Supporting Information

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Materials and Methods

Unless otherwise stated, all experiments were carried out with the SV40-transformed human fibroblast line MRC5V1, grown in Eagle's MEM with 10% or 15% FCS. For exposure to chemical mutagens, cells were plated at 5×10^5 cells per 6-cm dish and treated 24 h later in medium containing the mutagen for the indicated times. The medium was then removed, and the cells washed twice in PBS and incubated in fresh medium.

Generation of Stable Cell Lines Expressing Normal Levels of Proliferating Cell Nuclear Antigen (PCNA). MRC5V1 cells were transfected with DNA encoding wild-type PCNA or PCNA-K164R tagged with 6 histidine residues in pcDNA3.1. The cDNA constructs carried in addition the following silent mutations to render them refractory to targeting by the siRNA: wild type, GC CGA GAT CTC AGC CAT AT; mutant, GC **AGG** GA**C TTA TCT** CAT AT. G418-resistant clones were picked, expanded, and analyzed by immunoblotting for the level of expressed His-PCNA. Clones expressing His-PCNA at close to endogenous PCNA levels were selected for further analysis. One wild-type and one PCNA-K164R clone were used in subsequent studies.

siRNA Treatment. MRC5V1 cells were transfected with 20 nM ATR siRNA (Dharmacon SMARTpool) or with 5–10 nM RPA-siRNA (AACACUCUAUCCUCUUUCAUG) by using Hiperfect transfection reagent (Qiagen). Cells were UV-irradiated 72 h later. Cell lines expressing exogenous PCNA were treated with PCNA siRNA [GCCGAGAUCUCAGCCAUAUTT (Dharmacon)], polη siRNA (SMARTpool ON-TARGETplus), or both, in 24-well plates, and depletion of endogenous PCNA was analyzed 72 h later. For controls, the nontargeting siRNA pool (Dharmacon) was used.

Survival Experiments. Cells expressing His-PCNA (wild type or K164R) were treated with siRNA for 72 h. They were then trypsin-treated and plated for a further 12 h before UV-irradiation or treatment with MMS or camptothecin for 1 h. Fresh medium was added, and colonies were counted after 10 days.

Photoreactivation. Fifty percent confluent cells on 6-cm cell culture dishes were washed with PBS and UV-irradiated (20 J m^{-2}). Either immediately or after 6 h in growth medium, cells were washed with PBS, and 4 ml of Hanks' buffer without phenol red was added to the dishes. The cells were exposed from below at room temperature to photoreactivating light from two fluorescent tubes for 2 h. The dishes were placed on a Perspex sheet to prevent overheating. After photoreactivation, the cells were incubated further in growth medium.

Measurement of UV Photoproducts. Genomic DNA was extracted from UV-irradiated XP-A cells expressing photolyases (1) with or without photoreactivation treatment. Cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (6-4PPs) were measured by using a protocol described in ref. 2. Briefly, 5 μl containing 100 or 400 ng of DNA were spotted onto Hybond N^+ filters. The filters were incubated with either TDM-2 anti-CPD or 64M-2 anti-6-4PP antibody (3) for 1 h.

Flow Cytometry. Cells were harvested, washed twice in PBS, and fixed in ethanol. Fixed cells were washed in PBS–Tween, incubated with 0.5 mg/ml RNase A and 0.05 mg/ml propidium iodide for 1 h, and analyzed in a Becton-Dickinson FACSAria flow cytometer.

Western Blotting. Plates were washed twice in PBS and 150 μl of lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% SDS] added directly to the plates. Cells were harvested by scraping, incubated on ice for 10 min, and then sonicated to shear the DNA. Fifteen microliters was loaded onto 8% SDS/polyacrylamide gels. After electrophoresis, proteins were transferred to PVDF membranes and immunoblotted with antibodies to PCNA (PC10, 1:5,000; Santa Cruz Biotechnology), USP1 (1:3,000; supplied by Tony Huang, New York University, New York), ATR (1:500; Santa Cruz Biotechnology), RPA (1:1,000; Oncogene), Chk1 phosphorylated on S317 (1:1,000; Cell Signaling), or vimentin (1:5,000; Oncogene). Triton X-100 extraction of cells on the dishes was carried out as described in ref. 4.

1. Nakajima S, *et al.* (2004) UV light-induced DNA damage and tolerance for the survival of nucleotide excision repair-deficient human cells. *J Biol Chem* 279:46674–46677.
2. You YH, *et al.* (2001) Cyclobutane pyrimidine dimers are responsible for the vast majority of mutations induced by UVB irradiation in mammalian cells. *J Biol Chem* 276:44688–44694.
3. Mori T, *et al.* (1991) Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6′-4′) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. *Photochem Photobiol* 54:225–232.
4. Kannouche PL, Wing J, Lehmann AR (2004) Interaction of human DNA polymerase η with monoubiquitinated PCNA: A possible mechanism for the polymerase switch in response to DNA damage. *Mol Cell* 14:491–500.

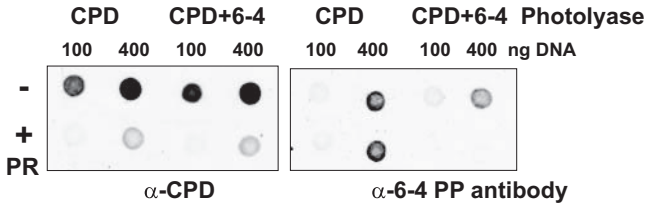

Fig. S1. Photoreactivation of UV lesions. XP-A cells expressing either CPD (*Left*) or both CPD and 6-4PP photolyase (*Right*) were irradiated with 0 or 20 J m^{-2} UV. Cells were immediately either sham-exposed (−) or exposed to visible light for 1.5 h (+PR). Photoproducts in the genome were measured with anti-CPD or anti-6-4PP antibody.

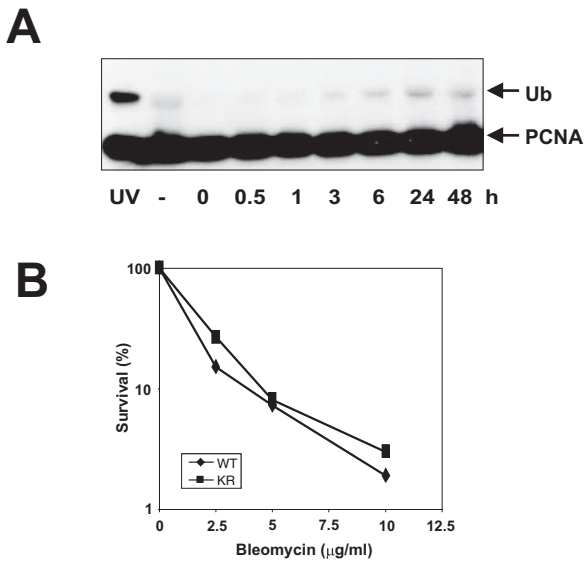

Fig. S2. Effect of bleomycin . (*A*) Cells were treated with 100 μg/ml bleomycin for 1 h and incubated for the indicated times. UV indicates cells exposed to UV (20 J m^{-2}) and incubated for 6 h, as a positive control. (*B*) Bleomycin survival curves of cells depleted for endogenous PCNA and expressing wild-type (WT) or mutant (KR) His-PCNA.

Table S1. Percentage of cells in different phases of the cell cycle with or without RPA knockdown (KD)

RPA status	G_1, %	S, %	G_2, %
No UV control	67	19	14
No UV RPA knockdown	58	21	20
UV control	71	15.5	13
UV RPA knockdown	67	17	15