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The human *REV1* gene codes for a DNA template-dependent dCMP transferase

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ABSTRACT

DNA is frequently damaged by various physical and chemical agents. DNA damage can lead to mutations during replication. In the yeast *Saccharomyces cerevisiae*, the damage-induced mutagenesis pathway requires the Rev1 protein. We have isolated a human cDNA homologous to the yeast *REV1* gene. The human *REV1* cDNA consists of 4255 bp and codes for a protein of 1251 amino acid residues with a calculated molecular weight of 138 248 Da. The human *REV1* gene is localized between 2q11.1 and 2q11.2. We show that the human REV1 protein is a dCMP transferase that specifically inserts a dCMP residue opposite a DNA template G. In addition, the human REV1 transferase is able to efficiently and specifically insert a dCMP opposite a DNA template apurinic/apyrimidinic (AP) site or a uracil residue. These results suggest that the REV1 transferase may play a critical role during mutagenic translesion DNA synthesis bypassing a template AP site in human cells. Consistent with its role as a fundamental mutagenic protein, the *REV1* gene is ubiquitously expressed in various human tissues.

INTRODUCTION

DNA damage can lead to mutations during replication. In the yeast *Saccharomyces cerevisiae*, it appears that the majority of induced mutations are generated through the damage-induced mutagenesis pathway (1,2). The required yeast genes in this pathway include: *RAD6*, *RAD18*, *REV1*, *REV3*, *REV6*, *REV7* and *NGM2* (1–7), most of which have been isolated by gene cloning. As expected, inactivating these mutagenesis genes dramatically decreases the mutation frequency following DNA damage (3,8).

Rad6 is a ubiquitin-conjugating enzyme (9) and forms a complex with Rad18 (10–12). It has been proposed that this complex may play an important role in the initial steps of the damage-induced mutagenesis pathway (10). Rev3 protein is a DNA polymerase (DNA polymerase ζ) capable of translesion DNA synthesis (13). In contrast to the replicative DNA polymerases, deletion of the yeast *REV3* gene does not lead to lethality (1). Hence, this polymerase is specifically required for damage-induced mutagenesis in yeast. Rev1 belongs to the UmuC family of proteins (14). It possesses a deoxycytidyl (dCMP) transferase activity in a template-dependent reaction, which can efficiently insert a dCMP opposite a template apurinic/apyrimidinic (AP) site (15). Yeast Rad30, an *Escherichia coli* DinB homolog, is another member of the UmuC family (14,16,17). However, unlike Rev1, Rad30 is not a component of the damage-induced mutagenesis pathway, but appears to be involved in a novel error-free lesion bypass mechanism (16,17). Most recently, Rad30 was shown to be a non-essential DNA polymerase (pol η) capable of error-free translesion DNA synthesis opposite a TT dimer *in vitro* (18). Apparently, the UmuC family of proteins are involved in different mechanisms in the damage tolerance response to unrepaired DNA lesions during replication.

It is only very recently that the damage-induced mutagenesis pathway in humans has been investigated. Two human homologs of the yeast *RAD6* gene have been identified: HHR6A and HHR6B (19,20). Additionally, hREV3 has been isolated as the human homolog of the yeast mutagenic DNA polymerase ζ (21,22). Thus, it is most likely that a damage-induced mutagenesis pathway similar to that in yeast is operational in humans. Given the genetic complexity of the yeast mutagenesis pathway, it is certain that more human mutagenesis genes remain to be identified. Since mutations are the building blocks of human cancers, understanding the damage-induced mutagenesis pathway in humans is a key to the understanding of carcinogenesis. Isolating the human mutagenesis genes and elucidating the activities of these gene products are essential steps in these studies.

In this report, we describe the isolation of a full-length cDNA representing the homolog of the yeast Rev1 mutagenesis protein. We determine the chromosomal location of the human *REV1* gene and show its ubiquitous expression in various human tissues. Furthermore, we demonstrate that the human REV1 protein is a dCMP transferase capable of inserting a dCMP opposite a template AP site.

MATERIALS AND METHODS

Materials

Human bone marrow and leukocyte cDNA libraries in λgt11 were purchased from Clontech Laboratories. A human T cell cDNA library in the ZAP Express vector and a human placenta genomic DNA library were purchased from Stratagene.

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Escherichia coli uracil-DNA glycosylase was obtained from New England BioLabs. The vector pUC19M1 was constructed by Deepak Rajpal (University of Kentucky, Lexington, KY) by adding *Bgl*II, *Eco*RV, *Nco*I and *Xho*I sites into the multiple cloning region of plasmid pUC19 immediately after the *Hin*dIII site. The plasmid vector PCR-Script was obtained from Stratagene. The *E.coli* strains XL1-Blue MRF′ and XLOLR were purchased from Stratagene. The yeast strain CL1265rev1Δ (*MATα rev1Δ arg4-17 leu2-3,112 his3-Δ1 trp ura3-52*) was derived from CL1265-7C (1) by deleting the *REV1* gene.

Isolation of human *REV1* cDNA

Based on a human EST sequence (GenBank accession number AA029147), a 59mer oligonucleotide (probe I), CATGGTAC-GAAAGCCTGGGGTCCTGTAGAAACTGCAAAATTTG-GAGGCCATGGAATTTG, was synthesized. After labeling with ^{32}P at its 5′ end by T4 polynucleotide kinase, the probe was used to screen human bone marrow and leukocyte cDNA libraries by plaque DNA hybridization (23). Seventeen positive clones were isolated from ~1.6 million independent clones. Each insert cDNA was either directly subcloned into the *Eco*RI site of pUC19 plasmid vector or amplified by PCR prior to plasmid subcloning using the λ phage-derived primers, CGGCAGTACAATGGATTTCCTT and CATCGCCATCT-GCTGCAC. These cDNA inserts were sequenced by the standard dideoxynucleotide chain termination method on both strands. The overlapping cDNA sequences yielded a partial cDNA sequence of 4.2 kb. Based on the 5′ region of this sequence, two 59mer oligonucleotides, CATTAGTTTTCT-CAATCTCAGCGGAAGATCTGTGTATCCATTAACATA-GATGGCAACTC (probe II) and CCACCCCATGTTTTC-CAGCCATCATTTTCAGCTCGCTTCCTCCATCCACCTC-GCCTCAT (probe III), were synthesized and used to screen a human T cell cDNA library. Approximately 40 cDNA clones were isolated and their insert sequences determined. Additional 5′ sequence of the human *REV1* cDNA was obtained from some clones. Combining the 5′ and 3′ sequences of various cDNA clones, the complete sequence of a 4255 bp human *REV1* cDNA was generated.

Construction of a full-length human *REV1* cDNA

The insert cDNA from two λgt11 clones with overlapping partial human *REV1* cDNA sequences was excised from the phage DNA with *Sal*I restriction endonuclease and subcloned into the plasmid vector pUC19M1. The resulting plasmids, pWL269 and pWL270, contain the 3′ and 5′ halves of the human *REV1* cDNA, respectively. The full-length human *REV1* cDNA was constructed by ligating a 1.7 kb *Xba*I–*Sph*I fragment from pWL270 and a 2.7 kb *Sph*I–*Sac*I fragment from pWL269 into the *Sac*I–*Xba*I sites of the plasmid vector pUC19. The resulting recombinant plasmid, pWL296, contained the full-length human *REV1* cDNA.

Isolation of human *REV1* genomic clones

A human *REV1* clone from the human T cell cDNA library was excised *in vivo* from the ZAP Express vector in *E.coli* XL1-Blue MRF′ cells infected with a M13 helper phage. The resulting packaged phagemid particles were used to infect *E.coli* XLOLR cells to convert the phagemid into a double-stranded plasmid containing the human *REV1* cDNA. The recovered plasmid was then digested with *Eco*RI restriction endonuclease, releasing the 0.8 kb human *REV1* cDNA insert (corresponding to the human *REV1* cDNA position –178 to +646) from the plasmid vector. After isolating it from an agarose gel, this cDNA fragment was used as the template to prepare ^{32}P-labeled DNA probes by randomly primed DNA synthesis. Approximately 2 million clones from a human placenta genomic DNA library were screened with the human *REV1* probes. Two clones were isolated. The DNA inserts of ~20 kb each were subcloned into the *Not*I site of PCR-Script vector and partially sequenced.

Northern blot analysis of the human *REV1* mRNA

A 59mer oligonucleotide probe, corresponding to the human *REV1* cDNA position –126 to –184, was synthesized and labeled with ^{32}P at its 5′ end by T4 polynucleotide kinase. A human mRNA blot (Invitrogen) was hybridized with the probe in a buffer containing 50% formamide, 0.25 M NaCl, 0.25 M sodium phosphate, pH 7.2, 1 mM EDTA and 5% SDS at 42°C for 18 h. The blot was washed with 15 mM NaCl, 1 mM sodium phosphate, pH 7.4, and 0.1 mM EDTA at 60°C for 1 h. The hybridized human *REV1* mRNA was visualized by auto-radiography at –80°C with an intensifying screen.

Detection of the human *REV1* expression

Expression of the *REV1* gene in various human tissues was detected by RT–PCR. Poly(A) mRNA samples were isolated from various human tissues and used for first strand cDNA synthesis by reverse transcriptase. These cDNA samples were then normalized against glyceraldehyde-3-phosphate dehydrogenase cDNA. Such human multiple tissue cDNA panels were purchased from Clontech Laboratories and used for PCR. Two PCR primers, CCCAGGAGGAGGATAAGGCTG and GTC-TTTGTAGGGTATTGACAAACTCAGTC, were used to amplify a 360 bp region of the human *REV1* cDNA. PCR reactions (20 μl) contained 0.4 ng cDNA, 5 pmol each of the primers, 2 mM $MgCl_2$, 20 mM Tris–HCl, pH 8.0, 50 mM KCl, 200 μM each of dATP, dCTP, dGTP and dTTP, and 1 U Taq DNA polymerase. After heating at 94°C for 30 s, 35 cycles of amplification were performed according to the following conditions: 20 s denaturation at 94°C, 30 s annealing at 60°C and 45 s extension at 68°C. Reaction products were separated by electrophoresis on a 1% agarose gel containing 0.5 μg/ml ethidium bromide.

Purification of the human REV1 protein

The 5′ 2.3 kb open reading frame of the human *REV1* cDNA was amplified from pWL296 by PCR, generating a DNA fragment flanked by *Xba*I (added by the 5′ PCR primer) and *Hin*dIII sites. This DNA fragment was cloned into the *Xba*I–*Hin*dIII sites of the yeast expression vector pEGLh6 (24). At its *Hin*dIII site, a 2 kb *Hin*dIII DNA fragment containing the missing 3′ end of the human *REV1* was then transferred from pWL296. The resulting plasmid pEGLh6-hREV1 codes for the full-length human REV1 protein tagged with six histidine residues at its N-terminus.

A yeast *rev1* deletion mutant strain was transformed with pEGLh6-hREV1 for regulated human *REV1* expression under the control of the *GAL1/10* promoter. Yeast cells containing pEGLh6-hREV1 were grown in minimum medium containing 2% sucrose to late logarithmic phase. Expression of the human

REV1 was induced by diluting the culture 10-fold in 16 l of YPG (2% Bacto-peptone, 1% yeast extract and 2% galactose) medium supplemented with sucrose to a final concentration of 0.5% and growth for 16 h at 30°C. Cells were collected by centrifugation at 6000 *g* for 10 min at 4°C and washed in water. After resuspending in an extraction buffer containing 50 mM Tris–HCl, pH 7.5, 600 mM KCl, 10% sucrose, 5 mM β-mercaptoethanol and protease inhibitors (25), cells were homogenized by zirconium beads in a Bead-beater (BioSpec Products) for 15 × 30 s pulses. The clarified extract (~100 ml) was loaded onto a Ni^{2+}-Sepharose column (10 ml) (Amersham Pharmacia Biotech), followed by washing the column with 100 ml of Ni buffer A (20 mM potassium phosphate, pH 7.2, 0.5 M NaCl, 20 mM imidazole, 10% glycerol, 5 mM β-mercaptoethanol and protease inhibitors). Bound proteins were eluted with a linear gradient of 20–135 mM imidazole (100 ml). The REV1 protein fractions were identified by western blots using a monoclonal anti-His antibody (Qiagene) and pooled. NaCl in the human REV1 sample was replaced with 50 mM KCl by passing the sample through a G-25 Sephadex column. Some protein precipitates were formed, which contained a significant amount of the human REV1 protein. The protein precipitates were recovered by centrifugation at 20 000 *g* for 10 min at 4°C and dissolved in a buffer containing 20 mM potassium phosphate, pH 7.2, 1 M KCl, 10% glycerol and 5 mM β-mercaptoethanol. This sample containing partially purified hREV1 was used for some activity assays. To further purify the human REV1 protein, the soluble fraction from the G-25 Sephadex column was loaded onto a FPLC Mono S HR 5/5 column (Amersham Pharmacia Biotech) that had been equilibrated in P buffer (20 mM KH_2PO_4, pH 7.4, 1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol and protease inhibitors) containing 50 mM KCl. The column was eluted with a linear KCl gradient from 50 to 500 mM in P buffer. The human REV1 eluted at ~190 mM KCl. The KCl concentration in the combined Mono S fractions was reduced to 50 mM by gel filtration through a G-25 Sephadex column, and subsequently loaded onto a FPLC Mono Q HR 5/5 column (Amersham Pharmacia Biotech). Column equilibration and elution conditions were as in the Mono S chromatography. The most pure human REV1 eluted at ~320 mM KCl.

Deoxycytidyl transferase assay

Deoxycytidyl transferase assays were performed essentially as described by Nelson *et al.* (15). The reaction mixture (10 µl) contained 25 mM potassium phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 0.1 mg/ml BSA, 10% glycerol, 5 mM dithiothreitol, 100 µM dNTP (dATP, dCTP, dGTP, dTTP or all four), 20 nM of 5′ end ^{32}P-labeled oligonucleotide primer annealed to an oligonucleotide template as indicated and protein sample. After incubation at 30°C for 30 min, reactions were terminated with 7 µl of stop solution (20 mM EDTA, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol). The reaction products were resolved on a 12% polyacrylamide gel containing 8 M urea and visualized by autoradiography. To obtain DNA substrate containing an abasic site, the uracil-containing substrate (10 pmol) was treated with 2 U *E. coli* uracil-DNA glycosylase at 37°C for 60 min. Under these conditions, the site-specific uracil residue was converted to an AP site in the template.

RESULTS

Cloning of the human *REV1* cDNA

Using the yeast Rev1 protein sequence, we searched the non-redundant GenBank CDS database for its homologs. A *Caenorhabditis elegans* hypothetical protein (ZK675.2) was identified. Alignment of 710 amino acid residues showed 27% identity and 44% similarity to the yeast Rev1. Thus, we conclude that this *C.elegans* protein is a homolog of the yeast Rev1. Using the *C.elegans* REV1 protein sequence, we subsequently searched the GenBank EST (expressed sequence tag) database and identified a human EST clone (GenBank accession number AA029147). Based on this clone, we searched the EST database again and identified two related EST clones (GenBank accession numbers AA393888 and T08134). Combining the three EST sequences, a partial 3′ cDNA sequence of the putative human *REV1* gene was obtained.

To isolate the full-length human *REV1* cDNA, we synthesized a 59mer oligonucleotide probe and screened three human cDNA libraries. As a result, an additional 5′ sequence of the putative human *REV1* gene was obtained. Subsequently, two additional 59mer oligonucleotide probes were synthesized and used to screen the human cDNA libraries. Forty cDNA clones were isolated, the largest of which contained an insert of 4 kb. The 5′ sequence of the putative human *REV1* gene was generated after sequencing. Finally, a cDNA clone containing both the 5′ and the 3′ sequences was reconstructed from partial cDNA clones (GenBank accession number AF151538). This cDNA (4255 bp) codes for a protein of 1251 amino acid residues with a calculated molecular weight of 138 248 Da and pI of 8.76. Upon searching the GenBank with our protein sequence, we identified the yeast Rev1 as its homolog ($P_N = 5 \times e^{-36}$). Hence, we conclude that our cDNA clone codes for a human homolog of the yeast mutagenesis protein Rev1. Accordingly, this cDNA and its gene is referred to as the human *REV1*.

The sequence context of the putative ATG start codon (CCACCATGA) in our human *REV1* clone matches well with the Kozak consensus sequence (CCACCATGG), which is commonly found surrounding the mammalian ATG initiator codon (26). However, the 5′ untranslated region of this human *REV1* cDNA does not contain an in-frame termination codon. Furthermore, two cDNA clones contained an intron-like sequence 5′ upstream of the position –10, in which the sequence context at the junction closely resembles the consensus sequence of the 3′ splicing site. These observations raised the question whether we have isolated the full-length human *REV1*. To answer this question, we first determined the size of the human *REV1* mRNA by a northern blot analysis. As shown in Figure 1, the human *REV1* mRNA was estimated to be 4.5 kb. This is in good agreement with the size of our cDNA clone (4.3 kb). Secondly, we screened a human genomic library using the human *REV1* cDNA as the probe. Two overlapping genomic clones were isolated. Sequencing these clones confirmed the presence of the 5′ splicing site and revealed multiple termination codons upstream of the cDNA sequence (GenBank accession number AF153594). These results show that we have isolated the full-length cDNA of the human *REV1*. Additionally, the results indicate that the first exon of the human *REV1* gene is non-coding.

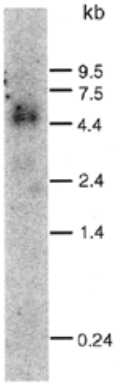

Figure 1. Northern blot analysis of the human *REV1* mRNA. An RNA sample prepared from normal human heart tissue was separated by electrophoresis and hybridized with a ^{32}P-labeled 59mer oligonucleotide probe specific to the human *REV1*, as described in Materials and Methods. The hybridized human *REV1* mRNA was visualized by autoradiography. The RNA size markers are indicated on the right (in kb).

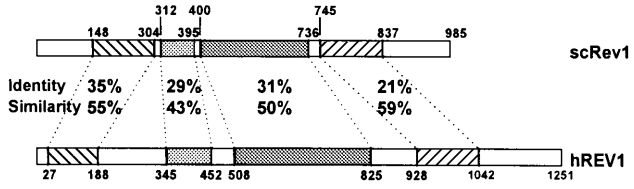

Figure 2. Conservation between the yeast and the human REV1 proteins. The yeast and the human REV1 protein sequences were aligned, and the significantly conserved regions of the proteins are schematically indicated by similarly shaded areas. The yeast Rev1 is shown at the top and the human REV1 at the bottom. The amino acid sequence identity and similarity within each conserved region are indicated.

In the human *REV1* cDNA, an out-of-frame ATG codon is located 32 nt upstream of the initiator codon, which could potentially direct the synthesis of a polypeptide of 12 amino acids. Translation from this first ATG codon would lead to an aborted human REV1 protein synthesis. Thus, the translational efficiency of the human *REV1* mRNA may be reduced.

Conservation of REV1 protein sequences from yeast to humans

Sequence alignment between the yeast and the human REV1 proteins revealed significant homology (Fig. 2). Four conserved regions were identified with amino acid sequence identities of 21–35% and similarities of 43–59% (Fig. 2). After we had cloned the human *REV1* cDNA, the *Arabidopsis thaliana* and the *Schizosaccharomyces pombe* REV1 homologs (GenBank accession numbers AC002342 and AL035548, respectively) were also identified from the genomic sequencing projects. Again, protein sequence conservation was found among these proteins (Fig. 3). Comparison of various REV1 proteins revealed a BRCT (BRCA1 C-terminus) domain at their N-terminal regions and five sequence motifs (Fig. 3).

Chromosomal localization of the human *REV1* gene

Using the human *REV1* cDNA as the probe, we isolated two human *REV1* genomic clones from a library. One clone contained a sequence tagged site (STS), EST164698 (GenBank accession number G25709), upstream from the 5′ end of the human *REV1* gene. The distance between this STS and the 5′ sequence of the human *REV1* cDNA was estimated to be 20 kb by PCR using either the genomic clone or the total genomic DNA isolated from human placenta (data not shown). The location of this STS was assigned to 512.6 cR from the top of chromosome 2 linkage group by radiation hybrid mapping of marker SGC33758 by the Whitehead Institute/MIT Center for Genome Research. On GeneMap'98, it was further mapped to physical position: 355.80 cR_{3000} ($P > 3.00$) between reference intervals D2S113–D2S176 (115.3–120.8 cM). These markers are localized between 2q11.1 and 2q11.2 on the cytogenetic ideogram. Therefore, we conclude that the human *REV1* gene is located between 2q11.1 and 2q11.2.

Expression of the *REV1* gene in human tissues

In yeast, the Rev1-involved mutagenesis pathway is a major mechanism for generating mutations after DNA damage. However, it is not known whether this pathway functions in various human tissues. Thus, we examined the expression of the *REV1* gene as an indication of the importance of this putative mutagenesis pathway in various human tissues. As shown in Figure 4, the human *REV1* expression was detected by RT–PCR in all of the 16 tissues examined. Hence, we conclude that the *REV1* gene is ubiquitously expressed in humans.

The human REV1 protein is a dCMP transferase

The yeast Rev1 protein possesses a deoxycytidyl transferase activity, which transfers a dCMP residue to the 3′ end of a DNA primer opposite a template G or an AP site (15). To determine whether the human REV1 protein is a dCMP transferase, we first partially purified the protein and then assayed for dCMP transferase activity. To facilitate detection and purification of the human REV1, we tagged the protein with six histidine residues at its N-terminus, and expressed it in yeast *rev1* deletion mutant cells. The tagged human REV1 was purified by affinity chromatography on a nickel–Sepharose column. As a control, we used *rev1* deletion mutant extracts for identical purification. Using a primed 40mer DNA template (Fig. 5A), we assayed the transferase activity of the partially purified human REV1. As shown in Figure 5B (lane 10), a transferase activity was detected that extended the ^{32}P-labeled primer by 2 nt opposite the two template G residues. In contrast, the control sample without the human REV1 did not contain any detectable transferase activity (Fig. 5B, lane 9), indicating that the transferase activity is specific to the human REV1 protein. To identify the nucleotides transferred opposite the template G residues, we performed the transferase assays with dATP, dCTP, dGTP or dTTP individually, rather than all four dNTPs together. Only dCTP supported the transferase activity (Fig. 5B, lane 4). Again, the transferase activity was not detected with the control sample without the human REV1 protein (Fig. 5B, lanes 1, 3, 5 and 7). The transferase activity was not observed opposite a template A, C or T (data not

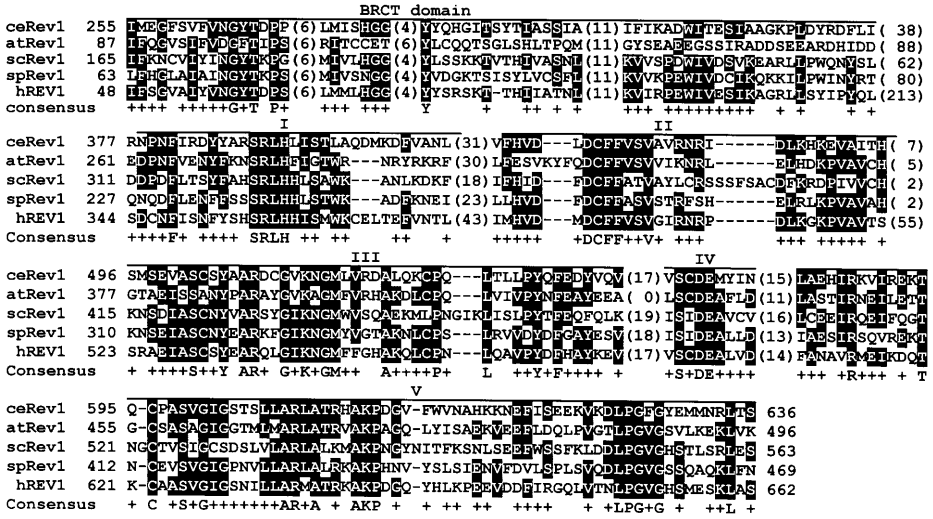

Figure 3. Conserved structural domain and sequence motifs of REV1 proteins from various biological sources. Several sequence features were identified by aligning REV1 protein sequences of various organisms as indicated. Identical amino acid residues are shown in reverse type. Similar amino acid residues are shown as '+' in the consensus sequence. Numbers in parentheses indicate gaps in the alignment. BRCT domain, the BRCA1 C-terminus domain; I–V, REV1 protein sequence motifs I–V. The REV1 sources are: ce, *C.elegans* (GenBank accession number Z46812); at, *A.thaliana* (GenBank accession number AC002342); sc, *S.cerevisiae* (GenBank accession number M22222); sp, *S.pombe* (GenBank accession number AL035548); h, *Homo sapiens* (GenBank accession number AF151538).

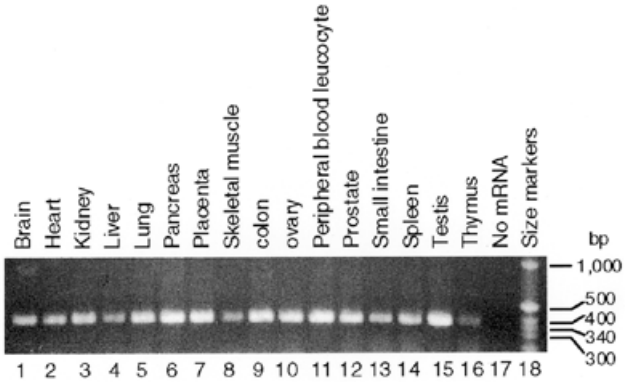

Figure 4. Expression of the *REV1* gene in various human tissues. First strand cDNA was synthesized from poly(A)$^+$ mRNA of various human tissues as indicated and normalized against the constitutive gene glyceraldehyde-3-phosphate dehydrogenase. A 360-bp DNA fragment corresponding to position +773 to +1132 of the human *REV1* cDNA was amplified by 35 cycles of PCR as described in Materials and Methods. DNA products were separated by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining. The size markers are shown on the right (in bp).

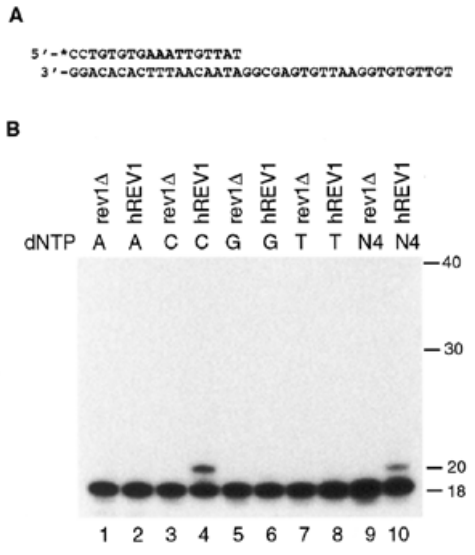

Figure 5. The dCMP transferase activity of the human REV1 protein. (**A**) The DNA substrate used for dCMP transferase assays. The 18mer primer was labeled with ^{32}P at its 5′ end. (**B**) Standard dCMP transferase assays were performed in the reaction buffer containing a single dNTP (A, C, G or T, lanes 1–8) or all four dNTPs (N4, lanes 9 and 10) as described in Materials and Methods. Protein samples used were 2 µl of the partially purified human REV1 (lanes 2, 4, 6, 8 and 10), or 2 µl of an identically purified protein fraction from the *rev1* deletion mutant cells (lanes 1, 3, 5, 7 and 9). DNA size markers are indicated on the right (in nt).

shown). Hence, we conclude that the human REV1 protein is a dCMP transferase, which transfers dCMP opposite a template G. Supporting this conclusion, the transferase activity co-purified with the human REV1 as revealed by western blots during nickel–Sepharose column chromatography (data not shown). In the control purification from *rev1* deletion mutant extracts, none of the fractions contained the transferase activity (data not shown).

To examine whether the transferase activity of the human REV1 functions opposite a template AP site, we prepared a site-specific uracil-containing template (Fig. 6A). Treatment

with uracil-DNA glycosylase completely converted the uracil-containing templates into AP site-containing templates, as revealed by the AP site cleavage with the *E.coli* endonuclease III (Fig. 6B, lane 2). Transferase activity of the human REV1 was detected opposite the template AP site (Fig. 6C, lane 9). A template U also supported the human REV1 transferase activity (Fig. 6C, lane 10). This is also observed with the yeast Rev1 protein (15). However, unlike the yeast protein, which utilizes the template AP site much more efficiently than the template U for its transferase activity (15), the human REV1 uses both the template AP site and uracil efficiently (Fig. 6C, compare lanes 9 and 10). To identify the nucleotides transferred opposite the template AP site or uracil, we performed the transferase assays with only one deoxynucleoside triphosphate, dATP, dCTP, dGTP or dTTP. As shown in Figure 6C (lanes 3 and 4), only dCTP supported the human REV1 transferase activity opposite the template AP site or uracil. These results show that the human REV1 protein is a template-dependent dCMP transferase that is active opposite a template G, U or AP site.

The yeast *REV1* gene had been deleted from the host cells used for the human REV1 expression and purification. Thus, the yeast Rev1 could not have contaminated our human REV1 protein preparations. Nevertheless, to provide further support to our conclusion that the human REV1 is a dCMP transferase, we purified the protein to apparent homogeneity (Fig. 7A and B). Again, a transferase activity opposite an AP site was observed with the pure human REV1 preparation (Fig. 7C). Additionally, we performed the transferase assay opposite a template G using the pure human REV1 protein. The dCMP transferase activity was detected (data not shown). These results show that the observed dCMP transferase activity is associated with the human REV1 protein.

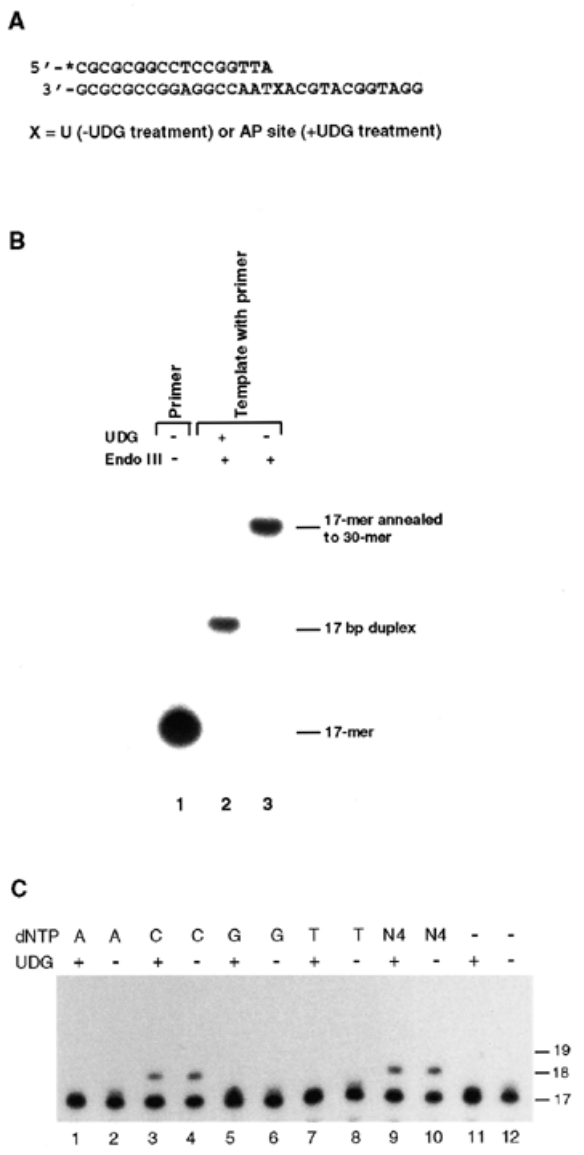

Figure 6. Transferase activity of the human REV1 protein opposite a template AP site. (**A**) The DNA substrates used for dCMP transferase assays. The X position is a U without uracil-DNA glycosylase (UDG) treatment, or an AP site with UDG treatment. The 17mer primer was labeled with ^{32}P at its 5′ end. (**B**) Complete conversion of uracil-containing templates into AP site-containing templates. After converting the site-specific uracil residue into an AP site by UDG treatment (UDG, +), the template (0.4 pmol) was incubated with 500 ng of *E.coli* endonuclease III at 37°C for 30 min (Endo III, +). Endo III cleaves DNA strand specifically at the AP site. The reaction products were separated by electrophoresis on a 15% native polyacrylamide gel and visualized by autoradiography. Lanes 1 and 3 are controls without any treatment or with Endo III treatment only, respectively. (**C**) Standard dCMP transferase assays were performed with 2 µl of the partially purified human REV1 in the reaction buffer containing a single dNTP (A, C, G or T, lanes 1–8) or all four dNTPs (N4, lanes 9 and 10) as described in Materials and Methods. UDG +, AP site template; UDG –, uracil-containing template. Lanes 11 and 12, control experiments without dNTPs in the reaction mixtures. DNA size markers are indicated on the right (in nt).

DISCUSSION

DNA damage-induced mutagenesis is an important cellular response to unrepaired DNA lesions during replication. The biological outcome of this pathway is enhanced cell survival and increased mutations following DNA damage. The yeast *S.cerevisiae* has served as the most informative model organism in studies of the damage-induced mutagenesis pathway in eukaryotes. Yeast genetic analyses have implicated at least seven genes in this mutagenesis pathway, including *REV1* (27)

We have isolated a full-length cDNA of the yeast *REV1* counterpart in humans. The REV1 protein is conserved from yeast to humans. Some regions share over 30% identity and more than 50% similarity between the yeast and the human proteins. The REV1 protein is additionally found in *S.pombe*, *C.elegans* and *A.thaliana*, with significant sequence homologies among them. Thus, the function of REV1 protein in the damage-induced mutagenesis pathway may have been preserved in various eukaryotic organisms during evolution. REV1 proteins of various sources all contain an N-terminal BRCT domain. It was originally identified in the breast cancer suppressor protein BRCA1 and subsequently found in some other proteins involved in cell cycle checkpoints, DNA repair and in recombination, such as Rad9, p53-binding protein, XRCC1 and DNA ligases III and IV (28–30). This structural domain is important in protein–protein interactions (31). Thus, it is likely that REV1 may interact with other proteins during damage-induced mutagenesis, although none of the REV1 interactions have been identified. Additionally, REV1 proteins contain several conserved sequence motifs (I–V), which

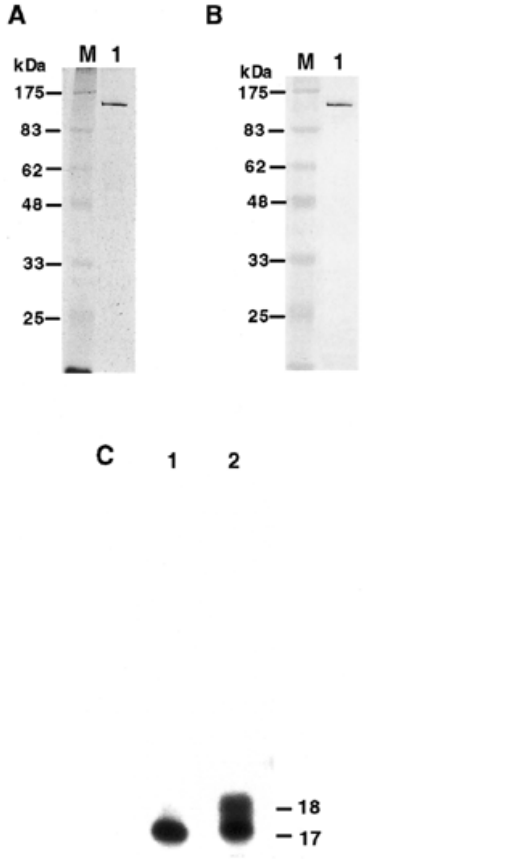

Figure 7. Pure human REV1 protein and its transferase activity. To confirm that the dCMP transferase activity is intrinsic to the human REV1, the protein was purified to apparent homogeneity as described in Materials and Methods. (**A**) The most pure Mono Q fraction was separated by electrophoresis on a 10% SDS–polyacrylamide gel using 10 µl of the sample. The His-tagged human REV1 protein was visualized by silver staining. Protein size markers (lane M) are indicated on the left (in kDa). (**B**) The identity of the human REV1 protein was confirmed by western blot using a monoclonal antibody against the His tag. (**C**) A transferase assay was performed using the AP site-containing template (see Fig. 6A) without (lane 1) or with (lane 2) the pure human REV1 protein (2 µl, ~10 ng) in a reaction volume of 5 µl at 30°C for 30 min. The reaction products were separated by electrophoresis on a 12% sequencing gel and visualized by autoradiography. DNA size markers are indicated on the right (in nt).

closely resemble those of *E.coli* UmuC-related proteins (14). Identifying these conserved motifs should be useful for future structure–function studies of REV1.

Examination of the 5′ untranslated region of the human *REV1* cDNA revealed the presence of an out-of-frame ATG at nucleotide position –35 which initiates an ORF of 12 codons and terminates at position +2. The stop codon of this mini ORF overlaps with the human *REV1* initiator ATG codon. The sequence context of this upstream ATG is close to the consensus Kozak sequence (26). Thus, it is likely that the translational efficiency of the human *REV1* message may be reduced by the presence of this upstream mini ORF. Structural features of the human *REV3* gene also suggest a low-level expression (21,22). These features imply that under normal growth conditions human cells may contain limited amounts of the mutagenesis proteins.

Most recently, by employing the yeast two-hybrid system Wixler *et al.* (32) identified a partial human cDNA whose polypeptide interacts with the cytoplastic domain of the α3A integrin subunit. This cDNA clone (alpha integrin interacting protein 80, AIBP80) corresponds to the 2.6 kb of the 3′ end of our human *REV1* cDNA, with a few sequence discrepancies. This sequence was localized by the Sanger Centre between 2q11.1 and 2q11.2, a region identical to our human *REV1* chromosomal location. Since integrins are transmembrane receptors that provide a link between extracellular matrix proteins such as laminins and the intracellular cytoskeleton (32), it is not obvious at present as to how such an integrin–REV1 interaction is involved in damage-induced mutagenesis. Thus, the physiological relevance of this protein interaction needs to be further examined.

We found that the human REV1 protein is a dCMP transferase capable of inserting a dCMP opposite a template AP site. This activity provides evidence supporting a role of the REV1 protein in damage-induced mutagenesis in humans. *In vitro*, the human REV1 dCMP transferase functions efficiently opposite a template AP site. Thus, the human REV1 transferase may play a critical role during mutagenic translesion DNA synthesis opposite a template AP site *in vivo*. Supporting this notion, Johnson *et al.* (33) recently demonstrated that AP site-induced mutagenesis in yeast requires the Rev1 protein. Our results also suggest that the damage-induced mutagenesis pathway will incorporate a C residue opposite an AP site during human DNA replication, regardless of the original base identity previously residing at the AP site. Since *REV1* is also needed for UV-induced mutagenesis in yeast (27) (D.Rajpal, X.Wu and Z.Wang, unpublished results), additional function of the protein must be involved during mutagenesis opposite other DNA lesions.

Yeast Rev1 is also a dCMP transferase (15). Thus, detection of the dCMP transferase activity of the human REV1 indicates that it is both a structural and a functional homolog of the yeast protein. Additionally, humans contain highly conserved homologs of the yeast mutagenesis proteins Rad6 (19,20) and Rev3 (21,22). Taken together, these observations clearly indicate the existence of a damage-induced mutagenesis pathway in humans in response to DNA lesions. Further supporting this conclusion, Gibbs *et al.* (21) showed that UV-induced mutagenesis in human cells requires the human REV3. We suspect that this damage-induced mutagenesis pathway is likely a fundamental and major mechanism for generating mutations in humans after DNA damage. Consistent with this hypothesis, we observed ubiquitous expression for both *REV1* and *REV3* (22) in various human tissues. The isolation of the human *REV1* cDNA and identification of its dCMP transferase activity should facilitate molecular and biochemical studies of the damage-induced mutagenesis pathway in humans.

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