Crystal Structure of Human REV7 in Complex with a Human REV3 Fragment and Structural Implication of the Interaction between DNA Polymerase ζ and REV1

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DNA polymerase ζ (Polζ) is an error-prone DNA polymerase involved in translesion DNA synthesis. Polζ consists of two subunits: the catalytic REV3, which belongs to B family DNA polymerase, and the noncatalytic REV7. REV7 also interacts with REV1 polymerase, which is an error-prone Y family DNA polymerase and is also involved in translesion DNA synthesis. Cells deficient in one of the three REV proteins and those deficient in all three proteins show similar phenotype, indicating the functional collaboration of the three REV proteins. REV7 interacts with both REV3 and REV1 polymerases, but the structure of REV7 or REV3, as well as the structural and functional basis of the REV1-REV7 and REV3-REV7 interactions, remains unknown. Here we show the first crystal structure of human REV7 in complex with a fragment of human REV3 polymerase (residues 1847–1898) and reveal the mechanism underlying REV7-REV3 interaction. The structure indicates that the interaction between REV7 and REV3 creates a structural interface for REV1 binding. Furthermore, we show that the REV7-mediated interactions are responsible for DNA damage tolerance. Our results highlight the function of REV7 as an adapter protein to recruit Polζ to a lesion site. REV7 is alternatively called MAD2B or MADA2L2 and also involved in various cellular functions such as signal transduction and cell cycle regulation. Our results will provide a general structural basis for understanding the REV7 interaction.

Large numbers of DNA lesions occur daily in every cell, and the majority of the DNA lesions stall replicative DNA polymerases. This results in the arrest of DNA replication, which causes lethal effects including genome instability and cell death.

Translesion DNA synthesis (TLS) releases this replication blockage by replacing the stalled replicative polymerase with a DNA polymerase specialized for TLS (TLS polymerase). It is generally considered that TLS includes two steps performed by at least two types of TLS polymerases, namely inserter and extender polymerases (reviewed in Refs. 1 and 2). In the first step, the stalled replicative polymerase is switched to an inserter polymerase such as Polη, Polκ, Polε, or REV1, which are classified as Y family DNA polymerases (3) and have different lesion specificity (reviewed in Refs. 4–8), and an inserter polymerase incorporates nucleotides opposite the DNA lesion instead of the stalled replicative polymerase. In the second step, an inserter polymerase is switched to the extender polymerase DNA polymerase ζ (Polζ), and then Polζ extends a few additional nucleotides before a replicative polymerase restarts DNA replication.

Polζ consists of the catalytic REV3 and the noncatalytic REV7 subunits. REV3 is classified as a B family DNA polymerase on the basis of the primary sequence. The catalytic activity of yeast REV3 is stimulated by yeast REV7 (9). Biochemical analysis has been done only for yeast REV3 but not mammalian REV3, because the molecular mass of human REV3 is larger (~350 kDa) than that of yeast REV3 (~150 kDa). Disruption of the mouse REV3 gene causes embryonic lethality accompanied by massive apoptosis (10–12), suggesting that the function of mammalian REV3 is essential for embryogenesis. Although REV7 is a smaller protein with a molecular mass of 24–28 kDa compared with REV3, the function of REV7 is less understood. REV7 is a member of the HORMA (Hop1, Rev7, and Mad2) family of proteins (13). REV7, which is alternatively called MADA2B or MADA2L2, appears to be involved in multiple cellular functions including not only TLS but also cell cycle regulation (14), bacterial infection (15), and signal transduction (16, 17).

In this study, we investigated the function of REV7 in TLS from structural analysis. Previous studies have reported that human REV7 interacts with the central region (residues 1847–1892) of human REV3 by yeast two-hybrid and in vitro interaction assays (18). Interestingly, human REV7 also interacts with the C-terminal region (residues 1130–1251) of human REV1 polymerase as...
shown by yeast two-hybrid, in vitro interaction and co-immunoprecipitation assays (19–21). Furthermore, human REV7 and human REV1 were co-expressed by Escherichia coli, and the REV7-REV1 complex was purified, whereas REV7 does not affect the polymerase activity of REV1 (22). The three yeast rev mutants and the triple mutant show very similar sensitivity to various genotoxic treatments (23–25). Furthermore, chicken DT40 cells deficient in one of the three REV proteins and those deficient in all three proteins show hypersensitivity to various genotoxic treatment including cisplatin (cis-diaminedichloroplatinum (II)), indicating the functional collaboration of the three REV proteins (26). However, these previous analyses failed to determine the mechanism underlying the protein-protein interactions on the atomic level, because they tried to analyze without data of the three-dimensional structures. In addition, it remains unclear whether mammalian REV1, REV3, and REV7 can form the Polζ-REV1 ternary complex. It has been considered that switching of DNA polymerase occurred at least twice in TLS: the switching from a stalled replicative polymerase to an inserter polymerase and from an inserter polymerase to the extender polymerase. Recently, the structural implications of the first polymerase switching have been reported (27). However, the mechanism underlying the recruitment of the extender polymerase to the lesion site and the second polymerase switching as well as the physical and functional interactions of REV1, REV3, and REV7 remains unclear. Here we report the first crystal structure of human REV7 in complex with a fragment of human REV3 (residues 1847–1898). The structure reveals the mechanism underlying Polζ formation and shows that the REV7-REV3 interaction unexpectedly provides a structural interface for REV1 binding. Furthermore, we show that these REV7-mediated interactions with REV1 and REV3 are responsible for DNA damage tolerance. Lastly, we propose a model of the structural interplay of REV1, REV3, and REV7 in TLS. Our results will provide a general structural basis for understanding the REV7 interaction in various cellular functions.

**EXPERIMENTAL PROCEDURES**

**Crystallographic Analysis of Human REV7 in Complex with REV3 Fragment**—In the present crystallographic study, REV7 with an R124A mutation, REV7(R124A), was used instead of wild type REV7, REV7(WT) (28). It has been shown that a human REV3 fragment (residues 1847–1892) interacts with human REV7 (18). Thus, based on the result of secondary structure prediction, we constructed the REV3 fragment carrying residues 1847–1898, REV3(1847–1898), for this crystallographic study (28). The REV7(WT)-REV3(1847–1898) complex was polydisperse and did not crystallize, whereas REV7(R124A)–REV3(1847–1898) complex was a monodisperse (28). In addition, REV7(R124A) efficiently binds REV3(1847–1898). Preparation and crystallization of the human REV7(R124A)-REV3(1847–1898) complex have been described before (28). In brief, recombinant human REV7(R124A) with an N-terminal hexameric His tag in complex with human REV3(1847–1898) was expressed in E. coli BL21(DE3) harboring the REV7(R124A)-REV3(1847–1898) co-expression vector. The protein was purified by nickel-Sepharose resin (GE Healthcare), HiTrap Q HP (GE Healthcare), and HiLoad Superdex200 (GE Healthcare). Monoclinic and tetragonal crystals of the REV7(R124A)-REV3(1847–1898) complex were obtained in different conditions. Heavy atom derivatives of monoclinic crystals were prepared by the soaking method using a solution of 10 mm ethylmercurithiosalicylate, 100 mm Tris-HCl, pH 7.5, 800 mm sodium formate, and 25% (w/v) polyethylene glycol 2000 monomethyl ether for 20 h.

X-ray diffraction data for native crystals were collected by using a Quantum 315 CCD detector (Area Detector Systems Corp.) on Beamline BL-5A at Photon Factory. X-ray diffraction data for derivative crystals were collected by using an FR-D in-house x-ray generator with an R-AXIS IV++ imaging plate detector (Rigaku). All of the diffraction data were processed with the program HKL2000 (29). The structure of the REV7(R124A)-REV3(1847–1898) complex was solved by the single isomorphous replacement method using the programs SOLVE and RESOLVE (30, 31). Model building was performed with the programs O (32) and COOT (33). Structure refinement was performed at 1.9 Å resolution with the programs CNS (34) and REFMAC (35). P2₁ crystal contains two REV7(R124A)-REV3(1847–1898) complexes in the asymmetric unit. The structure in the P4₂₁,₂ crystal was solved at 2.6 Å resolution by the molecular replacement method with the program MOLREP (36) using one of P2₁ structures. The structure was refined with a procedure similar to that of the monoclinic case. The data collection and refinement statistics are given in Table 1. The coordinates and structure factors have been deposited in the Protein Data Bank Japan.

**In Vitro Interaction Assays**—His-tagged REV7 protein was co-expressed with REV3(1847–1898) in E. coli BL21(DE3) as described before (28), and the cell lysate was applied to nickel-Sepharose resin (GE Healthcare). The beads were washed five times in a buffer (50 mm HEPES-NaOH, pH 7.4, 1.5 mm NaCl, and 20 mm imidazole), and bound proteins were analyzed by SDS-PAGE with Coomassie Brilliant Blue stain. For interaction assays between the REV7-REV3(1847–1898) complex and REV1, GST fused the C-terminal region of human REV1(1130–1251), GST-REV1(1130–1251), was overexpressed in E. coli JM109 by isopropyl β-D-thiogalactopyranoside induction (1 mm at 25 °C), and was purified by glutathione-Sepharose 4B resin (GE Healthcare) by a standard procedure. In pull-down assays of the His-REV7-REV3(1847–1898) complex and GST-REV1(1130–1251), purified GST-REV1(1130–1251) was incubated with His-REV7-REV3(1847–1898) complex bound to the nickel-Sepharose resin (GE Healthcare) at 4 °C for 1 h. The beads were washed five times in a buffer (50 mm HEPES-NaOH, pH 7.4, 1.5 mm NaCl, and 20 mm imidazole), and the bound proteins were analyzed by SDS-PAGE with Coomassie Brilliant Blue stain.

**Co-immunoprecipitation Assays with HEK293 Cells**—cDNA encoding wild type or mutant of human REV7 was inserted into a pEGFP-C2 vector (Clontech). cDNA encoding human REV1(826–1251) or REV3(1776–2044) (19) with an N-terminal FLAG sequence was inserted into a pcDNA3.1(+) vector (Invitrogen). For the co-immunoprecipitation assays, HEK293 cells were co-transfected with expression vectors for GFP-REV7 and FLAG-REV1(826–1251) or FLAG-REV3(1776–2044) using Lipofectamine 2000 (Invitrogen). The cells were disrupted in a buffer (20 mm HEPES-NaOH, pH 7.6, 300 mm NaCl, 0.1 mm EDTA, 10% glycerol, 1 mm dithiothreitol, 0.1%...
TWEEN 20, 1 mM phenylmethylsulfonyl fluoride, and 10 mg/ml leupeptin) with freeze and thaw cycles. After adjusting the NaCl concentration to 150 mM, the cell lysates were clarified by centrifugation. The supernatants were incubated first with protein G-Sepharose beads (Sigma) and then with mouse monoclonal anti-FLAG M2 antibody (Sigma) for 2 h. The antigen–antibody complex was immobilized on protein G-Sepharose beads, and the beads were washed three times in a buffer (20 mM HEPES-NaOH, pH 7.6, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, and 10 mg/ml leupeptin). The bound proteins were analyzed by SDS-PAGE and Western blotting with anti-FLAG antibody or rabbit polyclonal anti-GFP antibody (MBL).

Rapid Survival Assays Using Chicken DT40 Cells—For retrovirus infection, a pMSCV-IREs-GFP recombinant plasmid was constructed by ligating the 5.2-kb BamHI-NotI fragment from pMSCVhyg (Clontech) with the 1.2-kb BamHI-NotI fragment from pRES2-EGFP (Clontech). cDNA of chicken REV7 (GdREV7) was inserted between the BglII and EcoRI sites of pMSCV-IREs-GFP. Virus was prepared by using 293T cells and 1 μl of Gene Juice (Novagen), 1 μg of pMSCV-GdREV7-IREs-GFP, and 1 μg of pCL-Ampho. After the 293T cells were cultured with the above reagents and plasmids at 37 °C for 2 days, the cells were centrifuged, and the supernatant was stored at −80 °C. Retrovirus infection was done by centrifugation (3000 rpm, 30 min, 32 °C) of the DT40 REV7−/− cells (26) and the retroviral solution. A day after infection, expression of GFP was determined by a fluorescence-activated cell sorter. The cell number was calculated by the Cell Titer-Glo luminescent cell viability assay (Promega) according to the manufacturer’s instructions. We calculated the extent of cytotoxicity.

Figure Preparation—Fig. 1B was prepared with the programs Molscript (37) and Raster3D (38). Figs. 1D, 2A, and 3 (A and C) were prepared with the program PyMOL. Fig. 2C was prepared with the program TopDraw (39). All of the figures were modified by the programs PHOTOSHOP and ILLUSTRATOR (Adobe Systems).

RESULTS AND DISCUSSION

Overall Structure of Human REV7 in Complex with REV3 Fragment—We have determined the crystal structure of human REV7(R124A) in complex with human REV3 (1847–1898) in two different crystal forms (Table 1). The structures in two different forms are essentially identical (supplemental Fig. S1), and thus we describe the higher resolution structure as a representative structure. REV7 is composed of three α-helices (αA, αB, and αC), eight β-strands (β2, β3, β4, β5, β6, β7, β8, and β8′), and three 3_10 helices (3_10-1, 3_10-2, and 3_10-3) (Fig. 1, A–C). The REV3 fragment bound to REV7 comprises a β-strand (βREV3) and an α-helix (αREV3) (Fig. 1, A–C). Electron density of the N-terminal portion (residues 1847–1873 in the representative structure) of the REV3 fragment is not observed in all structures, indicating that the structure of the region is highly disordered. Three α-helices of REV7 are aligned on the same side of an anti-parallel β-sheet composed of β7′, βREV3, β6, β4, β5, and β8′ and are flanked with an anti-parallel β-sheet comprising β2 and β3 on the other side. The C-terminal region following β6 (residues 153–211) of REV7 wraps around the REV3 fragment, resulting in a knot structure.

Human REV7 shares 22% amino acid identity with human Mad2, another member of the HORMA family (13). Mad2 functions in spindle assembly checkpoint by binding directly to Mad1 (40–42). Mad2 undergoes a striking conformational change from the open (O-Mad2) to the closed (C-Mad2) form, in which the C-terminal region known as the “seatbelt” following Mad2 moves toward the edge of the ligand, and β1 is relocated. Concomitantly, β7 and β8 are rearranged to form β8′ and β8″ (43, 44) (Fig. 1A). Structural alignment between REV7 bound to the REV3 fragment and Mad2 bound to Mad1 shows a root mean square deviation value of 2.0 Å for 183 superimposable Ca atoms (Fig. 1D), indicating that the overall structure of REV7 in the REV7(R124A)-REV3(1847–1898) complex may be very similar to the structure of the closed Mad2 form. Conceivably, a large conformational change may occur in the seatbelt (residues 153–211) of REV7 upon interaction with the REV3 fragment.
In addition to the hydrogen bonds in the REV7, Tyr-63, and Trp-171 of REV7 are highly conserved of REV3(1847–1898) are observed. The aromatic residues Tyr-63 and Trp-171 stack with the aliphatic rings of Pro-1884 and Pro-1880, respectively (Fig. 2A). The secondary structure elements of human REV7 and REV3(1847–1898) are drawn above the sequences. The nomenclature of the secondary structures of REV7 is based on those of Mad2.

Interaction between REV7 and REV3 fragment. REV7 is shown in green, and REV3 is shown in yellow. Residues involved in the interaction are shown as stick models. Orange dots represent hydrogen bonds between REV7 and REV3. In vitro interactions between REV7 and REV3 fragment. His-REV7 mutant were co-expressed with REV3(1847–1898) in E. coli. Cell lysates were incubated on nickel-Sepharose beads. After washing, the bound proteins were resolved by SDS-PAGE with Coomassie Brilliant Blue stain. His-REV7(R124A) was used as a control binding to REV3(1847–1898) (lane 1). Y63A or W171A mutation significantly decreased the REV3 binding (lane 5 or 8). C, in vivo interactions between REV7 and REV3 fragment. GFP-REV7(WT) or GFP-REV7 mutant was co-expressed with FLAG-REV3(1776–2044) in HEK293 cells and incubated with Protein G beads. After washing, then proteins bound to beads were separated on SDS-PAGE and analyzed with Western blotting. Although GFP-REV7(Y63A) (lane 3) and GFP-REV7(W171A) (lane 4) retained binding affinity for FLAG-REV3(1776–2044), GFP-REV7(Y63A/W171A) showed no binding affinity for FLAG-REV3 and GFP-REV7(R124A) showed higher binding affinity for FLAG-REV3, as compared with GFP-REV7(WT) (lane 2). REV7(L186A) lacking REV1 interaction (see Fig. 3, C and D) retained REV3 interaction (lane 6).

The secondary structures of REV7 are denoted by colored bars: green (α-helix), blue (β-sheet), and red (β-strand). A, stereo view of structural details of the interactions between REV7 and REV3 fragment. REV7 is shown in blue and green, and REV3 is shown in yellow. B, overview of interactions between REV7 and REV3 fragment. C, stereo view of superimposed structures of the REV7-REV3 and Mad2-Mad1 complexes. The secondary structural elements are labeled. N- and C-terminal of REV7 and REV3 are also labeled. The colors of the structural elements correspond to those of B.D. The partial sequence of human Mad1 (residues 512–563) corresponding to REV3(1847–1898) is shown. The C-terminal region of REV7 (residues 153–211) corresponding to the seatbelt of MAD2 is shown in red ellipsoids. D, stereo view of superimposed structures of the REV7-REV3 and Mad2-Mad1 complexes. REV7, REV3, Mad2, and Mad1 are shown in blue, yellow, green, and pink, respectively. To simplify, a partial structure (residues 340–560) of Mad1 is shown.

The structure of human REV7 in complex with human REV3 fragment. A, secondary structures and structure-based sequence alignment of human REV7 and Mad2 and human REV3 and Mad1. The secondary structure elements of human REV7 and REV3(1847–1898) are drawn above the sequences. The colors of the elements correspond to those of B. The partial sequence of human Mad1 (residues 512–563) corresponding to REV3(1847–1898) is shown. The secondary structure elements of human Mad2 and Mad1 are drawn below the sequences. The identical and homologous residues are shown on black and gray backgrounds, respectively. The nomenclature of the secondary structures of REV7 is based on those of Mad2. B, overall structure of human REV7 in complex with REV3 fragment. Structure of human REV7 in complex with REV3 fragment is shown by ribbon representations. The secondary structural elements are labeled. N- and C-terminal of REV7 and REV3 are also labeled. The loops not located in the electron density (residues 155–160) are shown as dashed lines. REV7 is colored light blue and blue. REV3(1874–1895) is colored yellow. The C-terminal region of REV7 (residues 153–211) corresponding to the seatbelt of MAD2 is shown in green. Tyr-63 and Trp-171, which are responsible for interaction with REV3, are shown as stick models. Leu-186 and Gln-200, which are significantly involved in interaction with the C-terminal region of REV1, are also shown as stick models. C, topology diagram of the REV7-REV3 complex. The secondary structural elements are labeled. N- and C-terminal of REV7 and REV3 are also labeled. The colors of the structural elements correspond to those of B. Leu-186 and Gln-200 responsible for REV1 binding are shown by red ellipsoids. D, stereo view of superimposed structures of the REV7-REV3 and Mad2-Mad1 complexes. REV7, REV3, Mad2, and Mad1 are shown in blue, yellow, green, and pink, respectively. To simplify, a partial structure (residues 340–560) of Mad1 is shown.
the REV7-REV3 interaction is distinctly different from that underlying the Mad2-Mad1 interaction. In addition, the α-helix of REV3(1847–1898) might be required for interaction with REV7, because a REV3 fragment (residues 1847–1886) lacking the α-helix did not form a stable complex with REV7 (data not shown). This suggests that the van der Waal’s interactions by the α-helix of REV3(1847–1898) also contribute to the formation of the REV7-REV3 complex, Polζ.

Furthermore, to investigate the REV7-REV3 interaction in vivo, we carried out co-immunoprecipitation assays using HEK293 cells. Consistent with the in vitro results, REV7(Y63A/W171A) showed no binding affinity for REV3(1776–2044), although REV7(Y63A) and REV7(W171A) retained affinity (Fig. 2C, lanes 3–5). It is also noteworthy that the expression level of FLAG-REV3(1776–2044) in lane 5 of Fig. 2C is considerably lower compared with other lanes, even though the same amount of plasmid DNA was used for transfection in each cells, suggesting that REV7-unbound REV3(1776–2044) may be unstable in vivo. Interestingly, REV7(R124A) had markedly higher affinity for REV3(1776–2044), as compared with REV7(WT) (Fig. 2C, lane 2). This observation suggests that the R124A substitution stabilized the closed conformation of REV7. In fact, the analogous substitution in Mad2, Mad2(R133A), pushes the conformation toward the closed form and enables structure determination of the ligand-free C-Mad2 (45), although why this substitution stabilizes the closed form is less understood. The CD spectrum of the REV7(R124A)-REV3(1847–1898) complex is similar to that of the REV7(WT)-REV3(1847–1898) complex (28), whereas the CD spectrum of REV7(R124A) is distinct from that of REV7(WT) (supplemental Fig. S2). This observation indicates that REV7 also supposedly undergoes a significant structural change, in which the seatbelt region is expected to move upon the ligand binding as observed in Mad2.

**Structural Implication of the Interactions between Polζ and REV1—REV7 interacts with not only the central region (residues 1847–1892) of REV3 but also the C-terminal region (residues 1130–1251) of REV1** (reviewed in Ref. 46). The amino acid sequences of the REV7-interacting regions of REV3 and REV1 are not conserved, indicating that REV7 independently binds REV3 and REV1. However, it has remained unknown whether the human REV7-REV3 complex interacts with REV1. To investigate the physical interactions of the REV7-REV3(1847–1898) complex with REV1(1130–1251), we performed in vitro binding assay. Our results show that both the REV7(WT)-REV3(1847–1898) and REV7(R124A)-REV3(1847–1898) complexes interact with REV1(1130–1251) (Fig. 3B, lanes 1 and 2), indicating that REV7
simultaneously interacts with REV1(1130–1251) and REV3(1847–1898) using different interfaces of REV7.

To identify amino acid residues of REV7 responsible for interaction with REV1, we performed comprehensive alanine substitutions in the solvent-exposed residues of REV7 (Fig. 3A). Mad2 has two independent binding sites for different proteins; one is the sea belt region, where Mad1 interacts with Mad2, and the other is the αC helix, which is the binding site of O-Mad2 for checkpoint activation (47) or p31 3:1 for checkpoint inhibition (48). However, mutations in the αC helix in REV7 did not affect binding to REV1(1130–1251) (data not shown). Our results show that L186A or Q200A mutation significantly depletes REV1 binding, whereas those mutations have no effect on REV3 binding (Fig. 3B, lanes 3 and 4). The fact that Leu-186 and Gln-200 are exposed to solvent (Fig. 3C) indicates that these residues are directly involved in REV1 binding. Leu-186 and Gln-200 are present in β8’ and β8”, respectively (Fig. 3C). Therefore, the REV1-binding site is unprecedented and represents a novel interface for protein-protein interactions of HORMA family proteins. Consistent with this finding, Leu-186 and Gln-200 are not conserved in Mad2 (Fig. 1A), whereas they are conserved in yeast REV7. Leu-186 and Gln-200 of REV7 are positioned close to each other and are thus likely to provide a platform for REV1 binding on the anti-parallel β-sheet composed of β8’ and β8”. This observation implies that formation of the C-terminal β-sheet is significant for REV1 binding. To verify this idea, we performed further surveys by alanine substitution for residues that are relatively buried and seemingly involved in stabilizing the structure of the β-sheet. Consequently, we found that the Y202A mutation impaired the REV1 interaction (Fig. 3B, lane 5). Tyr-202 is located in β8” and directly interacts with both Leu-186 and Gln-200 (Fig. 3C). These results indicate that Tyr-202 stabilizes the REV1-binding platform.

To examine whether Leu-186REV7 is important for interaction with REV1 in vivo, we performed binding assays using human cells and showed that the L186A mutation greatly reduced the interaction with REV1(826–1251), although it did not reduce the interaction with REV3(1776–2044) (Fig. 2C, lane 6, and 3D, lane 4). On the other hand, the W171A mutation unexpectedly decreased the interaction with not only REV3(1776–2044) but also REV1(826–1251) (Fig. 3D, lane 3). Most interestingly, the R124A mutation, which stabilized the closed conformation of REV7, increased REV1 binding; furthermore, the double mutation R124A/W171A in REV7 brought back REV1 binding to the level of REV7(WT) (Fig. 3D, lanes 2, 3, and 5). Therefore, we conclude that REV1 interacts with the closed form of REV7 and that the REV7 REV3 interaction precedes the REV7 REV1 interaction during formation of the Pol-REV1 complex.

**Functional Role of REV7-mediated Interactions in DNA Damage Tolerance**—To examine the contribution of REV7-mediated interactions to DNA damage tolerance, we performed rapid survival assays using chicken DT40 cells. Chicken REV7 shares a high degree of amino acid identity with human REV7 (96%). To functionally analyze the REV7-mediated interactions, we expressed chicken REV7(WT) and REV7(Y63A/W171A) in DT40 REV7−/− cells and measured the sensitivity to cisplatin in the resulting reconstructed clones (Fig. 4). We chose cisplatin, because REV1−/−, REV3−/−, or REV7−/− DT40 cells show strong sensitivity to cisplatin (26, 49). The reconstitution of REV7−/− cells with the REV7 transgene completely normalized cellular sensitivity to cisplatin (white square). In contrast, expression of REV7(Y63A/W171A), which lacks both REV3 and REV1 interactions, had no impact on cellular sensitivity of REV7−/− cells to cisplatin (black triangle). Our results clearly indicate that REV7-mediated interactions are essential for resistance to the DNA damage caused by cisplatin, implying that formation of the Pol-REV1 complex is responsible for resistance and that REV7 functions as an adapter protein between REV3 and REV1 polymerases. 

**Recruitment of Pol- and Polymerase Switching**—Taking our results altogether, we can propose a model of the interactions involving REV1, REV3, and REV7 (Fig. 5A). In the absence of REV3, REV7 can adopt an open form. In the presence of REV3, REV7 undergoes structural rearrangement of the seat belt by REV3 binding, resulting in the formation of Pol7, where Tyr-63REV7 and Trp-171REV7 are crucial for the interaction (Fig. 2). The conformational change in the seat belt of REV7 provides an interface for REV1 interaction (Fig. 3C) and therefore enables formation of the Pol-REV1 complex. REV1, which is the inserter polymerase for an abasic lesion (50, 51), is also supposed to function as a scaffold protein for polymerase switching at a lesion site, because the C-terminal region (residues 1130–1251) of REV1 interacts with the other inserter polymerases, namely Pol7, Polk, and Pol (21).
that mouse PolK and Rev7 compete directly for binding to Rev1 (20). Based on our results, we can propose a model of the recruitment of Pol\(\zeta\) and the second polymerase switching from an inserter polymerase to the extender polymerase, as well (Fig. 5B). An inserter polymerase suitable for a DNA lesion could perform bypass synthesis after the first polymerase switching from a replicative polymerase to an inserter polymerase through interactions with ubiquitinated PCNA and/or the C-terminal region of REV1. Then Pol\(\zeta\) (REV7-REV3 complex) could be recruited to the lesion site by the interaction between REV7 and the C-terminal region of REV1, where the C-terminal \(\beta\)-sheet of REV7 is crucial. The REV7-REV1 interaction would release an inserter polymerase from the C-terminal region of REV1, and Pol\(\zeta\) subsequently could perform extension from the nucleotide inserted by an inserter polymerase (Fig. 5B). Depending on DNA lesions, it is considered that Pol\(\zeta\) performs both nucleotide insertion and extension. In this case, Pol\(\zeta\) is recruited to the lesion site through the REV7-REV1 interaction in a similar way. In contrast to the interaction of human REV7 and REV1, it has been reported that yeast Rev7 interacts with various regions of yeast Rev1 (the N-terminal BRCT domain; the polymerase-associated domain, which is alternatively called the little finger domain; and the C-terminal domain) (52–54). Furthermore, yeast Pol\(\eta\), an inserter polymerase, interacts with the polymerase-associated domain of yeast Rev1 (55). Therefore, the Rev1 interactions in yeast are complicated, and the interactions might diverge between yeast and human.

In this work, we have performed structural and functional analysis of REV7-mediated interactions, and clarified the structural basis of the REV7-REV3 interaction and obtained structural implication of Pol\(\zeta\)-REV1 interactions. We propose that REV7 functions as the adapter protein between REV3 and REV1 polymerases, thereby mediating the second polymerase switching. Human REV7 interacts with various proteins including the Shigella effector IpaB in bacterial infection and ELK1 and TCF4 in signal transduction (15–17). Those proteins have sequences similar to the REV7-binding region of human REV3, indicating that the mechanisms underlying the interactions of those proteins with REV7 are similar to that of the REV7-REV3 interactions described here. Thus, our finding will provide a general structural basis for understanding the interactions mediated by REV7 in various cellular functions.

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