Supporting Information

Khodyreva et al. 10.1073/pnas.1009182107

SI Experimental Procedures.

Purification of the Cross-Linked Protein-DNA Complex Using Streptavidin-Coated Beads. Preparative cross-linking was essentially as described under *Materials and Methods*. The volume of the reaction mixture was increased to 1.3 mL. Apurinic/apyrimidinic (AP)-DNA contained a biotinylated dUMP moiety introduced as described under *Materials and Methods*, "Preparation of linear duplex DNA." To increase the yield of cross-linked protein and decrease nonspecific binding of proteins to the streptavidin resin, we used bovine testis nuclear extract (BTNE). The final BTNE protein concentration in the reaction mixture for cross-linking was 2.8 mg/mL. After completion of the reduction step, magnetic beads with immobilized streptavidin (300 μL of the resin suspension prepared as recommended by the manufacturer) were added to the reaction mixture. The mixture was then incubated at room temperature for 60 min with constant mild agitation. The resin was washed six times by resuspension and sedimentation with a magnet using 300 μL of wash buffer (50 mM Tris-HCl, pH 8.0, and 2 M NaCl) and two times in 300 μL of 50 mM Tris-HCl, pH 7.5, and 75 mM NaCl. Then, 50 μL of gel-loading dye was added to the beads, followed by heating at 95 °C for 20 min. The sample was resolved by 10% SDS-PAGE followed by staining with Coomassie blue. Then, the wet gel was exposed to X-ray film overnight at 0 °C. A well-defined protein band that corresponded to the radioactive band was excised from the gel. The excised gel band was rinsed with 100 μL of water and destained by treatment with 50 μL of 25 mM NH_4HCO_3/50% CH_3CN for 20 min. The solution was decanted and 50 μL of the same solution was added. These steps were repeated until the stain was gone. After incubation with 50 μL of acetonitrile for 10 min and removal of the solvent, the gel slices were dried by vacuum centrifugation. Dried slices were rehydrated in 50 μL of 50 mM NH_4HCO_3 buffer containing trypsin (1 μM) for 1 h at 4 °C. Trypsinolysis was performed for 18 h at 37 ºC. Peptides were extracted from the gel 3 times in 25 μL of 50% acetonitrile/5% formic acid, dried, and dissolved in 5 μL 0.1% TFA (aqueous solution). The sample (1 μL) was mixed with 1 μL of the MALDI matrix material (saturated solution of α-cyano-4-hydroxycinnamic acid in a mixture of acetonitrile and 0.1% aqueous solution of TFA, 1:2 vol/vol). The REFLEX III mass spectrometer (Bruker Daltonics Inc.) was operated in the reflector mode at 23 kV acceleration. Spectra were obtained from an average of 600 laser shots over the m/z range 1,000–5,000. Peptide mass mapping was performed using the Mascot software and National Center for Biotechnology Information protein database at www.expasy.org.

Oligonucleotides. Synthetic oligonucleotides were obtained from the Laboratory of Medicinal Chemistry (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences) and Oligos Etc., Inc. Sequences of oligonucleotides were as follows:

(I) 5′-GGAAGACCCTGACGTTACCCAACTTAATCGCC-3′ – template;
(II) 5′-GGCGATTAAGTTGGG**U**AACGTCAGGGTCTTC-C-3′;
(III) 5′-GGCGATTAAGTTGGG**THF**AACGTCAGGGTCT-TCC-3′;
(IV) 5′-GGCGATTAAGTTGGG-3′;
(VI) 5′-p**THF**AACGTCAGGGTCTTCC-3′;
(VI) 5′-CTGCAGCTGATGCGC UGTACGGATCCCCGG-GTAC-3′;
(VII) 5′-GTACCCGGGGATCCGTACGGCGCATCAGCTG-CAG-3′);
(VIII) **5′-p**GCACGGCGCATCAGCTGCAG**AACAA**CTGC-AGCTGATGCGC-**THF**-GTGCGGATCCGGTGC**A-ACAA**GCACCGGATCC-3′
(IX) **5′-p**GCACGGCGCATCAGCTGCAG**AACAA**CTGCA-GCTGATGCGC-**U**-GTGCGGATCCGGTGC**AACAA**-GCACCGGATCC-3′
(X) **5′-p**GCACGGCGCATCAGCTGCAG**AACAA**CTGCAG-CTGATGCGC-**C**-GTGCGGATCCGGTGC**AACAA**GCA-CCGGATCC-3′
(XI) 5′-ATGCAAGCTTGGCGTAATCA-3′, 5′-CCAACGCT-CAACAGTAGGGC-3′, 5′-ATTCGGTCGCTGAGGC-TTGC-3′ – primers for M13mp19.

Preparation of Double-Hairpin DNA Substrates. ^{32}P-labeled or unlabeled oligonucleotide (IX to XI) that contained a double-hairpin sequence was annealed under the following conditions: 95 °C for 5 min; 65 °C for 30 min; cooling down from 65 °C to 10 °C at 1 °C/ min. The oligonucleotide was then ligated with T4 DNA ligase for 1 h at room temperature. Denaturing polyacrylamide gel electrophoresis was used to separate the ligated and unligated forms of the oligonucleotide. Then, autoradiography and UV shadowing was used to locate the ligated vs. unligated DNA. Gel slices were cut and smashed, and the ligated form of the DNA substrate was eluted with diffusion buffer (0.5 M ammonium acetate, 10 mM Mg^{2+}, 1 mM EDTA, 0.1% SDS) overnight at room temperature. Smashed gel was removed with a spin column (Pall Life Science, P/N ODGHPC34), and the DNA was precipitated with 0.3 M sodium acetate, two volumes of 100% ethanol and 70 μg/mL linear acrylamide at −80 °C for 2 h. The DNA pellet was washed once with 70% ethanol, lyophilized, and then resuspended in TE (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) buffer. The concentration of DNA was determined, and it was stored in small aliquots at −30 °C.

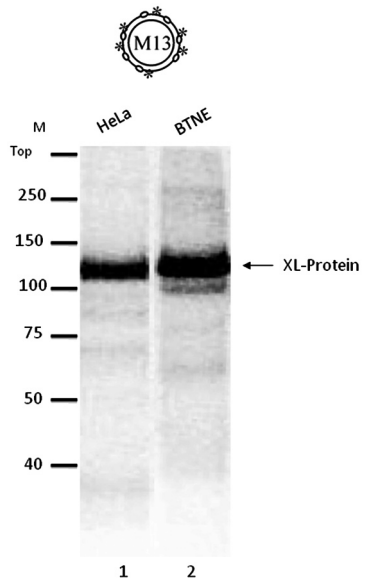

Fig. S1. $NaBH_4$ cross-linking of proteins in BTNE and HeLa whole cell extracts. The reaction conditions and product analysis were as described under *Materials and Methods*. ^{32}P-labeled AP site-containing circular DNA substrate (20 nM) was reacted with extracts (1.25 mg/mL) for 15 min at 37 °C. $NaBH_4$ was then added to a final concentration of 20 mM and further incubated on 0 °C for 30 min. Covalently cross-linked DNA–protein products with circular DNA were treated with benzonase (12 units) for 15 min at 37 °C. The cross-linked products (XL protein) were separated by SDS-PAGE (1). Representative phosphorimages illustrating the cross-linking of proteins in HeLa cell extract and BTNE are shown. The positions of protein markers and XL-protein are indicated. Schematic representation of DNA is illustrated at the top. The symbol '*' denotes the position of ^{32}P-label on DNA. The bubble like symbol denotes the AP site(s) in DNA.

1. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.

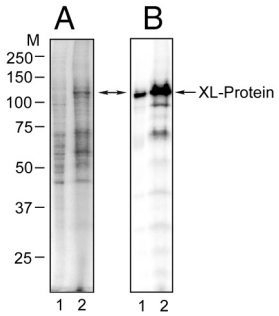

Fig. S2. Isolation and purification of the cross-linked protein–DNA complex. A reaction mixture (1.3 mL) containing BTNE (2.8 mg/mL) and [^{32}P]-labeled biotinylated AP-DNA (100 nM) was treated as described above under *Materials and Methods*. Following $NaBH_4$ cross-linking, the reaction mixture was further processed as described. (*A*) Coomassie blue stained gel. Lane 1, 10 μL aliquot of the reaction mixture; lane 2, eluant from beads. (*B*) Autoradiograph of the gel in *A*. Positions of ^{32}P-labeled protein–DNA band that was excised and subjected to mass spectrometry analysis and protein markers are indicated.

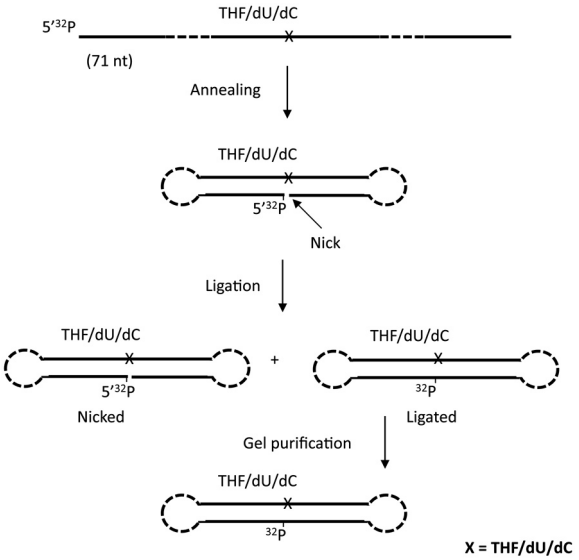

Fig. S3. Scheme for hairpin DNA substrate preparation. ^{32}P-labeled or unlabeled oligonucleotide (IX to XI) that contained a double-hairpin sequence was annealed under the following conditions: 95 °C for 5 min; 65 °C for 30 min; cooling down from 65 °C to 10 °C at 1 °C/ min. The oligonucleotide was then ligated using T4 DNA ligase for 1 h at room temperature. Denaturing polyacrylamide gel electrophoresis was used to separate the ligated substrate from the nicked form of DNA. Then, autoradiography and UV shadowing was followed to locate the ligated vs. unligated DNA. Gel slices were cut, smashed, and the ligated form of DNA substrate was eluted with diffusion buffer (0.5 M ammonium acetate, 10 mM Mg^{2+}, 1 mM EDTA, 0.1% SDS) overnight at room temperature. Smashed gel was removed with a spin column (Pall Life Science, P/N ODGHPC34), and the DNA was precipitated with 0.3 M sodium acetate, two volumes of 100% ethanol and 70 μg/mL linear acrylamide at −80 °C for 2 h. The DNA pellet was washed once with 70% ethanol, lyophilized, resuspended in TE buffer, and stored at −30 °C.

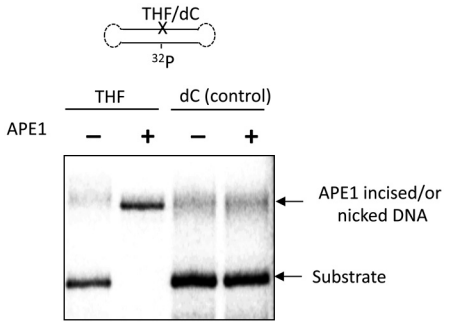

Fig. S4. AP endonuclease 1 (APE1) digestion of double-hairpin DNA substrates. An aliquot (20 nM) of each double-hairpin DNA substrate was reacted without (–) or with (+) 5 nM APE1. After incubation for 15 min at 37 °C, the addition of an equal volume of DNA gel-loading buffer terminated the reaction. All samples were heated for 3 min at 80 °C and analyzed in a 15% polyacrylamide gel containing 8 M urea (38). A phosphorimage of the gel is shown, depicting the positions of the intact circular hairpin DNA substrate and the APE1 incision product. The experiment was conducted either with THF-containing DNA or control DNA with dC instead of THF. These results indicated that our substrates contained only traces of nicked DNA. A schematic illustrating the structure and features of the substrate is shown at *Top*.

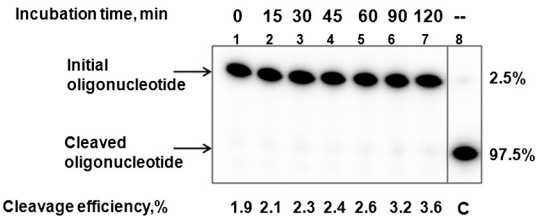

Fig. S5. Time dependence of spontaneous AP site-containing DNA cleavage. Labeled (32P-5′-end) linear DNA (100 nM) containing a natural AP site was incubated at 37 °C for the indicated periods (lanes 1–7). Aliquots were withdrawn, treated with NaBH4 and analyzed as described under *Materials and Methods*. Lane 8 represents a control sample where the AP site-containing DNA was treated with 0.1 M NaOH for 5 min at 75 °C, and this reflected the level of uracil excision by uracil-DNA glycosylase during preparation of the AP site-containing DNA. The percentage cleavage of the AP site-containing DNA is shown at the bottom. All eight lanes shown were from the same gel; some lanes from this gel are not shown because they do not add significantly to the information, and the order of the original lanes was adjusted.

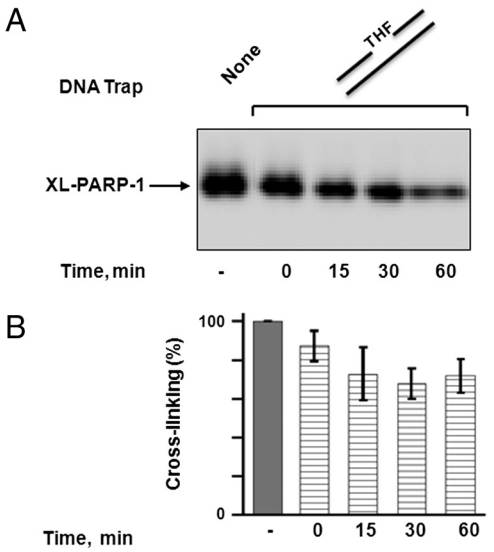

Fig. S6. Estimation of the stability of the poly(ADP-ribose) polymerase-1 (PARP-1)–AP site-containing DNA complex. The experiments are described under *Materials and Methods*. (*A*) Labeled linear DNA (100 nM) containing the natural AP site was preincubated with PARP-1 (200 nM) for 15 min at 37 °C. The mixture was then divided into portions: One was processed immediately for cross-linking (lane 1); the other was supplemented with excess DNA trap (unlabeled THF-DNA) and then further incubated at 37 °C for the indicated periods (lanes 2–4). Aliquots were withdrawn, treated with $NaBH_4$, and analyzed as described. A schematic representation of the DNA trap is illustrated at *Top*. The XL-PARP-1 is indicated. (*B*) Quantification summarizing the amount of cross-linked PARP-1 as a function of time of incubation in the presence of the trap. The yield of XL-PARP-1 in three experiments is similar to the experiment shown in panel *A*. In each independent experiment, the cross-linking in the absence of the trap was taken as 100%. The data are the mean ± SD, $n = 3$.

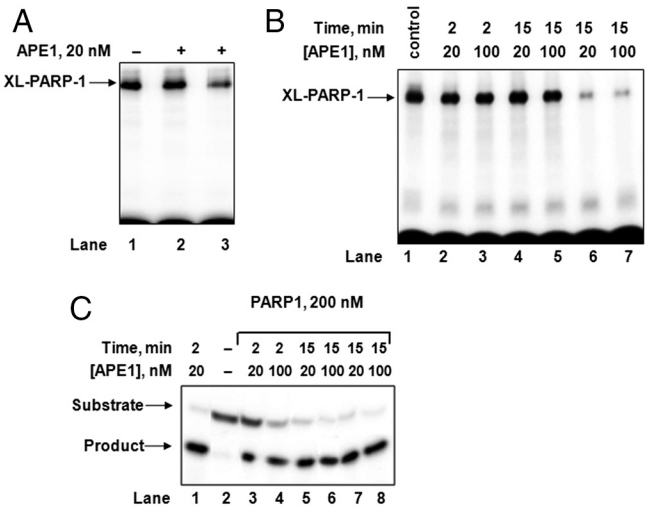

Fig. S7. Competition of PARP-1 and APE1 for AP site DNA. The experiments are described under *Materials and Methods*. (*A*) 5′end-labeled linear DNA (100 nM) containing the natural AP site was incubated with PARP-1 (200 nM) for 15 min at 37 °C in the absence of magnesium ions. APE1 was absent (lane 1) or present at 20 nM (lanes 2 and 3). Lane 2 represents a reaction mixture where PARP-1 was first preincubated with the AP site DNA for 15 min at 37 °C, and then APE1 was added, followed by incubation for additional 15 min at 37 °C. Lane 3, PARP-1 and APE1 were added simultaneously. All reaction mixtures were treated with $NaBH_4$ and analyzed as described. (*B*). Labeled linear DNA (100 nM) containing the natural AP site was incubated with PARP-1 (200 nM) for 15 min at 37 °C in the presence of 10-mM magnesium ions and absence of APE1 (lane 1) or presence of 20 nM APE1 (lane 6) or 100 nM APE1 (lane 7) for 15 min at 37 °C. Reaction mixtures were as follows (lanes 2–6): PARP-1 (200 nM) was first preincubated with AP site DNA (100 nM) for 15 min at 37 °C; then the reaction mixtures were supplemented with 20 nM APE1 (lanes 2 and 4) or 100 nM APE1 (lanes 3 and 5) and further incubated at 37 °C for the indicated periods. All reaction mixtures were treated with $NaBH_4$ and analyzed as described. Times of incubation with APE1 and concentrations of APE1 are indicated. (*C*) The experiment was performed as in *B*, but after $NaBH_4$ reduction the samples were analyzed by 20% PAGE-7M urea (38). Lane 1 represents cleavage of 100-nM AP site DNA substrate with 20 nM APE1 at 37 °C for 2 min. Lane 2, a control (incubation of AP site DNA for 15 min at 37 °C without proteins). Lanes 3–6, PARP-1 was first preincubated with AP site DNA at 37 °C for 15 min, and then 20 nM APE1 (lanes 3 and 5) or 100 nM APE1 (lanes 4 and 6) was added followed by incubation for the indicated periods at 37 °C. Lanes 7 and 8, PARP-1 and APE1 were added simultaneously and the reaction mixtures were incubated at 37 °C for 15 min.

Table S1. Observed masses in MALDI-TOF mass spectrum, which correspond to theoretical peptides of bovine PARP1

	$(M+H)^+_{obs}$	$(M+H)^+_{clc}$	Peptide	Position	Modifications	Missed cleavages
1	1,119.510	1,119.364	LNKKMEEVK	441–449		2
2	1,194.530	1,194.409	CMVKTQTPNR	324–333	MSO	1
3	1,234.650	1,234.543	SEKRMKLTLK	521–530		3
4	1,235.510	1,235.397	MEEVKEANIR	445–454	MSO	1
5	1,320.520	1,320.534	KEWVTPKEFR	334–343		2
6	1,322.550	1,322.583	CMVKTQTPNRK	324–334	MSO	2
7	1,322.550	1,322.623	ASCKKCKESIPK	19–30		3
8	1,334.680	1,334.511	DPIDVNYEKLK	790–800		1
9	1,335.580	1,335.413	TDVSGKGQDGVGSK	95–108		1
10	1,361.670	1,361.543	SWGRVGTVIGSNK	590–602		1
11	1,363.630	1,363.571	KMEEVKEANIR	444–454	MSO	2
12	1,365.590	1,365.702	SKLPKPVQNLIK	665–676		1
13	1,378.670	1,378.570	TTNFAGILSQGLR	868–880		0
14	1,389.670	1,389.640	STCKSCMEKIDK	126–137	MSO	2
15	1,390.640	1,390.587	SNRSTCKSCMEK	123–134	MSO	2
16	1,427.760	1,427.661	VEYAKSGRASCKK	11–23		3
17	1,487.670	1,487.750	YKPFKQLHNRR	850–860		2
18	1,494.690	1,494.734	KPPLLNNANSVQAK	750–763		0
19	1,508.750	1,508.773	LTGTANKASLCISTK	421–435		1
20	1,522.620	1,522.789	MAFMVESPMFDGK	35–47	MSO MSO	0
21	1,522.620	1,522.789	MAFMVESPMFDGK	35–47	MSO MSO	0
22	1,522.620	1,522.789	MAFMVESPMFDGK	35–47	MSO MSO	0
23	1,533.720	1,533.675	KGDEVDGIDEVTKK	212–225		2
24	1,533.720	1,533.675	GDEVDGIDEVTKKK	213–226		2
25	1,563.720	1,563.709	LYEEKTGNAWHSK	619–631		1
26	1,585.900	1,585.803	TQTPNRKEWVTPK	328–340		2
27	1,709.680	1,709.998	ACSTNDLKELLIFNK	258–272		1
28	1,738.760	1,739.058	SCMEKIDKGQVRLSK	130–144	MSO	3
29	1,748.950	1,748.928	GDEVDGIDEVTKKKSK	213–228		3
30	1,767.660	1,767.982	IEREGESQRYKPFK	841–854		2
31	1,790.670	1,791.029	MAESSDKLYRVEYAK	1–15		2
32	1,798.770	1,799.005	EDAIEHFMKLYEEK	610–623	MSO	1
33	1,993.940	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
34	1,993.940	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
35	1,993.940	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
36	1,993.940	1,994.415	KAMVEYEIDLQKMPLGK	686–702		2
37	1,994.690	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
38	1,994.690	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
39	1,994.690	1,994.303	DSIRMAFMVESPMFDGK	31–47	MSO MSO	1
40	1,994.690	1,994.415	KAMVEYEIDLQKMPLGK	686–702		2
41	2,210.820	2,210.652	AMVEYEIDLQKMPLGKLSK	687–705	MSO	2
42	2,210.820	2,210.652	AMVEYEIDLQKMPLGKLSK	687–705	MSO	2
43	2,412.210	2,412.590	MAETGGRTDVSGKGQDGVGSKTEK	88–111	MSO	3
44	2,707.510	2,707.174	IAPPEAPVTGYMFGKGIYFADMVSK	881–905	MSO	1
45	2,707.510	2,707.174	IAPPEAPVTGYMFGKGIYFADMVSK	881–905	MSO	1
46	2,810.730	2,811.174	GDAYYCTGDVTAWTKCMVKTQTPNR	309–333		2
47	2,826.790	2,827.174	GDAYYCTGDVTAWTKCMVKTQTPNR	309–333	MSO	2
48	2,840.810	2,841.159	VEMLDNLLDIEVAYSLLRGGSDDSSK	764–789		1
49	3,038.520	3,038.616	VVYPDKPQLGMVDCWYHPKCFVQKR	146–170		2
50	3,038.910	3,038.616	VVYPDKPQLGMVDCWYHPKCFVQKR	146–170		2