCAGC-3' (antisense). The product was digested with *Not*I and *Sal*I (New England Biolabs), subcloned into pTRE2 vector (Clontech) and verified by sequencing. The insert was then excised by *Eco*RI and *Xba*I digestion and subcloned into pEGFP-C1 vector (Clontech).

A human *DDB2* cDNA insert was isolated from the insect cell expression construct pFASTBac1-Fp48 (Wakasugi et al., 2001) after digestion with *Bam*HI and *Not*I, and subcloned into pTRE2 vector and subsequently into pCAGGS vector (Niwa et al., 1991) (a generous gift from Dr. Katsumi Yamashita, Kanazawa University) using *Kpn*I and *Not*I cloning sites.

Western Blotting. HeLa cells (5×10^5) were transiently transfected with 1 μg of the pEGFP-chDDB1 plasmid or pEGFP-C1 vector using Effectene™ transfection reagent (Qiagen) as described by the manufacturer. Fifty-six hours later, cells were washed with PBS twice and lysed in 150 μl of NP-40 lysis buffer (50 mM Tris-HCl (pH 7.5), 0.15 mM NaCl, 1% NP-40, 1% proteinase inhibitor cocktail (Roche)) for 30 min on ice. The lysates were centrifuged at 15000 rpm for 15 min at 4°C and the supernatants were used for Western blot analysis. Forty-eight

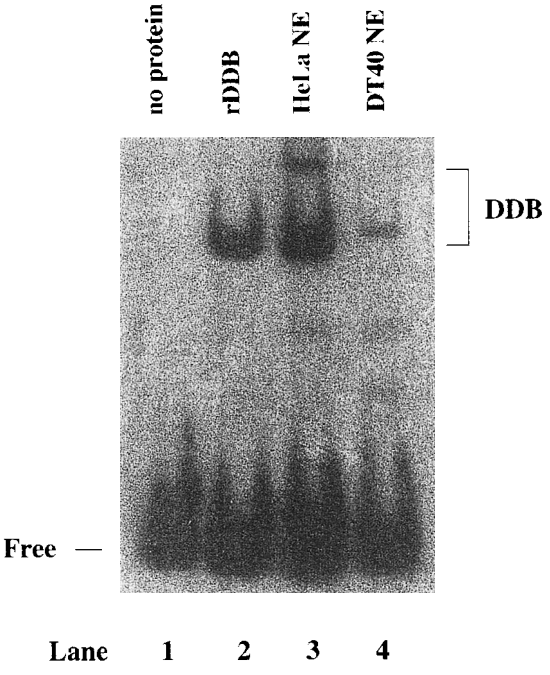
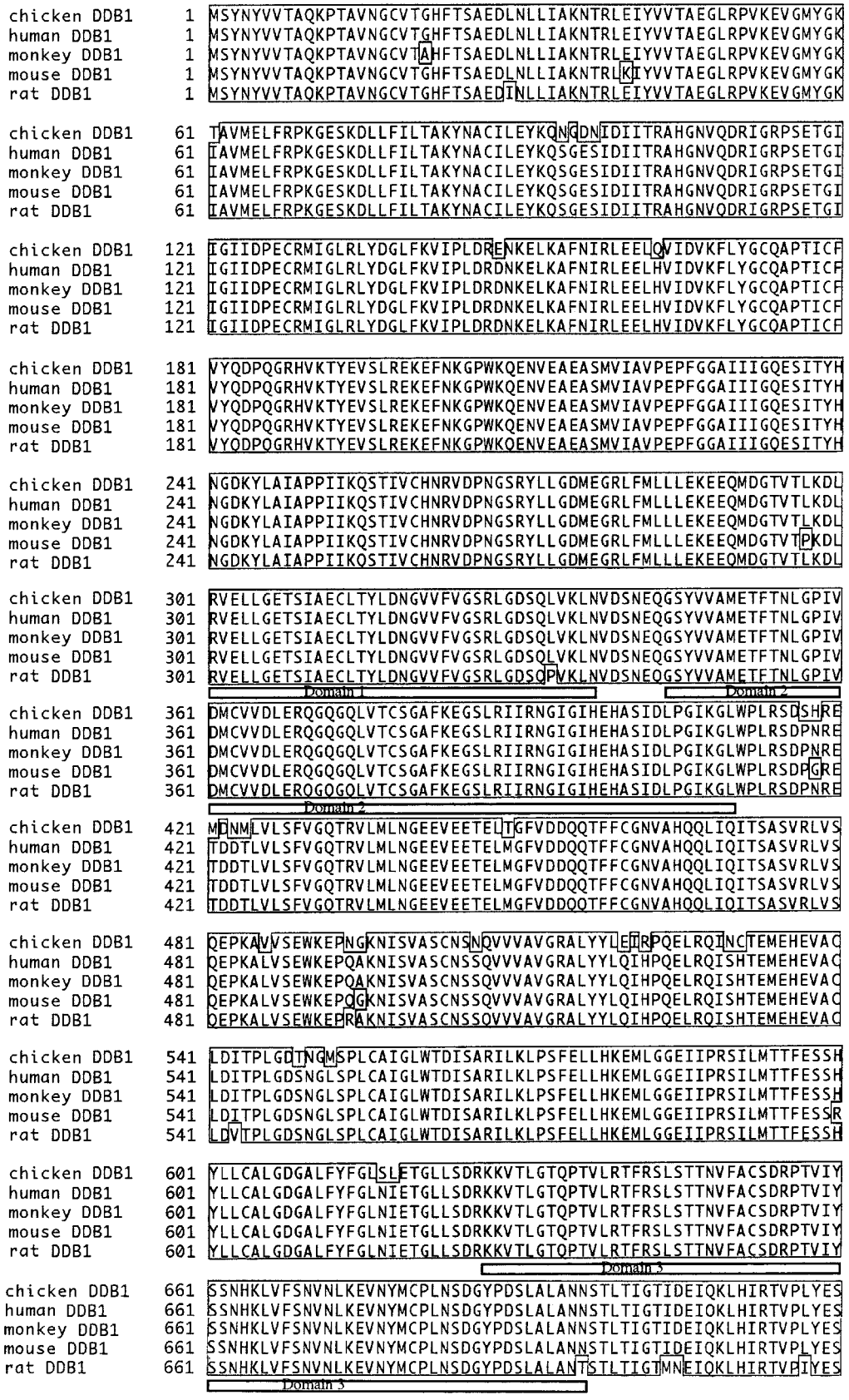


Fig. 2. DDB activity in chicken DT40 cells. Nuclear extracts (10 μg) or human recombinant DDB (13.2 ng) were incubated with ^{32}P-labeled 56-bp duplex DNA (2 fmol) containing a 6–4PP and analyzed by autoradiography after electrophoresis on a 5% nondenaturing polyacrylamide gel. Lane 1, no protein added; lane 2, recombinant DDB protein; lane 3, nuclear extract (NE) prepared from HeLa cells; lane 4, NE from DT40 cells.


chicken DDB1 1 MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTRLEIYVVTAEGLRPVKEVGMYGK
human DDB1 1 MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTRLEIYVVTAEGLRPVKEVGMYGK
monkey DDB1 1 MSYNYVVTAQKPTAVNGCVTAHFTSAEDLNLLIAKNTRLEIYVVTAEGLRPVKEVGMYGK
mouse DDB1 1 MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTRLKIYVVTAEGLRPVKEVGMYGK
rat DDB1 1 MSYNYVVTAQKPTAVNGCVTGHFTSAEDINLLIAKNTRLEIYVVTAEGLRPVKEVGMYGK
chicken DDB1 61 TAVMELFRPKGESKDLLFILTAKYNACILEYKQNGDNIDIITRAHGNVQDRIGRPSETGI
human DDB1 61 IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRIGRPSETGI
monkey DDB1 61 IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRIGRPSETGI
mouse DDB1 61 IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRIGRPSETGI
rat DDB1 61 IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIITRAHGNVQDRIGRPSETGI
chicken DDB1 121 IGIIDPECRMIGLRLYDGLFKVIPLDRENKELKAFNIRLEELQVIDVKFLYGCQAPTICF
human DDB1 121 IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF
monkey DDB1 121 IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF
mouse DDB1 121 IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF
rat DDB1 121 IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF
chicken DDB1 181 VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH
human DDB1 181 VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH
monkey DDB1 181 VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH
mouse DDB1 181 VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH
rat DDB1 181 VYQDPQGRHVKTYEVSLREKEFNKGPWKQENVEAEASMVIAVPEPFGGAIIIGQESITYH
chicken DDB1 241 NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTLKDL
human DDB1 241 NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTLKDL
monkey DDB1 241 NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTLKDL
mouse DDB1 241 NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTPKDL
rat DDB1 241 NGDKYLAIAPPIIKQSTIVCHNRVDPNGSRYLLGDMEGRLFMLLLEKEEQMDGTVTLKDL
chicken DDB1 301 RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSNEQGSYVVAMETFTNLGPIV
human DDB1 301 RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSNEQGSYVVAMETFTNLGPIV
monkey DDB1 301 RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSNEQGSYVVAMETFTNLGPIV
mouse DDB1 301 RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSNEQGSYVVAMETFTNLGPIV
rat DDB1 301 RVELLGETSIAECLTYLDNGVVFVGSRLGDSQPVKLNVDSNEQGSYVVAMETFTNLGPIV
Domain 1
Domain 2
chicken DDB1 361 DMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDSHRE
human DDB1 361 DMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDPNRE
monkey DDB1 361 DMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDPNRE
mouse DDB1 361 DMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDPGRE
rat DDB1 361 DMCVVDLERQGQGQLVTCSGAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDPNRE
Domain 2
chicken DDB1 421 MDNMLVLSFVGQTRVLMLNGEEVEETELTGFVDDQQTFFCGNVAHQQLIQITSASVRLVS
human DDB1 421 TDDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS
monkey DDB1 421 TDDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS
mouse DDB1 421 TDDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS
rat DDB1 421 TDDTLVLSFVGQTRVLMLNGEEVEETELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS
chicken DDB1 481 QEPKAVVSEWKEPNGKNISVASCNSNQVVVAVGRALYYLEIRPQELRQINCTEMEHEVAC
human DDB1 481 QEPKALVSEWKEPQAKNISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVAC
monkey DDB1 481 QEPKALVSEWKEPQAKNISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVAC
mouse DDB1 481 QEPKALVSEWKEPQGKNISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVAC
rat DDB1 481 QEPKALVSEWKEPRAKNISVASCNSSQVVVAVGRALYYLQIHPQELRQISHTEMEHEVAC
chicken DDB1 541 LDITPLGDTNGMSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSH
human DDB1 541 LDITPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSH
monkey DDB1 541 LDITPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSH
mouse DDB1 541 LDITPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSR
rat DDB1 541 LDVTPLGDSNGLSPLCAIGLWTDISARILKLPSFELLHKEMLGGEIIPRSILMTTFESSH
chicken DDB1 601 YLLCALGDGALFYFGLSLETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIY
human DDB1 601 YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIY
monkey DDB1 601 YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIY
mouse DDB1 601 YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIY
rat DDB1 601 YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRTFRSLSTTNVFACSDRPTVIY
Domain 3
chicken DDB1 661 SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYES
human DDB1 661 SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYES
monkey DDB1 661 SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYES
mouse DDB1 661 SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANNSTLTIGTIDEIQKLHIRTVPLYES
rat DDB1 661 SSNHKLVFSNVNLKEVNYMCPLNSDGYPDSLALANTSTLTIGTMNEIQKLHIRTVPIYES
Domain 3

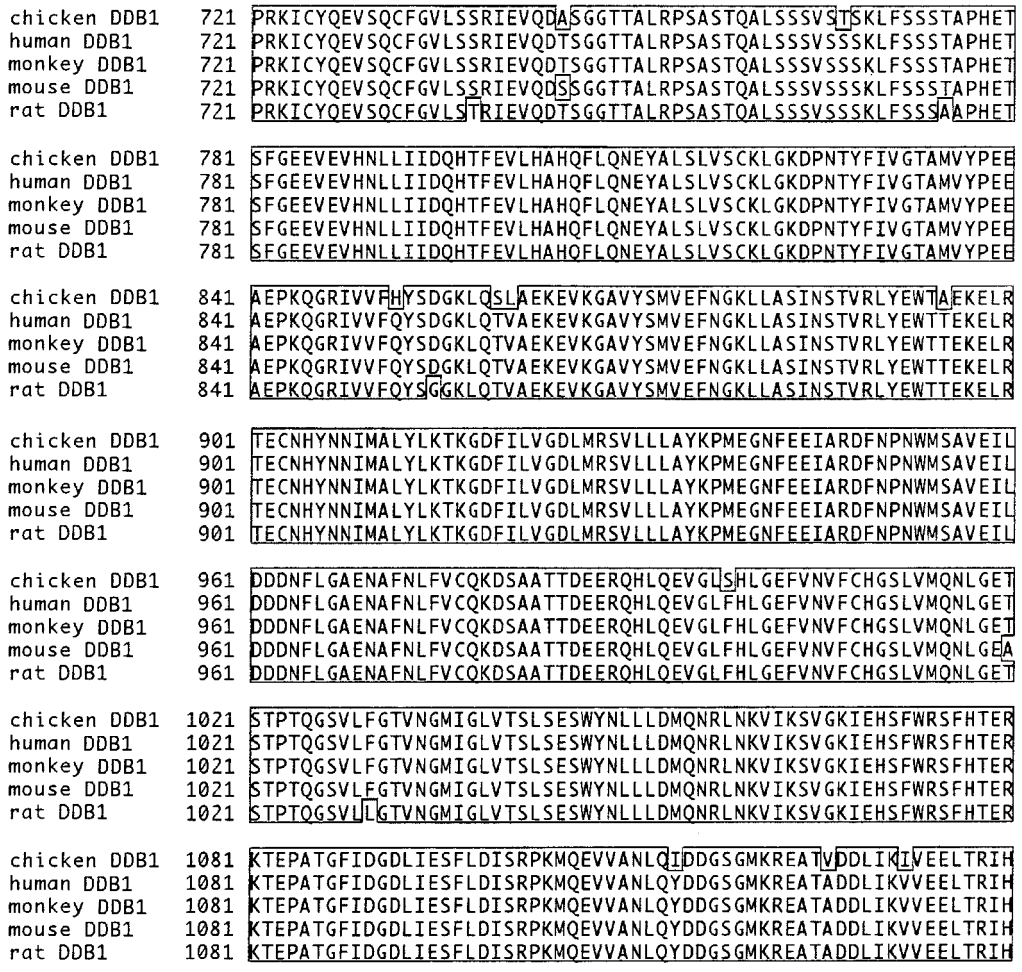


Fig. 3. Comparison of amino acid sequences of DDB1 deduced from chicken (this study), human (GenBank U18299), monkey (GenBank L20216), mouse (GenBank AF159853) and rat (GenBank AJ277077). Highly conserved domains 1, 2 and 3 shown in hatched boxes have been proposed by alignment of putative DDB1 homologs from human, mouse, *D. melanogaster*, *A. thaliana*, *C. elegans*, *D. dyscoideum* and *S. pombe* (Zolezzi and Linn, 2000).

μg of each lysates were resolved by SDS-polyacrylamide gel electrophoresis, transferred to an Immobilon-P membrane (Millipore), and probed with rabbit anti-GFP antibody (Clontech) followed by goat anti-rabbit IgG (H+L) conjugated with alkaline phosphatase (Zymed). Antibody binding was detected by incubating with AP buffer (100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$) containing BCIP/NBT color substrate (Promega).

GFP Fluorescence Microscopy. The following expression constructs were transfected into 2×10^5 HeLa cells in 35-mm dishes using Effectene™ transfection reagent: pEGFP-C1 (0.4 μg), pEGFP-chDDB1 (0.4 μg), pEGFP-C1/pCAGGS-F-hDDB2 (0.2 μg / 0.2 μg) or pEGFP-chDDB1/pCAGGS-F-hDDB2 (0.2 μg / 0.2 μg). Fifty-six hours later, fluorescence images were obtained with a Leica DMIRBE microscope equipped with a cooled CCD camera (CoolSNAP HQ, Photometrics).

RESULTS AND DISCUSSION

Repair Ability of UV-induced DNA Lesions and DDB Activity in Chicken DT40 Cells. In order to measure the repair ability of UV-induced CPD and 6-4PP in the chicken DT40 cell line, we irradiated the cells with 20 J/m^2 of UV and isolated their genomic DNA after 0, 2, 4 or 8 h. As shown in Fig. 1, CPD and 6-4PP in the DT40 genome decreased during the repair period, indicating that NER is active in the chicken cells. The repair rates of both photoproducts in DT40 cells were almost comparable to those in the human lymphoblastoid cell line, GM01953, although slightly slower in the earlier phase.

The previous report has shown that Chinese hamster cell lines have no detectable DDB activity due to the inactivation of *DDB2* gene by methylation (Hwang et al., 1998), whereas human, monkey, rat, and some but not all mouse cell lines show the DDB activity. We tested

whether the chicken DT40 cell line has DDB activity using an EMSA with ^{32}P-labeled DNA probe containing a single 6-4PP (Fig. 2). Nuclear extract prepared from human HeLa cells (Lane 3) and human recombinant DDB protein (Lane 2) were used as positive controls for the DDB activity. Nuclear extract from the chicken DT40 cells showed a retarded band (Lane 4), suggesting that DT40 cells possess the DDB activity. Under this condition, undamaged DNA probe conferred no retarded signal (data not shown). However, compared with HeLa nuclear extract, the activity was apparently reduced, probably due to the inability of DT40 to express the tumor suppressor p53 (Takao et al., 1999) since the expression of DDB2 is known to depend on p53 (Hwang et al., 1999). It should be also noted that the mobility of the shifted band appears to be somewhat slower than that with human DDB. A similar observation has been previously reported with mouse cell extract (Zolezzi and Linn, 2000).

Cloning and Sequence Analysis of the Chicken DDB1 cDNA. We searched the public chicken EST database (http//www.ri.bbsrc.ac.uk/cgi-bin/est-blast/blast.pl) for the human *DDB1* cDNA sequence and found one EST clone with a high homology to 5' region (1–272) of the human *DDB1* cDNA. Since the EST sequence was found in the DT40 transcripts by RT-PCR and sequencing, we designed a forward primer from the chicken EST sequence and a reverse primer from the human *DDB1* cDNA (GenBank U18299), and isolated a partial cDNA (154–2132) of the chicken *DDB1*. After the 5'- and 3'-RACE analyses, the 3621-bp cDNA sequence was obtained and an ORF of 3420 bp was found. The nucleotide sequence of the chicken DDB1 ORF and the deduced primary amino acid sequence have been registered in the DDBJ/EMBL/GenBank database (accession No. AB074-298).

Comparison of the Deduced Amino Acid Sequences between Chicken and Mammalian DDB1. The ORF encodes a polypeptide of 1140 amino acids, which is completely identical in size to the homologs of human, monkey, mouse, rat and *D. melanogaster*. The deduced amino acid sequence of the chicken DDB1 shares considerable homology to the mammalian DDB1 (97% identity to human, 97% to monkey, 96.8% to mouse and 96.1% to rat) (Fig. 3). Previous studies proposed three highly conserved domains based on the alignment of putative DDB1 homologs from human, mouse, *D. melanogaster*, *A. thaliana*, *C. elegans*, *D. dyscoideum* and *S. pombe* (Zolezzi and Linn, 2000). We confirmed that these three domains are completely conserved in the chicken *DDB1* cDNA sequence as well.

Northern Blot Analysis of *DDB1* Expression in the Chicken DT40 Cell Line. In order to verify the expression of *DDB1* in DT40 cells, 5' region (1–1150) of the chicken *DDB1* cDNA was used as a probe for Northern

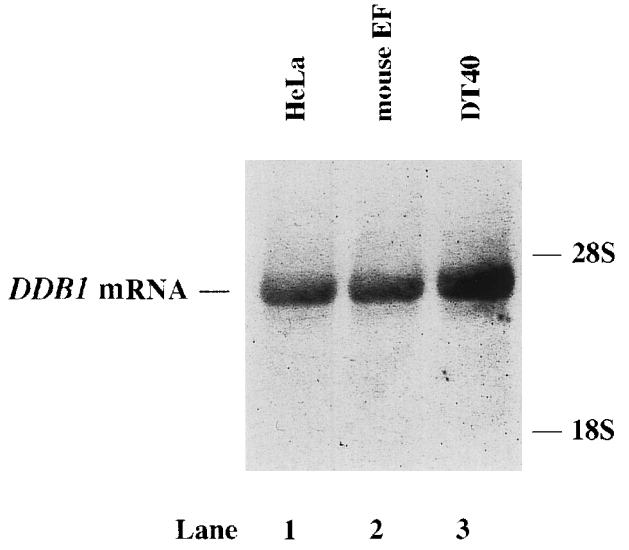


Fig. 4. Northern blot analysis of total RNA from human, mouse and chicken cells. Total RNA was isolated from human HeLa cells (lane 1), mouse embryonic fibroblasts (lane 2), or chicken DT40 cells (lane 3), and 20 μg of each were used for the separation on an agarose gel containing 6.6% formaldehyde. After electrophoresis and transfer to a nylon membrane, the blot was probed with the partial cDNA of the chicken *DDB1* labeled with DIG.

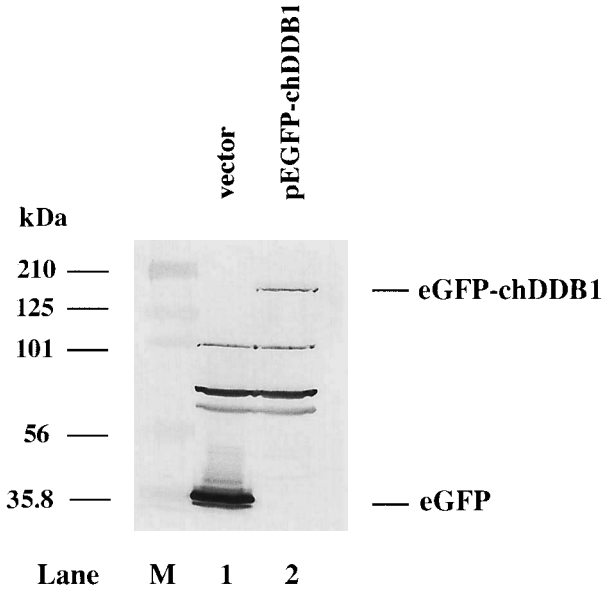


Fig. 5. Transient expression of the chicken DDB1 tagged with eGFP in HeLa cells. HeLa cells were transfected with pEGFP-C1 vector (lane 1) or pEGFP-chDDB1 plasmid (lane 2). After 56-h incubation, cell lysates were prepared and used for Western blot analysis with anti-GFP antibody.

blot analysis (Fig. 4). DT40 cells showed a single transcript of ~ 4.3 kb (Lane 3), which is identical to that observed in human HeLa cells (Lane 1) and mouse embryonic fibroblasts (Lane 2). This expression pattern was also similar to the previous data with human and monkey cells (Takao et al., 1993). This result indicates that the chicken cDNA probe cross-hybridizes to mammalian *DDB1* transcripts, consistent with their high sequence conservation among the three species.

Expression and Subcellular Localization of Chicken DDB1 in Human Cells. We tried to express the chicken recombinant DDB1 in human cells. The full-length cDNA of the chicken *DDB1* was subcloned in frame into a mammalian expression vector pEGFP-C1 encoding for enhanced GFP (eGFP) and transiently transfected into HeLa cells. Western blot analysis showed that the fusion protein of eGFP and DDB1 was expressed in human HeLa cells and its mobility in SDS-PAGE seems to correspond with its predicted molecular weight (Fig. 5, Lane 2).

To examine the subcellular localization of the chicken DDB1, fluorescence images of the transfected HeLa cells were analyzed. eGFP alone showed a uniform distribution in the nucleus as well as the cytoplasm (Fig. 6A), whereas eGFP-chDDB1 primarily localized in the cytoplasm (Fig. 6B), consistent with the previous results with the human DDB1 (Shiyanov et al., 1999a; Liu et al., 2000). We wanted to know whether coexpression of human DDB2 affects the localization of chicken DDB1, since DDB2 has been shown to play a critical role in the nuclear localization of DDB1 in human cells (Shiyanov et al., 1999a). Cotransfection of pEGFP-chDDB1 with pCAGGS-F-hDDB2 led to a dramatic change of the eGFP-chDDB1 localization from the cytoplasm to nucleus (Fig. 6D), while the human DDB2 expression conferred no change in the localization pattern of eGFP alone (Fig. 6C). These results indicate that the human DDB2 is capable of making a complex with the chicken DDB1 and promoting its nuclear entry.

Although we have not tested the activity of the heterologous DDB complex yet, DDB1 appears to be an evolutionary conserved protein structurally as well as functionally, suggesting its fundamental role in higher eukaryotes. The precise roles of DDB1 are still unknown. No natural mutation has been found so far in

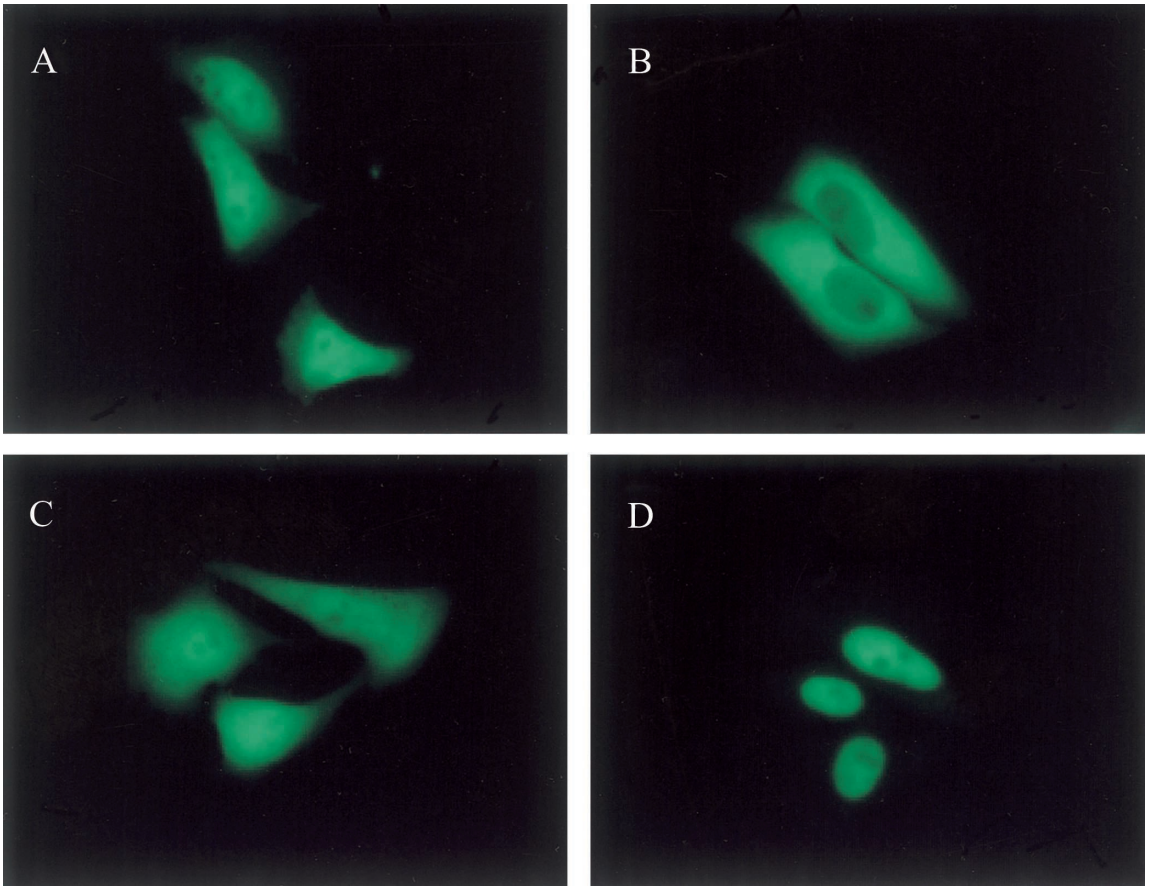


Fig. 6. Subcellular localization of the chicken DDB1 in HeLa cells. HeLa cells were transfected with pEGFP-C1 vector alone (A), pEGFP-chDDB1 alone (B), pEGFP-C1 and pCAGGS-F-hDDB2 (C) or pEGFP-chDDB1 and pCAGGS-F-hDDB2 (D), and observed after 56 h under a fluorescence microscope.

the cDNA of human *DDB1*. It has been recently reported that knockout mutant of *DDB1* in *Schizosaccharomyces pombe* manifests an impairment in colony-forming ability, elongated phenotype, and abnormal nuclei (Zolzzi et al., 2002). Since chicken DT40 cells show the unique highest efficiency in the targeted integration (Sonoda et al., 1998), the chicken *DDB1* cDNA cloned in this study would be valuable for the investigation in the chicken DT40 knockout model.

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