Molecules and Cells

SUPPLEMENTARY MATERIALS AND METHODS

1. DNA PREPARATION

DNA preparation for DNA curtain assay

The DNA preparation for DNA curtain assay was performed by following the previous protocol (Cheon et al., 2019). All oligomers were synthesized from Bioneer (South Korea) and listed in Supplementary Table S1. For DNA curtain assays, lambda phage (λ-) DNA was annealed with oligomers complementary to *COS* sequences in 10 mM Tris-HCl [pH 7.5] and 100 mM NaCl. 15 nM of λ-DNA was mixed with 1 μM of COS_biotin_R and 1 μM COS_comp for single-tether DNA curtain. For annealing, each mixture was heated at 65°C for 10 min and then slowly cooled down to 23°C. The nicks were then sealed by T4 DNA ligase (NEB). After the ligases were heat-inactivated, the excessive oligomers were removed by S-400 spin column (Illusta MicroSpinTM S-400; GE Healthcare).

DNA preparation for single-molecule photobleaching assay

The plasmid (5,455 bp) derived from pET28a(+) containing 1× Widom 601 sequence was used as a template for polymerase chain reaction (PCR) to generate 147 bp and 269 bp DNA fragments. Biotinylated DNA fragments containing Widom 601 sequence were prepared by PCR with primers listed in Supplementary Table S1. For biotinylated 25, 40, and 80 bp duplexes containing the core Widom 601, complementary sets of oligomers were synthesized and then hybridized in 10 mM Tris-HCl [pH 7.5] and 100 mM NaCl (Supplementary Table S1).

DNA preparation for electrophoretic mobility shift assay

For 80 bp duplex DNA containing the core Widom 601, complementary oligomers (Widom601_80 /Widom601_80_comp) were synthesized and hybridized (Supplementary Table S1).

2. BULK BIOCHEMICAL ASSAYS

Magnetic bead pull-down assay

0.05 mg of streptavidin-coated magnetic beads (Dynabeads M-280 streptavidin; Invitrogen) were transferred to an Eppendorf tube, which was placed on a magnet. The supernatant was discarded, and the beads were resuspended in 200 μl wash buffer (20 mM Tris-HCl [pH 8.0] and 100 mM NaCl). This bead washing step was repeated three times. The beads were resuspended in 5 μl of 5 nM of biotinylated 269 bp DNA containing Widom 601 in washing buffer. The DNA molecules were conjugated with the beads at 23°C for 10 min. The DNA-conjugated magnetic beads were prepared by removing unbound DNA molecules by three-time washing with washing buffer.

The DNA-conjugated magnetic beads were mixed with 2 μM Abo1 or pre-incubated 2 μM Abo1 and 5 μM H3-H4 dimer in total 15 μl reaction buffer (50 mM Tris-HCl [pH 8.0], 100 mM NaCl, 2 mM DTT, 1 mM ATP, 2 mM $MgCl_2$, 0.05 mg/ml BSA, and 0.005% Tween 20) at 23°C for 30 min. Then the beads were pull downed by a magnet, and proteins unbound to DNA were taken from the supernatant. The beads were washed with 200 μl reaction buffer and the washing solution was also saved. The beads were resuspended in 15 μl reaction buffer, and the DNA bound proteins were eluted by treating DNase I (D5319-2; Sigma) at 23°C for 10 min. The proteins in the supernatant, the washing solution, and the elution were analyzed by 10% SDS PAGE (100 V for 95 min at 23°C). The gel was stained by Coomassie.

3. SINGLE-MOLECULE DNA CURTAIN ASSAYS

Total internal reflection fluorescence microscope (TIRFM)

Prism-type total internal reflection fluorescence microscope (TIRFM) was custom-built with Nikon Eclipse Ti-2. The solid-state 488-nm for YOYO-1 and Alexa488 or 637 nm laser for Cy5 (200 mW, OBIS, Coherent Laser) was used to excite fluorescent dyes. The laser beam was incident through a prism to increase the incident angle beyond the critical angle, so that the laser beam was totally reflected at the boundary between the surface of fused-silica slide and buffer, generating the ~250 nm high evanescent field. The fluorescence signals from the dyes were collected by 60× water-immersion objective lens (CFI Plan Apo VC 60XWI; Nikon) and then imaged by an EM-CCD camera (iXon 897; Andor Technology). To block laser beams, long-pass filters suitable for fluorescent dyes were used. The data were collected by the NIS-Element software (Nikon).

Flowcell preparation and DNA curtain building-up

Nano-patterned slides were fabricated based on the previous protocol (Cheon et al., 2019). Briefly, piranha cleaned fused-silica

slides having two holes were spin-coated with e-beam resistor (950k PMMA). Electron-beam lithography made Charlie-Brown patterns, which were developed in a developer (MiBK:IPA 1:3). 40 nm of chromium was evaporated by electron-beam evaporator, and then patterned slides were completed by lift-off in acetone. The patterned slides were thoroughly cleaned by successive treatments with 2% Hellmanex III (Sigma) for at least one day, 99% ethanol for 2 h, and 1 M sodium hydroxide for 20 min. The slides were rinsed with DI water between each cleaning step and then finally stored in DI water until use. A microchannel was constructed by assemble the patterned slide and a coverslip with a double-sided tape. Nanoports (IDEX) were glued on the holes to link the flowcell with a syringe pump-based fluidic system. The flowcell was rinsed with DI and with lipid buffer (20 mM Tris-HCl [pH 8.0] and 100 mM NaCl) successively. Liposomes consisting of DOPC (1,2-dioleoyl-*sn*-glycero-phosphocholine), 0.5% biotinylated-DPPE (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl)), and 8% mPEG 2000-DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]) (Avanti-Polar Lipids) were deposited on the microchannel surface. The lipid bilayer was spread on the surface by 20 min incubation after washing out free liposomes. BSA buffer (40 mM Tris-HCl [pH 8.0], 50 mM NaCl, 2 mM $MgCl_2$, and 0.4% BSA) was added for further surface passivation, and then 0.025 mg/ml of streptavidin in BSA buffer was injected and bound to biotins on lipids. The unbound streptavidin was washed out. For single-tether DNA curtain, 10-100 pM of λ-DNA tagged only with biotin at one end was added into the flowcell and anchored on the biotinylated lipid via streptavidin. The flowcell was connected with the syringe-based fluidic system and then placed on TIRFM. Under the continuous buffer flow, DNA molecules were moving along the flow and then were stretched and aligned at the chromium nano-barriers. All DNA curtain experiments were performed at 23°C.

Single-tether DNA curtain assay for H3-H4 dimer, H2A-H2B dimer and octamer deposition by Abo1

The formation of DNA curtains was firstly ensured by staining λ-DNA with an intercalating agent YOYO-1, which was excited by the 488 nm laser. To exclude unexpected interference of YOYO-1 with histone deposition by Abo1, YOYO-1 was removed from λ-DNA by washing with Abo1 buffer (50 mM Tris-HCl [pH 8.0], 100 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT, 1.6% glucose, and 0.1 × gloxy) supplemented with 20 mM $MgCl_2$ and 200 mM NaCl. 10 nM Abo1 and 25 nM Cy5-labeled H3-H4 dimers (25 nM Alexa488-labeled H2A-H2B dimers or 25 nM octamers with Cy5 at H2A) were pre-incubated in Abo1 buffer supplemented with a nucleotide cofactor on ice for 15 min and then were injected into the DNA curtains. Upon the arrival of the maximum amount of proteins at the DNA curtains, the flow was stopped, and the proteins were incubated with DNA molecules for 15 min. The binding of H3-H4 dimers (H2A-H2B dimers or octamers) to DNA was visualized by resuming the flow to stretch down λ-DNA into the evanescent field. We also tested the binding of H2A-H2B dimers to DNA in the presence of H3-H4 dimers. 25 nM unlabeled H3-H4 dimers and 25 nM Alexa488-labeled H2A-H2B dimers were simultaneously incubated with 10 nM Abo1, and then the mixture was injected into the flowcell. In addition, unlabeled H3-H4 dimers were pre-loaded to DNA by Abo1 in the flowcell as described above, and then we injected Alexa488-labeled H2A-H2B which was pre-incubated with Abo1. To avoid the photobleaching of fluorescent dyes, we turned off the laser during incubation in the flowcell.

Data analysis

All DNA curtain images taken by NIS-Elements (Nikon) were transformed into TIFF format and then were analyzed by ImageJ (NIH). Kymographs for individual DNA molecules were created, and the fluorescence signals disappearing in the absence of flow were chosen. The fluorescence intensity profile was then obtained by taking line profiles of five frames and averaging them in ImageJ. The fluorescence intensity profile was fitted by multiple Gaussian functions. The center coordinates of peaks were collected to build up a histogram for binding distribution of H3-H4 dimers. The peak intensities were divided by minimum intensity to estimate the binding number of H3-H4. At each peak position, the number of H3-H4 dimers bound to DNA was calculated. The histogram was built up with 1 kbp bin size.

4. SINGLE-MOLECULE PHOTOBLEACHING ASSAY

For the single-molecule photobleaching assay, we firstly laid 0.2 mg/ml streptavidin on the flowcell surface (Fig. 2A). Then lipid bilayer without biotinylated lipids was promoted on the surface to inhibit nonspecific adsorption of proteins. 300 pM of biotinylated DNA substrates containing 1× Widom 601 sequence were anchored on slide surface through streptavidin. After washing unbound DNA molecules out, 300 pM Abo1 and 750 pM Cy5-labeled H3-H4 dimers, which were pre-incubated on ice for 15 min, were injected into the flowcell in the Abo1 buffer with 1 mM ATP. Likewise, for *S. cerevisiae* CAF-1, 30 pM of *S. cerevisiae* CAF-1 and 75 pM of Cy5-labeled H3-H4 dimers were pre-incubated in CAF-1 buffer (25 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 0.02% Tween-20, and 0.5 mM TCEP) on ice for 15 min, followed by the injection into the flowcell. After 15 min incubation inside the flowcell at 23°C, residual proteins were flushed out in the Abo1 buffer with 1 mM ATP for Abo1 and without $MgCl_2$ for CAF-1. Then fluorescence images were taken by TIRFM under the continuous illumination of 637 nm laser until fluorescence signals were photobleached. Images were analyzed by a home-built analysis software. The first 10 frames of image were averaged, and overall background was subtracted. Fluorescence puncta were selected only when the puncta were well-fitted by 2-dimensional Gaussian function. The fluorescence trajectory from each punctum was extracted. The photobleaching steps were manually analyzed from each intensity trajectory.

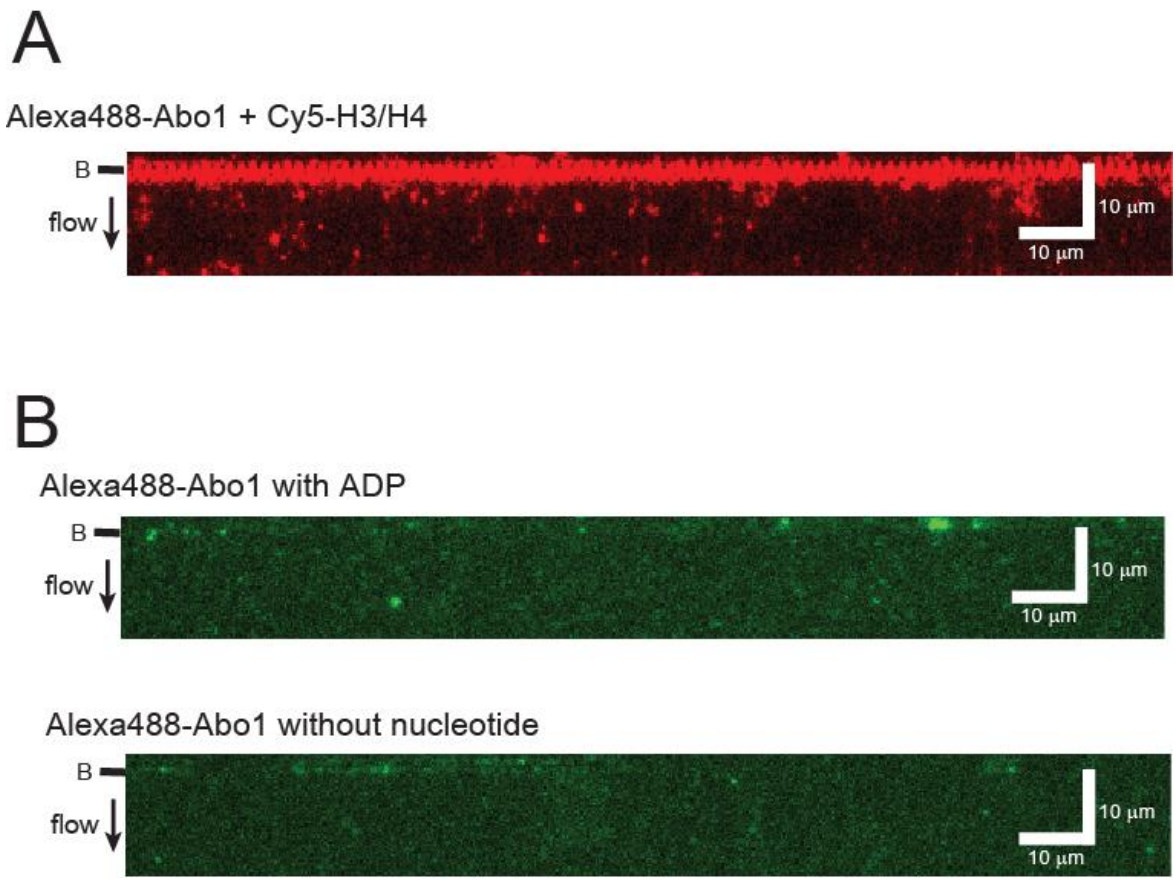
SUPPLEMENTARY REFERENCE

Cheon, N.Y., Kim, H.S., Yeo, J.E., Schärer, O.D., and Lee, J.Y. (2019). Single-molecule visualization reveals the damage search mechanism for the human NER protein XPC-RAD23B. Nucleic Acids Res. *47*, 8337-8347.

Supplementary Table S1. List of oligomers

Name	Sequences
Widom601_269_F	5’-AAG AAG GAG ATA TAA TAG AAT TCC C-3’
Widom601_269_R_biotin	5’-[Biotin]-ATC TCA GTG GTG GTG G-3’
Widom601_147_F_biotin	5’-[Biotin]-ACA TCG AGA ATC CCG GTG-3’
Widom601_147_R	5’-CGG ATG TAT ATA TCT GAC ACG T-3’
Widom601_80_biotin	5’-[Biotin]-GTC GTA GAC AGC TCT AGC ACC GCT TAA ACG CAC GTA CGC GCT GTC CCC CGC GTT TTA ACC GCC AAG GGG ATT ACT CCC TA-3’
Widom601_80_comp	5’-TAG GGA GTA ATC CCC TTG GCG GTT AAA ACG CGG GGG ACA GCG CGT ACG TGC GTT TAA GCG GTG CTA GAG CTG TCT ACG AC-3’
Widom601_80	5’-GTC GTA GAC AGC TCT AGC ACC GCT TAA ACG CAC GTA CGC GCT GTC CCC CGC GTT TTA ACC GCC AAG GGG ATT ACT CCC TA-3’
Widom601_40_biotin	5’-[Biotin]-CCGCTTAAACGCACGTACGCGCTGTCCCCCGCGT TTTAAC-3’
Widom601_40_comp	5’’-GTTAAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGC GG-3’
Widom601_25_biotin	5’-[Biotin]-GCTTAAACGCACGTACGCGCTGTCC-3’
Widom601_25_comp	5’-GGACAGCGCGTACGTGCGTTTAAGC-3’
COS_biotin_R	5’-[Phosphate]-AGG TCG CCG CCC-[Biotin]-3’
COS_comp	5’-[Phosphate]-GGG CGG CGA CCT-3’

Supplementary Fig. S1. Interaction between Abo1 and H2A-H2B dimer. (A) DNA curtain image for loading of H2A-H2B dimers along with Abo1 and H3-H4 dimers. (B) DNA curtain image for loading of H2A-H2B dimers by Abo1 after H3-H4 dimers are already loaded by Abo1.



Supplementary Fig. S2. Interaction between Alexa488-labeled Abo1 and DNA. (A) DNA curtain image for loading activity test of Alexa488-labeled Abo1. Alexa488-labeled Abo1 can deposit Cy5-labeled H3-H4 dimer (red puncta). (B) DNA curtain images for DNA binding of Alexa488-labeled Abo1 in the presence of ADP (top) or in the absence of nucleotide (bottom).