Supporting Information

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SI Materials and Methods

Materials. MEM and New Zealand FCS were obtained from Invitrogen. Erythro-9-2-hydroxy-3-nonyladenine was a gift from Staffan Eriksson (Swedish University of Agricultural Sciences, Uppsala, Sweden), and Immucillin H was a gift from Vern Schramm (Yeshiva University, New York, NY). Ethidium bromide (EtBr), deoxynucleosides, and deoxynucleotides were purchased from Sigma-Aldrich. The following mouse monoclonal antibodies were used: anti-cyclobutane pyrimidine dimers (CPDs) (clone TDM-2; CosmoBio), anti-(6,4) photoproducts (6-4PPs) (clone 64M-2; CosmoBio), and anti–phospho-histone H2AX (Ser139) (clone JBW301; Millipore).

Analytical Procedures. Intracellular concentrations of the four dNTPs were determined by an enzymatic assay (1) modified as described recently (2). The number of mtDNA copies was determined by real-time PCR (3) as described previously (4).

Depletion of mtDNA. To deplete cells of mtDNA, EtBr (5) was added at a final concentration of either 20 or 50 ng/mL to medium supplemented with 1 mM sodium pyruvate and 50 μg/mL of uridine. For cycling cells, cells were transplanted every third day in the EtBr-containing medium containing 10% FCS. To obtain quiescent mtDNA-depleted cultures, the cells were incubated in EtBr-containing medium with 10% FCS before they reached confluence, followed by a second 7-d incubation in EtBr medium with 0.1% dialyzed FCS. With both cycling and quiescent cells, treatment was stopped by removing the EtBr medium, washing the monolayer with warm PBS, and adding fresh medium without EtBr.

UV Irradiation of Cells. Cell monolayers were irradiated with a 254-nm UVS-11 mineral light lamp at a fluency rate of 2 J/m^2/sec. Then fresh medium was added, and the cells were returned to culture conditions.

Analysis of Photoproducts After UV Irradiation. Dot blot immunoassays (6) were performed to determine the relative amounts of CPDs and 6-4PPs in total genomic DNA from cells collected at different times after UV irradiation. Genomic DNA was isolated using the Puregene Core Kit B (Qiagen). After equal amounts of DNA were denatured from each sample by boiling for 5 min, the samples were placed on ice, and equal volumes of Tris-EDTA buffer and 20× sodium chloride-sodium phosphate-EDTA buffer were added. Using a dot-blot apparatus (BioRad), each sample was blotted in triplicate (10 ng DNA/dot for CPDs and 100 ng DNA/dot for 6-4PPs) onto nitrocellulose membranes previously soaked in 6× SSC. DNA was fixed to the membranes by heating for 2 h at 80 °C, after which the membranes were blocked in PBS/0.2% Tween 20 containing 5% (wt/vol) blocking agent (GE Healthcare) for 1 h at room temperature. The membranes were then incubated with either the CPD-specific monoclonal antibody TDM-2 (dilution 1:6,000) or the 6-4PP–specific monoclonal antibody 64M-2 (dilution 1:6,000) overnight at 4 °C. After washing with PBS-Tween, the membranes were incubated with anti-mouse HRP-conjugated Ig (dilution 1:20,000) for 1 h at room temperature. After further washing, the signals were developed with the ECL Advanced Chemiluminescence Kit (GE Healthcare) in accordance with the manufacturer's instructions. The relative intensity of each signal was determined using a Kodak 440CF one-dimensional imaging station.

Fluorometric Analysis of DNA Unwinding. Fluorometric analysis of DNA unwinding (FADU) (7) was performed using five different buffer solutions: solution B, composed of 0.25 M myoinositol, 1 mM $MgCl_2$, and 10 mM Na-phosphate buffer (pH 7.2); solution C, 9 M urea, 10 mM NaOH, 5 mM cyclohexanediaminetetraacetate, 0.1% SDS; solution D, 0.45 vol/vol solution C in 0.2 M NaOH; solution E, 0.40 vol/vol solution C in 0.2 M NaOH; and solution F, 1 M glucose and 14 mM dithiotreitol. For FADU analyses, the cells were trypsinized at fixed time points after UV irradiation, counted, and washed twice with cold PBS. At each time point, the cells were suspended in solution B and divided (triplicate samples of 3.5×10^5 cells/0.1 mL of solution B) into each of three sets of tubes: *B*, blank samples, completely unwound DNA; *T*, total fluorescence of native DNA, and *P*, samples for determination of DNA unwinding rate. After addition of 0.1 mL of solution C, all samples were incubated on ice for 10 min. Samples *T* were neutralized with 0.2 mL of solution F with mixing. Then 0.05 mL of solution D and 0.05 mL of solution E were added very gently and without mixing to each triplicate sample *B*, *P*, and *T*. After a 30-min incubation on ice, samples *B* were sonicated for 15 min at 50 W in a Fisher 300 sonicator and then incubated for 1 h at room temperature. Samples *P* were incubated in parallel for 1 h at 16 °C, and samples *T* were kept on ice for 1 h. Denaturation was stopped by adding 0.2 mL of solution F and chilling on ice.

The percentage of dsDNA was estimated by staining with EtBr (0.5 μg/mL in 13.3 mM NaOH), which selectively binds to dsDNA. Fluorescence was read in a Jasko 821-FP spectrofluorimeter (excitation, 520 nm; analyzer, 590 nm). The % dsDNA values (*D*) were calculated from the fluorescence of *B*, *T*, and *P* samples using the equation $D = (P - B)/(T - B)$.

γH2AX Determination by Flow Cytometry. For flow cytometry analysis (8), cells that had been fixed in 70% ethanol and stored at 4 °C until analysis were washed in PBS, centrifuged, resuspended in 1 mL of cold PBS/4% FCS/0.1% Triton X-100 (T-PBS) and placed on ice for 10 min to rehydrate. They were then centrifuged, resuspended in 0.2 mL of mouse monoclonal anti-γH2AX antibody (1:500 in T-PBS), and incubated for 2 h at room temperature under shaking. After rinsing in T-PBS and centrifugation, cell pellets were resuspended in 0.2 mL of secondary antibody (donkey anti-mouse Alexa Fluor 488, 1:200 in T-PBS) and shaken for 1 h at room temperature. The cells were rinsed in T-PBS and counterstained with 50 μg/mL of propidium iodide in 1 mL of PBS containing RNase A (0.1 mg/mL) for 1 h at 37 °C. Samples of ∼25,000 cells were analyzed with a BD dual-laser FACSCantoII flow cytometer. Data were analyzed using BD FACSDiva software.

γH2AX Determination by Immunofluorescence Microscopy. Cells were grown in 35-mm thin-bottomed Petri dishes for high-end microscopy (Ibidi), fixed with 2% paraformaldehyde/0.3 M sucrose/0.5% Triton X for 20 min on ice, and blocked with MAXblock blocking medium (Active-Motif) for 1 h at 37 °C. The fixed cells were incubated with mouse monoclonal anti-γH2AX antibody (1:500) for 1 h at 37 °C. After three 10-min washes with PBS + 0.05% Tween 20, the cells were incubated with donkey anti-mouse Alexa Fluor 488 (1:500) for 1 h at 37 °C. The cells were counterstained with 20 ng/mL of DAPI for 20 min at room temperature, and after washing with PBS-Tween, the coverslips were mounted for fluorescence microscopy. The immunostained cells were visualized using a Leica TCS SP5 confocal microscope equipped with a 63× oil immersion objective.

1. Sherman PA, Fyfe JA (1989) Enzymatic assay for deoxyribonucleoside triphosphates using synthetic oligonucleotides as template primers. *Anal Biochem* 180: 222–226.
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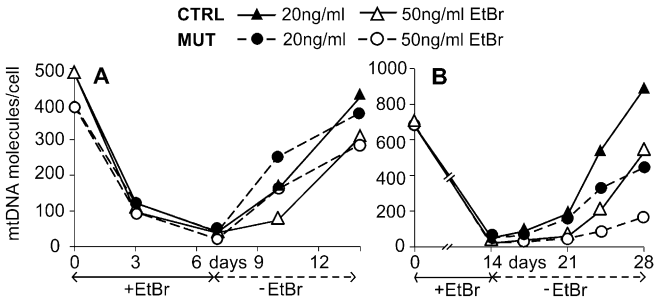
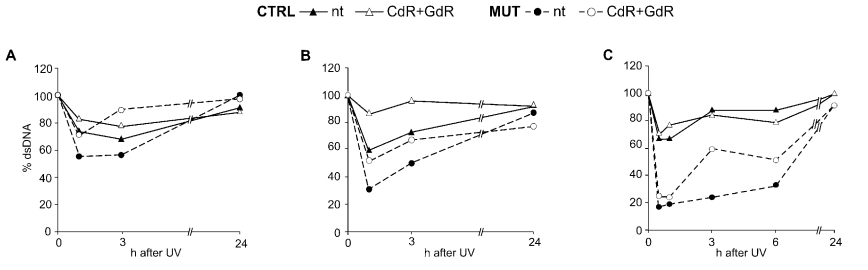


Fig. S1. Depletion and recovery of mtDNA in cycling and quiescent fibroblasts. Mutant and control fibroblasts were treated with EtBr 20 or 50 ng/mL for 7 d (cycling cells) or 14 d (quiescent cells), followed by a recovery period in the absence of the drug. The copy number of mtDNA (mtDNA molecules/cell) was determined at time intervals by real-time PCR. (*A*) Cycling cells, recovery period in 10% FCS. (*B*) Quiescent cells, recovery period in 0.1% FCS.



	dCTP		dGTP	
	nt	CdR+GdR	nt	CdR+GdR
CTRL	3.07	5.16	0.36	1.18
MUT	2.43	5.59	0.3	1.2

	dCTP		dGTP	
	nt	CdR+GdR	nt	CdR+GdR
CTRL	2.65	4.46	0.32	0.99
MUT	1.06	5.63	0.24	0.81

	dCTP		dGTP	
	nt	CdR+GdR	nt	CdR+GdR
CTRL	3.24	5.21	0.36	1.17
MUT	0.88	3.66	0.19	0.66

Fig. S2. FADU analysis of UV-induced DNA repair in quiescent mutant and control cells maintained for various periods in low serum; effects of deoxynucleosides. The cells were kept for 4 d (*A*), 7 d (*B*), or 11 d (*C*) in low serum before exposure to UV irradiation (12 J/m^2). The time course of DNA repair was analyzed by FADU as recovery of dsDNA. In each experiment, half of the cultures received 5 μM CdR + GdR 18 h before UV irradiation and over the next 24 h. The tables show dCTP and dGTP pool sizes (pmol/10^{6}cells) measured at the time of irradiation. nt, cells not treated with deoxynucleosides.

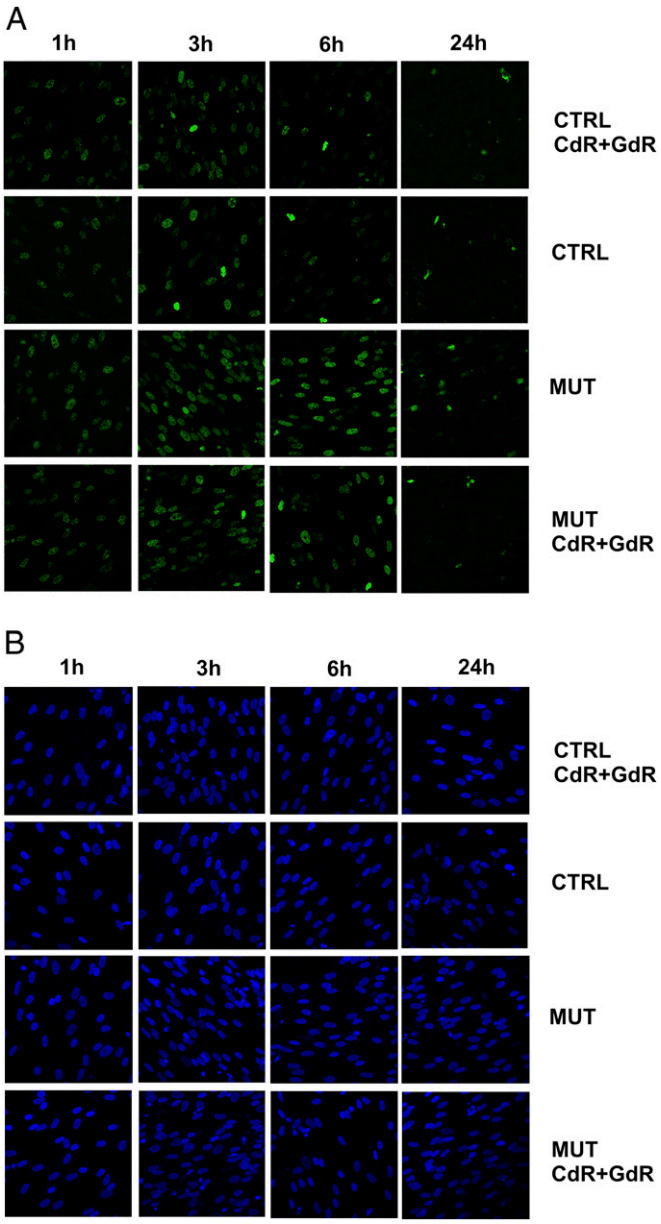


Fig. S3. Deoxynucleosides affect the heterogeneity of the UV-induced H2AX phosphorylation in quiescent mutant cells. We maintained control and mutant fibroblasts in the absence or presence of CdR + GdR for 7 d in low serum and for 24 h after exposure to UV irradiation (12 J/m^2). (*A*) γH2AX was detected by immunofluorescence analysis at the indicated times. (*B*) Nuclear DNA was counterstained with DAPI. In both cell lines, the γH2AX signal produced dim nuclear foci at 1 h after irradiation. After 3 h, most cells had brighter foci, and a few cells showed a far stronger pan-nuclear staining. After 6 h, the signal had declined in the control cells, whereas the mutant cells maintained the bright nuclear foci and the pan-nuclear staining. In both cell lines, the γH2AX signal virtually disappeared by 24 h. In the presence of deoxynucleosides, the γH2AX fluorescence pattern of mutant cells was closer to that of the control cells.

Table S1. Deoxynucleotide pools in quiescent mutant and control fibroblasts incubated with deoxynucleosides

	dNTP pmoles/10^6 cells							
	Control				Mutant			
Treatment	dCTP	dGTP	dTTP	dATP	dCTP	dGTP	dTTP	dATP
None	1.8	0.4	2.0	2.3	1.3	0.3	2.5	1.4
5μM CdR	2.6	0.5	3.3	1.7	3.8	0.4	3.7	1.4
1μM GdR	1.3	0.7	1.6	1.6	0.9	0.7	1.8	1.1
5μM GdR	1.3	1.0	1.8	1.8	0.7	1.0	1.7	1.3
5μM CdR + 1μM GdR	2.5	0.7	3.1	1.6	2.9	0.7	3.1	1.3
5μM CdR + 5μM GdR	2.4	0.8	2.9	1.6	3.1	0.9	2.9	1.7

Cells were maintained for 7 days in low serum and incubated for 18 h with deoxynucleosides before pool extraction.