Interactions in the Error-prone Postreplication Repair
Proteins hREV1, hREV3, and hREV7*

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Most mutations after DNA damage in yeast Saccharomyces cerevisiae are induced by error-prone translesion DNA synthesis employing scRev1 and DNA polymerase ζ that consists of scRev3 and scRev7 proteins. Recently, the human REV1 (hREV1) and REV3 (hREV3) genes were identified, and their products were revealed to be involved in UV-induced mutagenesis, as observed for their yeast counterparts. Human REV7 (hREV7) was also cloned, and its product was found to interact with hREV3, but the biological function of hREV7 remained unknown. We report here the analyses of precise interactions in the human REV proteins. The interaction between hREV1 and hREV7 was identified by the yeast two-hybrid library screening using a bait of hREV7, which was confirmed by in vitro and in vivo binding assays. The homodimerization of hREV7 was also detected in the two-hybrid analysis. In addition, the precise domains for interaction between hREV7 and hREV1 or hREV3 and for hREV7 homodimerization were determined. Although hREV7 interacts with both hREV1 and hREV3, a stable complex formation of the three proteins was undetectable in vitro. These findings suggest the possibility that hREV7 might play an important role in regulating the enzymatic activities of hREV1 and hREV3 for mutagenesis in response to DNA damage.

An error-free DNA replication system is required to pass accurate genetic information on to the next generation. However, various kinds of DNA damage induced by endogenous and exogenous factors impair this replication ability and cause genetic alterations, resulting in cancer predisposition (1). Cells have excellent systems for avoiding these genetic alterations by removing and repairing the damaged lesions before DNA replication for maintaining the genetic stability of the organism; these systems include base excision repair, nucleotide excision repair, mismatch repair, and recombination repair (2, 3). If a lesion on template DNA escapes these repair systems, a polymerase may stall at this point and start synthesis again downstream, resulting in a single strand gap in the DNA, which can be repaired by postreplication repair. Usually, recombination repair in postreplication repair can fix this gap without base substitution, but when this repair does not happen, DNA synthesis by a bypass formation across the lesion called translesion synthesis (TLS)1 may take place to fill the gap. This TLS may be held in the last resort for DNA repair because mutations can be induced during this step (for reviews, see Refs. 4–6).

In budding yeast Saccharomyces cerevisiae, the scRAD30 gene, the product of which is DNA polymerase η, is involved in the error-free TLS that can replicate DNA through cis-syn thymine-thymine (T-T) dimer in an error-free manner (7–10), whereas the scREV1, scREV3, and scREV7 genes are involved in the error-prone TLS that frequently induces mutations at the damaged lesions (for reviews, see Refs. 11–13). It is known that most mutations induced after UV irradiation are caused by the products of these three REV genes. scRev1 protein is a terminal deoxycytidyl transferase that induces a dCMP opposite an absic site (14, 15). This protein displays a weak homology with the bacterial UmuC protein, a component of Escherichia coli DNA polymerase pol V (UmuD’C complex), which is involved in damage-induced mutagenesis in an error-prone TLS manner (16–18). scRev1 is now a member of the UmuC/DinB/Rev1/Rad30 superfamily of polymerase, most of which are involved in TLS (for reviews, see Refs. 19 and 20). scRev3 protein has a DNA polymerase domain and interacts with scRev7 protein to form DNA polymerase ζ, which can replicate DNA past a cis-syn T-T dimer in an error-prone manner (21–23). Although scRev7 interacts with scRev3 to increase the polymerase activity of scRev3 about 20–30-fold (23), the actual function of scRev7 protein is not yet known.

The human homologs of these genes involved in error-free and error-prone TLS were identified recently. Human DNA polymerase η, which is the product of the gene responsible for the variant form of xeroderma pigmentosum, can pass through the lesion of cis-syn T-T dimer in an error-free manner, like its yeast counterpart (24–27). However, it was shown that human DNA polymerase η copies undamaged DNA with a much lower fidelity, indicating its limited enzymatic activity for damage tolerance (28, 29). hREV1 and hREV3 were identified by searching the human expressed sequence tags homologous with scREV1 and scREV3 (30–35). The human cells expressing high levels of hREV1 or hREV3 antisense mRNA fragments grow normally but show less mutagenic properties after UV irradiation, suggesting that hREV1 and hREV3 are involved in UV-induced mutagenesis (31, 34). Recombinant hREV1 protein

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1 The abbreviations used are: TLS, translesion synthesis; GST, glutathione-S-transferase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; IVTT, in vitro transcription-translation; aa, amino acid(s).
shows terminal deoxycytidyl transferase activity, as does scRev1 (30, 32). hREV7 was identified in the two-hybrid library screening using a bait of hREV3. Interaction between hREV3 and hREV7 was confirmed in vitro binding assay, indicating the existence of DNA polymerase ζ complex in human cells, but the function of hREV7 is not yet known, nor is that of its yeast counterpart (36).

We describe here the analyses of interactions in the human REV proteins hREV1, hREV3, and hREV7. The interaction between hREV1 and hREV7 was identified by two-hybrid library screening using a bait of hREV7 and was confirmed by both in vitro and in vivo binding assays. Such interaction has not yet been shown in yeast. In addition, the homodimerization of hREV7 was also detected in the two-hybrid assay. We determined the precise interaction domains of hREV1 and hREV7, hREV3 and hREV7, and hREV7 homodimerization. Although we investigated a stable complex formation of these three proteins, it was undetectable in vitro. These results suggest that hREV7, which interacts with either hREV1 or hREV3, might play an important role in regulating the hREV1 and hREV3 enzymatic activities for damage tolerance and mutagenesis.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—For yeast two-hybrid assay, full-length and truncated fragments of hREV7 cDNA were placed into vector pAS2-1 (CLONTECH), and full-length and truncated fragments of hREV1 cDNA and truncated fragments of hREV3 cDNA were placed into vector pACT2 (CLONTECH). For in vitro binding assay, full-length hREV1 cDNA and full-length hREV7 cDNA were placed into vector pET2d4 (Novagen) to produce radiolabeled or nonradiolabeled hREV1 and hREV7 proteins, and truncated fragments of hREV1 cDNA and full-length hREV7 cDNA were placed into vector pGEX4T-2 (Amersham Pharmacia Biotech) to produce glutathione S-transferase (GST) fusion proteins. For in vivo binding assay, full-length hREV1 cDNA tagged with the FLAG sequence at its C terminus and a truncated fragment of hREV3 cDNA tagged with the FLAG sequence on its C terminus were placed into vector pCDNA3.1 (+) (Invitrogen) to express the FLAG-tagged hREV1 and hREV3 proteins in cells. The DNA fragment cloned into each vector was produced by PCR with Pfu DNA polymerase (Stratagene).

**Yeast Two-hybrid Assay**—The pAS2-1/hREV7 plasmid, which contains full sequence of the hREV7 coding region, was used for yeast-two-hybrid library screening to isolate the hREV7 interacting proteins. Yeast two-hybrid library screening was performed in the Y190 yeast strain using MATCHMAKER 2 two-hybrid system (CLONTECH) according to the manufacturer’s protocol. Twenty-five ml 3-amino-1,2,4-triazole was added to selective medium lacking tryptophan, leucine, and histidine to inhibit His3p expression activated by GAL4 DNA binding domain fused hREV7 alone. We screened about 5 × 10^6 independent clones of the human testis cDNA library constructed in vector pACT2 (CLONTECH) on 150-mm dishes. Sequential qualitative β-galactosidase assay was performed to eliminate false positives according to the manufacturer’s instructions (CLONTECH). Liquid culture assay for β-galactosidase activity was also performed to check the interactions quantitatively according to the manufacturer’s protocol (CLONTECH). The β-galactosidase activity was determined by taking the average of values in three independent experiments. pACT2 library plasmids in the positive clones were extracted and subjected to sequencing. Double-strand sequencing of the inserts in pACT2/library plasmids extracted from positive clones was performed by a cycle sequencing program using the dye-deoxynucleotide kit and purified using a Qiagen plasmid kit (Qiagen), and the purified plasmids were subjected to sequencing. 5′-Rapid amplification of cDNA ends and reverse transcription-PCR were also performed to confirm the sequence of hREV1. The λEMBL3 human genomic library (CLONTECH) was screened using hREV1 cDNA as a probe to isolate the genomic clones of hREV1. The phage DNAs of positive clones were purified using a Qiagen lambda kit, and the DNAs were sequenced directly to elucidate the intron-exon boundaries of hREV1. The size of each intron was determined first by genomic PCR using the sets of primers on exons and was confirmed with the genomic sequence available in the data base. To determine the chromosomal location of hREV1 locus, sets of PCR primers were designed to amplify gene specific genomic fragments and 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/Massachusetts Institute of Technology 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 membranes were purchased from CLONTECH and were hybridized with hREV1 cDNA or human β-actin cDNA probe using standard methodologies.

**Cell Culture and Reagents**—HeLa cells were grown in RPMI medium supplemented with 10% fetal bovine serum. For a transient transfection experiment, HeLa cells were grown on a 10-cm culture dish and transfected with 10 μg of pcDNA3.1 (+) or pcDNA3.1 (+)/hREV3-S-FLAG plasmid by using GenePORTER transfection reagent (Gene Therapy Systems) according to the manufacturer’s protocol. Cells were harvested 72 h after transfection for further analysis.

**Antibodies**—Rabbit polyclonal anti-hREV7 antibody was produced by immunization with keyhole limpet hemocyanin-conjugated peptide containing the C-terminal 19 amino acids of hREV7, and affinity-purified as described previously (36). Mouse monoclonal anti-FLAG M2 antibody was purchased from Sigma.

**Western Blot Analysis**—Harvested cells were disrupted in cell lysis buffer (20 mM Hepes, pH 7.6, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 5 μg/ml pepstatin A) with freeze and thaw cycles. The cell lysates were clarified by centrifugation (15,000 × g) for 10 min, and then they were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto a polyvinylidine difluoride membrane (Millipore). After blocking with 5% bovine serum albumin in TBST buffer (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20), the membranes were probed with anti-rabbit or anti-mouse IgG secondary antibody, followed by incubation with anti-rabbit or anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (Dako). After intensive washing, the antigen-antibody complexes were visualized using the ECL Western blotting detection reagent (Amersham Pharmacia Biotech) according to the manufacturer’s instructions (CLONTECH).

**In Vivo Protein-Protein Interaction Assay**—GST fusion proteins were expressed in E. coli transformed with pGEX4T-2 plasmids with the induction of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. After incubation for several hours at 30°C, E. coli cells were harvested and disrupted in bacterial lysis buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 50 μg/ml leupeptin) with sonication. The bacterial lysates were centrifuged at 15,000 × g for 10 min, and the GST fusion proteins were immobilized on glutathione-Sepharose beads (Amersham Pharmacia Biotech). Radiolabeled proteins were synthesized with [35S]methionine 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 Guerette et al. (37). Briefly, 25 μl of glutathione-Sepharose beads containing 5 μg of a GST fusion protein or GST protein (alone) were incubated with radiolabeled proteins at 4°C for 2 h in 200 μl of binding buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 0.1% Tween 20, 0.75 mg/ml bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin). The beads were washed 5 times with the binding buffer, and bound proteins were eluted by boiling in 1× sample buffer and analyzed on SDS-PAGE gels followed by autoradiography.

**Identification of cDNA Sequence, Genomic Structure, and Chromosomal Location of hREV1**—To obtain the complete cDNA sequence of hREV1, the λTriplEx human testis cDNA library (CLONTECH) was screened using a PCR probe derived from the insert of pACT2/C76 (CLONTECH) and purified using a Qiagen plasmid kit (Qiagen), and the purified plasmids were subjected to sequencing. 5′-Rapid amplification of cDNA ends and reverse transcription-PCR were also performed to confirm the sequence of hREV1. The λEMBL3 human genomic library (CLONTECH) was screened using hREV1 cDNA as a probe to isolate the genomic clones of hREV1. The phage DNAs of positive clones were purified using a Qiagen lambda kit, and the DNAs were sequenced directly to elucidate the intron-exon boundaries of hREV1. The size of each intron was determined first by genomic PCR using the sets of primers on exons and was confirmed with the genomic sequence available in the data base. To determine the chromosomal location of hREV1 locus, sets of PCR primers were designed to amplify gene specific genomic fragments and 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/Massachusetts Institute of Technology 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 membranes were purchased from CLONTECH and were hybridized with hREV1 cDNA or human β-actin cDNA probe using standard methodologies.
(Sigma) or a control mock antibody for 2 h. The antigen-antibody complex was immobilized on protein G-Sepharose beads, and the beads were washed five times in the same lysis buffer. The bound proteins were eluted by boiling in 1× sample buffer and subjected to SDS-PAGE and Western blotting with anti-hREV7 or anti-FLAG antibody.

RESULTS

Cloning and Characterization of the Human Homolog of scREV1—To identify the interacting proteins of hREV7, we performed a yeast two-hybrid library screening using hREV7 as a “bait.” Approximately $5 \times 10^9$ independent clones in the human testis cDNA expression library constructed in vector pACT2 (CLONTECH) were screened on selective medium, and sequential β-galactosidase assay was performed to eliminate false positives. Several positive yeast clones were detected in this screening. pACT2/library plasmids were extracted from the positive yeast clones, and the library DNA fragments in the pACT2 plasmids were subjected to sequencing. Three of them, C76, C156, and C234, were shown to be derived from the same gene, which showed significant homology with scREV1. We screened a human testis cDNA library constructed in vector pACT2 and checked the interaction of truncated hREV1 proteins with full-length hREV7. In addition to the qualitative colony color system, we performed quantitative liquid culture β-galactosidase assay for all of the constructs. As shown in Fig. 2B, full-length hREV1 and hREV1-1, -2, -5, -6, -8, and -10 truncated proteins displayed both a qualitative and quantitative interaction with hREV7, whereas hREV1-3, -4, -7, and -9 truncated proteins displayed no interaction. The minimum interaction domain of hREV1 was revealed to be within amino acid residues 1130–1251. In the same way, the domain of hREV7 for interaction with hREV1 was determined by using the full-length and deletion mutants of hREV7 cDNA cloned in vector pAS2-1, which expressed hREV7 proteins fused to GAL4 DNA binding domain (Fig. 2C). Full-length hREV7 and hREV7-6, -8, and -9 truncated proteins showed both a qualitative and quantitative interaction with full-length hREV1, whereas hREV7-1, -2, -3, -4, -5, and -7 truncated proteins did not. These results indicate that hREV1 interacts with the region of amino acid residues 21–155 of hREV7.

The interaction was also confirmed by in vitro and in vivo binding assays. A GST-hREV7 fusion protein containing full-length hREV7 was produced in bacteria and was purified by binding to glutathione-Sepharose beads (Amersham Pharmacia Biotech). Radiolabeled full-length hREV1 was synthesized using IVTT system (Promega) and tested for binding to GST-hREV1 compared with a control containing the GST moiety alone. We found that the IVTT hREV1 bound the GST-hREV7 fusion protein, but not GST alone (Fig. 3A). Conversely, the interaction between GST-fused hREV1 protein and non-radio-labeled IVTT hREV7 protein, which could be detected by Western blotting with anti-hREV7 antibody, was checked in vitro. Fig. 3B shows the specificity of anti-hREV7 antibody. Western

![Figure 1](image-url)
blot analysis using an anti-hREV7 antibody showed one major product in a HeLa cell lysate, the size of which was the same as that of the IVTT product of hREV7. In *in vitro* binding assay, IVTT hREV7 bound GST-hREV1-5 fusion protein, containing amino acid residues 826–1251, but not GST-hREV1-3 and GST-hREV1-4, containing amino acid residues 1–386 and 387–825.

**FIG. 2.** *Yeast two-hybrid analyses for interaction between hREV1 and hREV7.* A, yeast transformants grown on selective plates in the yeast two-hybrid assay. The yeast Y190 strain was transformed by the combination of plasmids as indicated. hREV7 and hREV1 proteins were expressed in yeast as GAL4 DNA binding domain fusion protein and GAL4 transcription activation domain fusion protein, respectively. Growth on the –His selective plate indicates interaction. B, binding domain of hREV1 with hREV7. The full-length and truncation mutants of hREV1 were expressed and examined for their interaction with full-length hREV7 in yeast two-hybrid system with both qualitative and quantitative β-galactosidase assays. The values of β-galactosidase activity are indicated in Miller units. The full-length hREV1 and hREV1-1, -2, -5, -6, -8, and -10 truncation mutants (shaded bars) displayed qualitative interaction and high β-galactosidase activity, indicating that the binding domain of hREV1 with hREV7 resides in the region of amino acid residues 1130–1251. C, binding domain of hREV7 with hREV1. The full-length and truncation mutants of hREV7 were examined for their interaction with full-length hREV1 as described above. The results indicated that all of the domains of hREV7 for interaction with hREV1 and hREV3 and for hREV7 homodimerization reside in the same region of amino acid residues 21–155.

**FIG. 3.** *In vitro and in vivo analyses for interaction between hREV1 and hREV7.* A, *in vitro* binding of GST-hREV7 with IVTT hREV1. GST and GST-hREV7 fusion protein immobilized on glutathione-Sepharose beads were incubated with radiolabeled IVTT full-length hREV1 protein in binding buffer, and bound proteins were subjected to SDS-PAGE and autoradiography. Interaction of IVTT hREV1 is indicated by comparing binding with GST-hREV7 fusion protein versus GST alone. B, Western blot analysis of hREV7. GST and GST fusion proteins immobilized on glutathione-Sepharose beads were incubated with nonradiolabeled IVTT hREV7 protein and HeLa cell lysate and probed with anti-hREV7 antibody. One major band was detected in HeLa cell lysate, the size of which was the same as that of the IVTT product of hREV7, indicating the specificity of anti-hREV7 antibody. C, *in vitro* binding of GST-hREV1 truncation mutants with IVTT hREV7. GST and GST fusion proteins immobilized on glutathione-Sepharose beads were incubated with nonradiolabeled IVTT hREV7 protein in binding buffer, and bound proteins were subjected to SDS-PAGE and Western blotting with anti-hREV7 antibody. Only GST-hREV1-5 (aa 826–1251) interacts with IVTT hREV7, not GST-hREV1-3 (aa 1–386) and GST-hREV1-4 (aa 387–825). Each input lane in A and C contains 20% of the amount of protein used in the binding reaction. D, in *vivo* binding of FLAG-tagged hREV1 and hREV7. The top panel shows the blot with anti-FLAG antibody, and the bottom panel shows the blot with anti-hREV7 antibody. Endogenous hREV7 protein co-precipitated with FLAG-tagged hREV1 protein overexpressed transiently in HeLa cells, indicating the interaction between hREV1 and hREV7 in *vivo*. Ig, immunoglobulin light chain.
indicating the interaction between hREV3 and hREV7 in vivo. Endogenous hREV7 co-precipitated with FLAG-tagged hREV3 when the binding domain of hREV3 with hREV7 resides in the region of amino acid residues 1847–1892. B, in vivo binding of FLAG-tagged hREV3 truncation mutant with hREV7. The top panel shows the blot with anti-FLAG antibody, and the bottom panel shows the blot with anti-hREV7 antibody. Endogenous hREV7 co-precipitated with FLAG-tagged hREV3-8 (aa 1776–2044) protein overexpressed transiently in HeLa cells, indicating the interaction between hREV3 and hREV7 in vivo. Ig, immunoglobulin light chain. C, homodimerization of hREV7 in vitro. GST and GST-hREV7 fusion proteins were examined for their interaction with radiolabeled IVTT full-length hREV7 protein as described in Fig. 2A. GST-hREV7 bound IVTT hREV7, but not GST alone, indicating the homodimerization of hREV7. Input lane contained 20% of the amount of protein used in the binding reaction.

respectively (Fig. 3C). In vivo binding assays, HeLa cells were transfected transiently with the pcDNA3.1(+)/FLAG-hREV1 plasmid, which was designed to express full-length hREV1 protein tagged with FLAG on its N terminus. The cells were harvested 72 h after transfection and disrupted in the cell lysis buffer with freeze and thaw cycles. Their lysates were immunoprecipitated with anti-FLAG antibody (Sigma) or a control mock antibody, and the precipitated proteins were subjected to Western blotting with anti-hREV7 or anti-FLAG antibody. The endogenous hREV7 co-precipitated with FLAG-tagged hREV1 when anti-FLAG antibody was used for immunoprecipitation, whereas neither hREV7 nor FLAG-tagged hREV1 was detected when the mock antibody was used for immunoprecipitation, indicating that hREV7 interacts with hREV1 in vivo (Fig. 3D). These results are consistent with the yeast two-hybrid data and confirm the interaction between hREV1 and hREV7.

Interaction between hREV3 and hREV7—As shown in a previous publication (36), hREV3 interacts with hREV7, just as their yeast counterparts, scRev3 and scRev7, do. We determined the precise interaction domains of hREV3 and hREV7 by the two-hybrid assay. We made truncated hREV3 cDNA fragments and cloned them in vector pACT2, which expressed hREV3 truncated proteins fused to GAL4 transcription activation domain in yeast host strain (Fig. 4A). We found that full-length hREV3 displayed both a qualitative and quantitative interaction with hREV3 in vivo (Fig. 3D). These results are consistent with the yeast two-hybrid data and confirm the interaction between hREV1 and hREV7.

The minimal interaction domain of hREV3 was shown to be within the region of amino acid residues 1847–1892 (Fig. 4A). Full-length and truncated hREV7 cDNAs cloned in vector pA2S1 and hREV3-3 truncated cDNA cloned in vector pACT2 then were used to determine the domain of hREV7 for interaction with hREV3. Full-length hREV7 and hREV7-6, -8, and -9 truncated proteins showed both a qualitative and quantitative interaction with hREV3-3 truncated protein, but hREV7-1, -2, -3, -4, -5, and -7 truncated proteins did not. This indicates that hREV7 interacts with hREV3 within the region of amino acid residues 21–155 of hREV7, which is the exact same region in which hREV7 interacts with hREV1 (Fig. 2C). However, the levels of the β-galactosidase activity for interaction between hREV3 and hREV7 truncation proteins were different from those for interaction between hREV1 and hREV7 truncation proteins. For example, the activity for the interaction between hREV3 and hREV7-6 is much higher than that between hREV1 and hREV7-6 (Fig. 2C). This finding suggests that the binding affinity for hREV7-hREV3 complex is different from that for hREV7-hREV1 complex, although
Fig. 6. Summary of the interactions in human REV proteins and a model of functions of REV protein complexes. Three forms of complexes, hREV1-hREV7, hREV3-hREV7, and hREV7-hREV7 homodimer, may be present in human cells. An interesting model for the mechanism of error-prone TLS in human cells could be that hREV7 usually exists in the form of homodimers, and hREV1 or hREV3 in the form of monomers, which have less TLS activity, in the regular condition without DNA damage. When the error-prone TLS is necessary at a DNA lesion after DNA damage, it is possible that hREV7 may dissolve the homodimer and forms the complex with hREV1 or hREV3, which then may have high TLS activity of terminal deoxycytidyl transferase or lesion bypass polymerase, respectively, in order to synthesize the DNA through the lesion. The sequence homology of hREV1 and scRev1 and that of hREV3 and scRev3 are also shown. Note that the binding domain of hREV1 with hREV7 does not show homology with scRev1, suggesting that the interaction between REV1 and REV7 is specific in humans. For a study of the homology between hREV3 and scRev3, see Ref. 34.

the same region of hREV7 interacted with both hREV1 and hREV3. The interaction between hREV3 and hREV7 was also checked by in vivo binding assay. HeLa cells were transiently transfected with the pcDNA3.1(+)/hREV3-8-FLAG plasmid, which was designed to express hREV3-8 truncated protein containing the hREV3 amino acid residues 1776–2044 tagged with FLAG on its C terminus, and the cells were harvested 72 h after transfection and disrupted in the cell lysis buffer with freeze and thaw cycles. Their lysates were immunoprecipitated with anti-FLAG antibody or a control mock antibody followed by Western blotting with anti-hREV7 or anti-FLAG antibody. The endogenous hREV7 co-precipitated with FLAG-tagged hREV3-8 in this assay, indicating an interaction between hREV3 and hREV7 in vivo (Fig. 4B). The in vitro binding assay was shown in a previous publication (36). These in vitro and in vivo results are consistent with the binding data in the yeast two-hybrid assay and confirm the association between hREV3 and hREV7.

Homodimerization of hREV7—In the yeast two-hybrid assay using a bait of hREV7, we isolated three additional positive clones, C45, C54, and C95, the pACT2/library plasmids of which had the fragments derived from hREV7, indicating a homodimerization of hREV7. The interaction domain of hREV7 for homodimerization was also determined by the yeast two-hybrid assay qualitatively and quantitatively using pAS2-1/hREV7 truncation mutants and pACT2/hREV7 plasmids. The result showed that the interaction domain for hREV7 homodimerization was also located within the region between amino acid residues 21 and 155, the same region for the interaction with hREV1 or hREV3 (Fig. 2C). The hREV7 homodimerization was confirmed by the in vitro binding assay (Fig. 4C). GST-hREV7 fusion protein bound radiolabeled IVTT hREV7. These binding analyses revealed that hREV7 binds hREV1 and hREV3 and also forms a homodimer.

hREV1, hREV3, and hREV7 Together Do Not Form a Stable Complex—Because it was shown that hREV7 binds both hREV1 and hREV3, we investigated a stable complex formation of hREV1, hREV3, and hREV7. We first checked the interaction between hREV1 and hREV3 by the two-hybrid and in vitro binding assays, which showed no interaction in the two proteins (data not shown). Then we analyzed a complex forma-
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suggest that hREV1 may be somewhat different from scRev1 in its enzymatic properties.

DISCUSSION

The systems used by yeast *S. cerevisiae* for recovery from damage in DNA was intensively investigated using deletion mutants. Of the three major pathways of DNA repair, RAD3 excision repair, RAD6 postreplication repair, and RAD52 recombination repair, the RAD6 postreplication repair pathway is the least investigated because of its diversity (11). The RAD3 pathway should be error-free. However, the RAD6 pathway contains both error-free repair and error-prone repair; the latter is called mutagenesis. scRev1, scRev3, and scRev7 are the main proteins involved in the error-prone pathway, and their functions are now being actively investigated. scRev1, which is a terminal deoxyctydyl transferase, and DNA polymerase ε, which consists of scRev3 and scRev7, demonstrate mutagenic properties via TLS (15, 23). scRev7 interacts with scRev3 to form DNA polymerase ε and facilitates the polymerase activity of scRev3, but interaction of scRev7 with scRev1 has not yet been identified in yeast. The investigation of human DNA repair genes has mainly followed that of their yeast counterparts and has made it clear that the homologous genes between human and yeast have similar functions. Human genes involved in the error-prone TLS pathway also appear to have similar functions to those of yeast genes. hREV1 has terminal deoxyctydyl transferase activity, as does scRev1. Human cells overexpressing high levels of hREV1 or hREV3 anti-sense RNA fragments show less mutagenic properties after UV irradiation, indicating that hREV1 and hREV3 function in an error-prone manner (30, 31, 34). However, little is known about the function of hREV7, except for its interaction with hREV3 (36). In the present study, we performed the yeast two-hybrid assay to identify proteins interacting with hREV7, expecting that it would give us clues to investigate the hREV7 function. As a consequence, we identified an interaction between REV1 and REV7 in human, which is not found in yeast, indicating that hREV7 interacts not only with hREV3 but also with hREV1. In addition, it turned out that the interaction domain of hREV1 with hREV7 is present in its C-terminal region, which scRev1 lacks. These findings suggest that the C-terminal region of hREV1, which shows no homology with any other proteins of the umuC/dimB/Rev1/Rad30 superfamily, including scRev1, may have a significance for its enzymatic activity and that hREV7 may be involved in the significance. We also determined the precise interaction domains of hREV3 and hREV7, both of which are shown to be present in the regions conserved in scRev3 and scRev7, suggesting that the function of DNA polymerase ε may be exactly conserved among species.

It now appears that hREV7 interacts with hREV1 and hREV3, and also forms a homodimer, but these three proteins do not form a stable complex together. These findings lead us to consider that hREV7 may play an important role in the TLS process of both DNA polymerase ε and hREV1. Although there is no direct evidence of the polymerase activity of hREV3 in human cells, hREV7 may facilitate the polymerase activity of hREV3, as observed for scRev7. Also, it is possible that hREV7 may modulate the terminal deoxyctydyl transferase activity of hREV1. Upon further consideration, an interesting model for the mechanism of error-prone TLS in human cells could be proposed; hREV7 may usually exist in the form of homodimers, whereas hREV1 or hREV3 may be present in the form of monomers, which have less TLS activity, in the regular condition without DNA damage. When the error-prone TLS is necessary at a DNA lesion after DNA damage, hREV7 may dissolve its homodimer and form the complex with hREV1 or hREV3, leading to the high TLS activity to synthesize DNA through the lesion (Fig. 6). Alternatively, it is also possible that hREV7 dimers may complex with hREV1 or hREV3. Further investigations are necessary to clarify the roles of these protein complexes in the error-prone TLS pathway.

One model of the function of DNA polymerase ε was recently reported, which shows that DNA lesion with (6–4) T-T photoproduct or abasic site is replicated by the combined action of human DNA polymerase ε and yeast DNA polymerase ε (38, 39). DNA polymerase ε is the product of *hRAD30*, the second human homolog of *scRAD30*, which replicates undamaged DNA in a highly error-prone manner. DNA polymerase ε incorporates a nucleotide opposite a lesion of (6–4) T-T photoprod-uct or abasic lesion but cannot bypass this lesion. The bypass of this lesion occurs when DNA polymerase ε is combined with DNA polymerase ε, suggesting that DNA polymerase ε functions as a mispair extender. This suggestion is supported by the fact that the abasic lesion is bypassed by the combined action of DNA polymerase ε and scRev1 or DNA polymerase δ in yeast (15, 40). Although several molecules were recently identified to function as lesion-bypass DNA polymerases in humans, including DNA polymerase ε, η, λ, and κ, it is not known yet how cells can distinguish these proteins and use them properly to maintain the genetic information (25, 26, 34, 38, 41–43). One suggestion in the case of *E. coli* is that the cell uses a pool of translesional DNA polymerases to bypass DNA lesions in response to the diversity of existing DNA damage (44–46).

Unlike the case of scRev3, which is not essential to yeast survival, it was reported recently that the disruption of mouse Rev3 causes early embryonic lethality (47–49). This finding suggests that Rev3 is necessary for cell proliferation during embryonic development in mammals. Because mRev3 is about twice as large as scRev3, one possibility is that mRev3 has some essential function other than TLS (50). The other possibility is that DNA damage caused by endogenous factors may be frequent during embryonic development, and DNA polymerase ε may be necessary for TLS past such damaged DNA lesions in the embryo. We did not find mutations of the hREV3 and hREV7 genes in many human tumor cell lines or in clinical tumors tested in a previous study, suggesting that these two genes may be essential for cell survival (36). It is important to note that the main role of DNA polymerase ε is to maintain the genetic information by TLS, rather than “mutagenesis.” More attention should be paid to the damage tolerance function of DNA polymerase ε or hREV1 than to their mutagenic function in future investigations.

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