A Human REV7 Homolog That Interacts with the Polymerase ζ Catalytic Subunit hREV3 and the Spindle Assembly Checkpoint Protein hMAD2*

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Widespread alteration of the genomic DNA is a hallmark of tumors, and alteration of genes involved in DNA maintenance have been shown to contribute to the tumorigenic process. The DNA polymerase ζ of Saccharomyces cerevisiae is required for error-prone repair following DNA damage and consists of a complex between three proteins, scRev1, scRev3, and scRev7. Here we describe a candidate human homolog of S. cerevisiae Rev7 (hREV7), which was identified in a yeast two-hybrid screen using the human homolog of S. cerevisiae Rev3 (hREV3). The hREV7 gene product displays 22% identity and 53% similarity with scREV7, as well as 23% identity and 54% similarity with the human mitotic checkpoint protein hMAD2. hREV7 is located on human chromosome 1p36 in a region of high loss of heterozygosity in human tumors, although no alterations of hREV7 or hREV3 were found in primary human tumors or human tumor cell lines. The interaction domain between hREV3 and hREV7 was determined and suggests that hREV7 probably functions with hREV3 in the human DNA polymerase ζ complex. In addition, we have identified an interaction between hREV7 and hMAD2 but not hMAD1. While overexpression of hREV7 does not lead to cell cycle arrest, we entertain the possibility that it may act as an adapter between DNA repair and the spindle assembly checkpoint.

DNA damage is induced by a variety of endogenous and exogenous factors (1). Such DNA alterations include reactive oxygen damage, deamination, loss of nucleotides, nucleotide modifications, and DNA strand breaks. DNA damage repair has evolved to cope with these environmental and mutagen-induced DNA alteration and plays a central role in maintaining the genetic stability of the organism (2, 3).

Extensive studies of bacterial and yeast systems have identified components of the DNA repair machinery. In the yeast Saccharomyces cerevisiae, pyrimidine dimers induced by UV radiation damage are corrected by the RAD3 excision repair, the RAD6 postreplication repair, and the RAD52 recombinational repair pathways (for a review see Ref. 4). Interestingly, the removal of UV damage by the RAD6 pathway occurs by both error-free and error-prone (mutagenic) mechanisms (5, 6). The mutagenic repair of UV damage has been shown to require the UV revertible genes, REV1, REV3, and REV7, a lesion bypass polymerase complex consisting of a deoxycytidyl-transferase (Rev1), a polymerase catalytic subunit (Rev3), and a polymerase accessory protein (Rev7) (for review see Refs. 7 and 8). This polymerase complex has been termed polymerase ζ.

Both the genetic and biochemical evidence suggest that the polymerase ζ is capable of translesion DNA synthesis across abasic sites, pyrimidine dimers, and modified nucleotides (9–11). In the case of abasic sites, the Rev1 deoxycytidyl transferase appears to introduce a cytosine opposite the lesion (9). The Rev1 gene product displays weak homology with bacterial UmuC protein, which functions in damage-induced SOS mutagenesis in Escherichia coli (12). The bacterial RecA-activated UmuDζC in concert with polymerase III appears to perform a similar function to the yeast polymerase ζ (13, 14). The Rev3 gene product contains several of the conserved sequence motifs found in DNA polymerases and appears to form a complex with Rev7 protein to construct the DNA polymerase ζ complex (7, 15). The Rev7 gene product has no reported similarities to any known proteins, and its function is unknown (16).

Recently, hREV3, the likely human homolog of S. cerevisiae Rev3 (scRev3), was identified and found to be almost twice the size of the scRev3 product (17–19). Human cells expressing an hREV3 antisense RNA fragment grew normally but appeared slightly more sensitive to UV and displayed little or no UV-induced mutagenesis (18), suggesting that hREV3 might function in a similar way to scRev3. The human homologs of scRev1 and scRev7 have not been reported.

Chromosomal instability is thought to be one of several underlying causes of cancer development (20–22). Moreover, spindle assembly checkpoint genes have been found to play important roles in maintaining chromosome integrity (for a review, see Refs. 23–25). The spindle assembly checkpoint appears to prevent the early onset of anaphase in cell cycle until all of the mitotic spindles are attached to the kinetochores on chromosomes and all of the chromosomes are aligned properly at the metaphase plate. Mutants of the spindle assembly checkpoint have been identified in S. cerevisiae based on a defect in M phase cell cycle arrest after treatment with a microtubule-depolymerizing drug, which include the following: MAD (mitotic arrest-deficient) 1–3 and BUB (budding uninhibited by benzimidazole) 1–3 (26–29). In addition, serial studies in S.

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cerevisiae have also identified other genes that appear to take part in the M phase cell cycle arrest and include MPS1 (monopolar spindle 1), CDC55 (cell division cycle 55), and CDC28 (cyclin-dependent kinase) (30–32).

The human homologs of these S. cerevisiae spindle assembly checkpoint genes have also been identified. hMAD2 was isolated in a screen for high copy number suppressors of thiamin-bendazole sensitivity in yeast cells lacking CBF1, a component of the kinetochore (33). hMAD1 was isolated as a cellular target of the human T-cell lymphotrophic virus-1 oncprotein Tax using a yeast two-hybrid screen (34). hBUB1 and hBUBR1 were isolated by expressed sequence tag (EST)2 search as homologous genes with S. cerevisiae scBUB1 (35), whereas hBUB3 was isolated in an EST search for homolog(s) of scBUB3 (36). Although these genes have been identified in human cells as possible spindle assembly checkpoint genes, their definitive relationship to the spindle assembly process is not fully understood. Interestingly, rare mutations of hBUB1 have been found in colorectal tumors (35).

Here, we describe the identification of a candidate for hREV7, the human homolog of the scRev7 protein. Interestingly, hREV7 displays significant homology to hMAD2. The hREV7 gene was initially identified based on its strong interaction with hREV3 using the yeast two-hybrid system. We have additionally characterized the interaction regions between hREV7 with hREV3 using a combination of yeast two-hybrid and GST fusion in vitro binding systems and further demonstrate hREV7 interaction with hMAD2 but not hMAD1. Although we found no effect on G2-M arrest by overexpression hREV7 in human cells, these results appear to support the notion that the human mutagenic bypass DNA polymerase ζ contacts the spindle assembly checkpoint via hMAD2, which in turn associates with hMAD1 and/or the CDC20/APC complex.

MATERIALS AND METHODS

Cloning of the hREV3 cDNA—The sequence of scREV3 was used to screen the Human Genome Sciences computer data base with the TFASTA computer software designed by the Genetics Computer Group (University of Wisconsin, Madison, WI). One EST clone (GenBank accession no. R12903) was found to have significant homology with scREV3. PCR primers were designed based on the EST sequence, and PCR-amplified fragments from human peripheral blood cDNA were used as probes in the screening of a human cDNA library. Serial screenings of human testis ADR2 cDNA library (CLONTECH) were performed by the conventional plaque hybridization method to obtain the complete cDNA sequence of hREV3. The phage DNAs of isolated positive clones were purified using the QIAGEN Lambda Kit (Qiagen). Double strand sequencing of all of the positive clones was performed by an automated Applied Biosystems sequencer model 377 (Applied Biosystems). The sequence of the 5′-terminal end was determined by 5′-rapid amplification of cDNA ends (RACE) method using Marathon-Ready human testis cDNA (CLONTECH). The RACE products were cloned into pBluescript SK(+) (Stratagene) and sequenced. All sequences were confirmed by reverse transcription (RT)-PCR.

Yeast Two-hybrid Assay—Yeast two-hybrid screening was performed in the Y190 yeast strain using the Matchmaker Two-hybrid System 2 (CLONTECH) according to the manufacturer’s protocol. Twenty-five mlt 3-aminostriazole was added to the medium to inhibit His3p expression activated by GAL4 DNA binding domain fusion hREV3 alone. We screened 5 × 10⁶ independent clones on 150-mm dishes for each screening. Quotient β-galactosidase assay was performed to estimate false positives according to the manufacturer’s instructions. Liquid culture assay for β-galactosidase activity was also performed to check the interactions quantitatively according to the manufacturer’s protocol.

2 The abbreviations used are: EST, expressed sequence tag; IVTT, in vitro transcription-translation; GST, glutathione S-transferase; PCR, polymerase chain reaction; RT, reverse transcriptase; ORF, open reading frame; APC, anaphase-promoting complex; RACE, rapid amplification of cDNA ends; bp, base pair(s).

pACT2 vectors in the positive clones were extracted and subjected to sequencing.

Identification of cDNA Sequence, Genomic Structure, and Chromosomal Location of hREV7—Human testis agt11 cDNA library (CLONTECH) was screened using the PCR probe derived from the insert of pACT2/hREV7 plasmid by the conventional plaque hybridization method to obtain the full-length hREV7 cDNA. The phage DNAs from isolated positive clones were purified using the QIAGEN Lambda Kit (Qiagen) and sequenced. 5′-RACE and RT-PCR were also performed to confirm the sequence. Human genomic cosmid library (Stratagene) was screened using hREV7 cDNA as a probe, isolated positive clones were purified using the QIAGEN Plasmid Kit (Qiagen), and the DNA was sequenced directly to elucidate the intron-exon boundaries of hREV7. To determine the chromosomal location of hREV7, sets of PCR primers were designed to amplify gene-specific genomic fragments, and they were used for PCR screening of the GeneBridge 4 radiation hybrid mapping panel (Research Genetics). The result of the screening was submitted to the Whitehead Institute/MIT Center for Genome Research and was analyzed with the statistical program RHMAP.

Northern Blot Analysis—Human multiple tissue Northern blot and human cancer cell line Northern blot were purchased from CLONTECH and were hybridized with hREV7 cDNA or human β-actin cDNA probe using standard methodologies (37).

Mutation Analysis—For cell lines in which RNA was available, the full-length open reading frame (ORF) of hREV7 was amplified by RT-PCR using SuperScript reverse transcriptase (Life Technologies, Inc.) and Pfu DNA polymerase (Stratagene). The PCR products were purified via a PCR purification kit (Qiagen) and were sequenced directly with internal primers. In the cell lines and tumors where only genomic DNA was available, the exons that contain the ORF sequences of hREV7 were amplified individually with Pfu DNA polymerase, and the PCR products were purified and then sequenced directly by using internal primers. All of the sequences were compared with that of wild type hREV7.

Cloning of hMAD1 and hMAD2 cDNAs—The full-length ORFs of hMAD1 and hMAD2 cDNAs were amplified by RT-PCR with Pfu DNA polymerase using pairs of gene-specific primers that were designed according to the published sequences (GenBank accession no. U33922 and U31278). The PCR products were cloned in the pGEX vector (Amersham Pharmacia Biotech) and pET vector (Novagen) and sequenced in their entirety. These vectors were used for protein expression and in vitro protein-protein interaction assays.

In Vitro Protein-Protein Interaction—GST fusion proteins were expressed in E. coli and were immobilized on glutathione-agarose beads (Amersham Pharmacia Biotech). Radiolabeled proteins were synthesized using a coupled in vitro transcription-translation (IVTT) system according to the manufacturer’s instructions (Promega). Protein-protein interaction was examined by a method similar to that of Guertette et al. (38). Briefly, 25 μl of glutathione-agarose beads containing 5 μg of a GST fusion protein or GST protein (alone) were incubated with radiolabeled proteins at 4 °C for 1 h in 200 μl of buffer A (50 mM Tris, 0.5 M NaCl, 5 mM EDTA, 10% glycerol, 0.75 μg/ml bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin) or buffer B (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.75 mg/ml bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin). The beads were washed 3× in the same binding buffer, and bound proteins were eluted by boiling in 1× sample buffer and analyzed on SDS-polyacrylamide gels followed by autoradiography.

Antibody Production—Rabbit polyclonal anti-hREV7 antibody was produced by immunization with keyhole limpet hemocyanin-conjugated peptide containing hREV7, and affinity-purified as described previously (39).

Western Blot—Cells were disrupted in lysis buffer (20 mM Hepes, pH 7.6, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM dithiotreitol, 0.1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 5 μg/ml pepstatin A) with freeze and thaw cycles. The cell lysate was electrophoresed by SDS-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane (Bio-Rad), probed with a anti-hREV7 antibody, and visualized using the ECL Western blotting detection reagent (Amersham Pharmacia Biotech).

Cell Cultures and Reagents—U2OS cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. ErkR23 cells transfected with pVgRXR that has an ecdysone receptor gene and has an ecdysone-inducible expression system (Invitrogen). A plasmid, pMD/hREV7, that con-
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RESULTS

Cloning of hREV3—A search of GenBank™ and the Human Genome Sciences data base revealed the presence of human ESTs that had significant homology with scREV7 (GenBank™ accession no. R12903). An initial screen of a human testis cdNA library (Clontech) performed using an EST-R12903-specific probe produced several clones that did not appear to contain the full-length hREV3 cdNA. We performed additional screens using successive N-terminal probes in a serial screening of human testis cdNA library. In addition, 5'-RACE was also performed using human testis cdNA to determine the 5' sequences. We identified three different 5'-RACE species, two of which appeared identical to previously published alternative splice products (Fig. 1, A and B) (18). In addition, we identified a larger 5'-RACE product that contained an insertion of 107 bp compared with Fig. 1B (Fig. 1C). Thus, there appear to be three splice variants in hREV3 transcript, the smallest one containing an ORF of 9,390 bp (Fig. 1A) and the other two containing an ORF of 9,159 bp (Fig. 1, B and C). The smallest transcript contains an additional 77 amino acid at the N terminus of hREV3 and appears to display the highest homology with that of scREV7. A complete cdNA of the largest 150 bp insert was amplified by RT-PCR in some cell lines where RNA was available, while in the other cell lines and tumor samples, all exons containing the ORF sequence were amplified by PCR using genomic DNAs as templates and sequenced directly. We found no mutations of the hREV7 gene in any of the cell lines or tumor samples.

Identification of hREV7—To examine the function of hREV3, we performed a yeast two-hybrid search for interacting proteins. Because the entire hREV3 gene was found to be too large for efficient expression by the yeast system, we divided it into three parts: hREV3a (containing amino acid residues 1–949), hREV3b (residues 949–1,775), and hREV3c (residues 1,776–3,130). These fragments were subcloned separately in pAS2–1 vectors that express a GAL4 DNA binding domain fusion of hREV3a, -b, and -c proteins (pAS2–1/hREV3a, pAS2–1/hREV3b, and pAS2–1/hREV3c, respectively) (see Fig. 4B). Each vector was used as a "bait" for screening a human testis cdNA expression library constructed in pACT2. We screened approximately 5 × 10^6 independent clones for each construct and performed sequential β-galactosidase assays. Two positive clones were identified from the pAS2–1/hREV3c only, and both inserts appeared to be derived from the same gene. We screened a human testis λgt11 cdNA library by using the probe of one insert and obtained a complete cdNA of 1,163 bp containing a 633-bp ORF with a predicted protein of 211 amino acid residues and an expected molecular mass of 24 kDa. There was an in-frame STOP codon at −150 bp. The amino acid sequence of this gene product displayed 23% identity and 53% similarity with that of scREV7 using the standard GCG analysis (Fig. 2). Interestingly, this gene product also displayed 23% identity and 54% similarity with hMAD2, one of the spindle assembly checkpoint genes (Fig. 2). Based on its similarity to scREV7 and its interaction with hREV3, it is likely that the gene is the human homolog of scREV7 (hREV7).

Characterization of hREV7—To obtain the genomic locus, a human genomic cdNA library was screened using hREV7 cdNA as a probe. We isolated six cosmids clones, and their DNAs were purified and partially sequenced. Intron-exon structure was determined from the sequences of both cdNA and genomic locus, which revealed that the hREV7 locus contains nine exons covering a region of 6.5 kilobase pairs (Fig. 3A). The hREV7 ORF starts in exon 2 and ends in exon 9. Northern hybridization analysis using a human multiple tissue Northern blot showed ubiquitous expression of a single 1.3-kilobase hREV7 mRNA in all tissues, with the highest level in testis followed by thymus, spleen, and peripheral blood leukocyte (Fig. 3B). Western blot analysis using an anti-hREV7 antibody against a U2OS cell lysate showed one major product that was the same size as the hREV7 IVTT product (Fig. 3D). A minor product of approximately 19 kDa was observed in both immune and preimmune serum.

Using the GeneBridge 4 radiation hybrid mapping panel, hREV7 was found to be located on human chromosome 1p36, a region of high loss of heterozygosity in some types of human tumors (40–44). This result prompted us to search for mutations of hREV7 in human tumor cell lines and tumor samples. Northern blot analysis did not reveal any alterations in the hREV7 transcript compared with that of human normal tissues (Fig. 3C), although the expression appeared to vary considerably between several of these tumor cell lines. We performed sequence analysis of hREV7 on 12 neuroblastoma cell lines, 11 melanoma cell lines, 18 breast tumor cell lines, 20 clinical breast tumor samples, 9 colon tumor cell lines, and 13 clinical colon tumor samples. Full-length ORFs were amplified by RTPCR in some cell lines where RNA was available, while in the other cell lines and tumors, all exons containing the ORF sequence were amplified by PCR using genomic DNAs as templates and sequenced directly. We found no mutations of the hREV7 gene in any of the cell lines or tumor samples.

Interaction between hREV3 and hREV7—To confirm the interaction between hREV3 and hREV7, we constructed "reversed" two hybrid vectors containing hREV7 fused to the GAL4 DNA binding domain and hREV3c fused to the GAL4 transcription activation domain. A positive interaction between hREV3 and hREV7 was easily demonstrated (Fig. 4A). To determine the interaction regions of hREV3 with hREV7, we subcloned the fragments of hREV3 (Fig. 4B) in pACT2 vector.
and examined the interaction between full-length hREV7. In addition to the qualitative colony color system, we performed quantitative liquid culture β-galactosidase assays on all of the constructs (Fig. 4B). We found that hREV3c, hREV3d, and hREV3g fragments displayed both a qualitative and quantitative interaction with hREV7, while hREVa, hREVb, and hREVf displayed no interaction. These results suggest that the interaction between hREV3 and hREV7 is located in a region between amino acid residues 1776 and 2195.

To confirm the interaction between hREV3 and hREV7, we developed a GST/IVTT assay system. A GST fusion containing hREV7 protein was produced in bacteria and purified by binding to glutathione-agarose beads (Amersham Pharmacia Biotech). Radiolabeled hREV3 fragments (Fig. 4C) were synthesized by IVTT (Promega) and tested for binding to GST-hREV7 compared with a control containing the GST moiety alone. We found that only the hREV3j fragment, which contained hREV3 amino acid residues 1776–2455, bound the GST-hREV7 fusion protein (Fig. 4C). Conversely, IVTT hREV7 protein was found to bind a GST-hREV3g fragment containing amino acid residues 1776–2195 (Fig. 4D). These results are consistent with the yeast two-hybrid data and suggest that hREV7 associates with hREV3 in a minimal region of hREV3 that encompasses amino acid residues 1776–2195.

Interaction of hREV7 with hMAD2—Because the amino acid sequence of hREV7 displayed significant homology with hMAD2 protein and most spindle assembly checkpoint proteins appear to interact with one another, we examined the interaction between hREV7 with hMAD1 and hMAD2 (Fig. 5). We found that IVTT hREV7 protein bound the GST-hMAD2 fusion protein but not GST alone, and conversely IVTT hMAD2 protein bound the GST-hREV7 fusion protein but not GST alone (Fig. 5A). These results suggest that hREV7 may interact with hMAD2.

The observed interaction between hREV7 and hMAD2 suggested the possibility of an even more complex interaction that might include hREV3. To test this possibility, we used a GST-hMAD2 fusion protein and IVTT hREV7 and hREV3 (fragment j containing the hREV7 interaction region). We observed no interaction between the control GST protein and IVTT of either hREV7 or hREV3j (Fig. 5B). Moreover, the GST-hREV7 interacted strongly with the IVTT hREV3j fragment, while the GST-hMAD2 exhibited a moderate interaction with IVTT hREV7 (Fig. 5B). Interestingly, the GST-hMAD2 appeared to interact with IVTT hREV3j, and the inclusion of IVTT hREV7 in this mix reproducibly increased the interaction of hREV7 with GST-hMAD2 (in the presence of hREV3j) (Fig. 5B). These results suggest that hREV3, hREV7, and hMAD2 appear to be capable of forming a stable triprotein complex.

Since hMAD2 is known to interact with hMAD1, we speculated that it might be an additional member of the hREV3/hREV7/hMAD2 complex. While we could easily demonstrate interaction between GST-hREV7 and IVTT hMAD2 and GST-hMAD2 and IVTT hMAD1, we found no interaction between GST-hREV7 and IVTT hMAD1 (Fig. 5C). Moreover, the inclusion of both IVTT hMAD1 and IVTT hMAD2 with GST-hREV7 resulted in precipitation of IVTT hMAD2 only (Fig. 5C, last lane). These results suggest that an interaction between GST-
hREV7 with IVTT hMAD2 is incapable of additionally co-precipitating hMAD1. The lack of interaction between hMAD1 would appear to further strengthen the argument that the candidate hREV7 is not a functional homolog of MAD2. We entertain the possibility that hREV7 and hMAD1 are competitors for binding to hMAD2.

Overexpression of hREV7 Does Not Affect the Cell Cycle—This interaction between hREV7 and hMAD2 suggested that hREV7 might affect the spindle assembly checkpoint. In a screen of stable transfectants, we identified three inducible cell lines containing hREV7 controlled by the ecdysone promoter (Invitrogen). The expression levels prior to induction and after induction were determined by Western analysis. In the case of one of these cell lines (EcR293/hREV7–59), the expression prior to induction was nearly undetectable (background expression of endogenous hREV7) and increased >100-fold upon induction (data not shown). To test the effect of overexpressing hREV7 on the cell cycle, we induced expression and harvested cells at 0, 24, 48, and 72 h. The fractions of cells in G1, S, and G2/M were determined by fluorescence-activated cell sorting analysis. We found no alteration of the cell cycle upon overexpression of hREV7 protein (data not shown).

DISCUSSION

Two polymerase systems appear to exist in eucaryotic cells that are capable of bypass synthesis across damaged nucleotides in DNA (8): polymerase \( \eta \) (composed of Rev1, Rev3 and Rev7) and polymerase \( \zeta \) (the product of the RAD30 gene) (9–11, 45, 46). The human homolog of Rad30, hRAD30, has recently been identified as the gene responsible for xeroderma pigmentosum (XP) variant (XP-V), a rare hereditary skin cancer predisposition syndrome (47, 48). This result suggests that polymerase bypass synthesis is an important component of the cellular DNA repair and genome maintenance functions.

Here we have identified the likely human homolog of scRev7, hREV7, the accessory subunit of polymerase \( \eta \). This identification is based on its lack of interaction with hMAD1 and strong interaction with the catalytic core of polymerase \( \zeta \), hREV3, which we previously identified and cloned from the EST data base. An enhancement of polymerase \( \zeta \) catalytic activity is required to confirm a functional role for this candidate hREV7 and is in progress.

We have also detailed an interaction region on hREV3 (residues 1776–2195) that appears responsible for the strong association with the catalytic core of polymerase \( \zeta \), hREV3, which we previously identified and cloned from the EST data base. An enhancement of polymerase \( \zeta \) catalytic activity is required to confirm a functional role for this candidate hREV7 and is in progress.

Despite an intensive search, we have found no alterations of either hREV3 or

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hREV7 in human primary tumors or human tumor cell lines. These results either suggest that inactivation of hREV3 or hREV7 does not promote tumorigenesis or that one or both of these genes are essential for cellular survival.

The efficiency of error-free translesion synthesis by polymerase γ across UV-induced thymine-thymine dimers appears to be more than 70% in vitro (46). The S. cerevisiae polymerase γ can also replicate a thymine-thymine dimer or N2-acetylaminofluorene lesion, but the efficiency of this translesion synthesis is only about 10% (10, 11). Moreover, approximately 3% of the REV3 bypass synthesis contained mutations. These results support the notion that polymerase γ is the likely polymerase responsible for the RAD6 error-prone UV repair pathway (8). It is interesting to note that the “fill-in” synthesis associated with double strand break repair in S. cerevisiae can also be mutagenic, and this mutagenic repair is dependent on scREV3 (49).

The function of scREV7 in polymerase ζ is unknown. However, the polymerase activity of scRev3 is approximately 20–30 times more efficient when scRev7 is present (10). We consider several functions for hREV7: 1) targeting DNA polymerase ζ to lesion-containing DNA; 2) maintaining the structure/function of DNA polymerase ζ; and 3) controlling the cell cycle or cellular conditions that promote appropriate DNA polymerase ζ function in vivo.

The study of spindle assembly checkpoint is an active area of research, and many genes have been implicated in this complicated process (50). hMAD2 is believed to be the key component of the spindle assembly checkpoint. The MAD2 protein has been shown to bind MAD1 and also forms a complex with CDC20-APC to prevent activation of APC (34, 51). Recently, a sequence conservation (HORMA) implicated in a variety of protein-protein interactions as well as oligomerization was identified in comparisons of scMad2, scRev7, scHop1 (a meiotic-synaptonemal complex component), and several other proteins (52, 53). The hREV7 protein contains this HORMA domain, which may ultimately be the region responsible for interaction with hREV3 and/or hMAD2.

Overexpression of Schizosaccharomyces pombe spMad2 or S. cerevisiae scMsp1 or scBub1 results in cell cycle arrest at M phase (50, 54, 55). We produced stable transfectants capable of regulated hREV7 overexpression. Examination of three such cell lines that showed dramatic overexpression of hREV7 has revealed no alterations of the cell cycle. We have not eliminated the possibility of an effect of hREV7 overexpression on damage-induced M-phase arrest. We consider the possibility that hREV7 may act as an adapter for DNA repair and spindle assembly checkpoint. This idea is based on the strong interaction between hREV7 and both the bypass polymerase catalytic subunit hREV3 and the mitotic spindle assembly checkpoint protein hMAD2. Moreover, we have demonstrated that these three proteins appear capable of forming a multiprotein complex in vitro. Unfortunately, the lack of available and/or useful antibody reagents has precluded demonstration of an interaction between hREV7 and hMAD2 in vivo. Thus, this in vitro interaction must be regarded with some skepticism. Details of any role for hREV7 in polymerase ζ activity and/or the spindle assembly checkpoint await further study.

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Figure 5. Interactions among hREV3, hREV7, hMAD2, and hMAD1. A, interaction of IVT-hREV7 with GST-hMAD2 and, conversely, that of IVT-hMAD2 with GST-hREV7. B, interaction of GST-hMAD2 with IVT-hREV3 and IVT-hREV7. Note that hMAD2 interacts with hREV7 and hREV3. Moreover, when GST-hMAD2, IVT-hREV3, and IVT-hREV7 are incubated together, both IVT-hREV3 and IVT-hREV7 precipitate with GST-hREV7, indicating that these three proteins appear to form a stable complex. C, interaction of GST-hREV7 with IVT-hMAD2 and IVT-hMAD1. Note that hREV7 interacts with hMAD2 but not hMAD1, while hMAD2 interacts with hMAD1. However, when GST-hREV7, IVT-hMAD2, and IVT-hMAD1 are incubated together, only IVT-hMAD2 precipitates with GST-hREV7, indicating that these three proteins do not form a stable complex. Each input lane contains 20% of the amount of protein used in the binding reaction.
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