Luijsterburg et al., http://www.jcb.org/cgi/content/full/jcb.200909175/DC1

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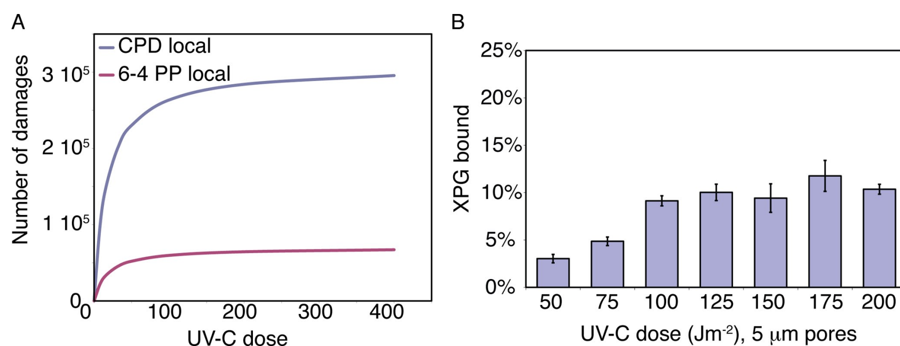

Figure S1. **Locally inflicted lesions.** (A) Estimate of the amount of 6-4 PPs produced at different UV fluencies (including experimental conditions used in this study) based on extrapolation of previous data by Perdiz et al. (2000). (B) UV-C dose ($J.m^{-2}$) response curve (measured in percentage of protein bound in the locally irradiated area) of UV135 cells expressing XPG-EGFP locally irradiated through 5 µm irradiated at UV-C fluencies ranging from 50 to 200 $J.m^{-2}$.

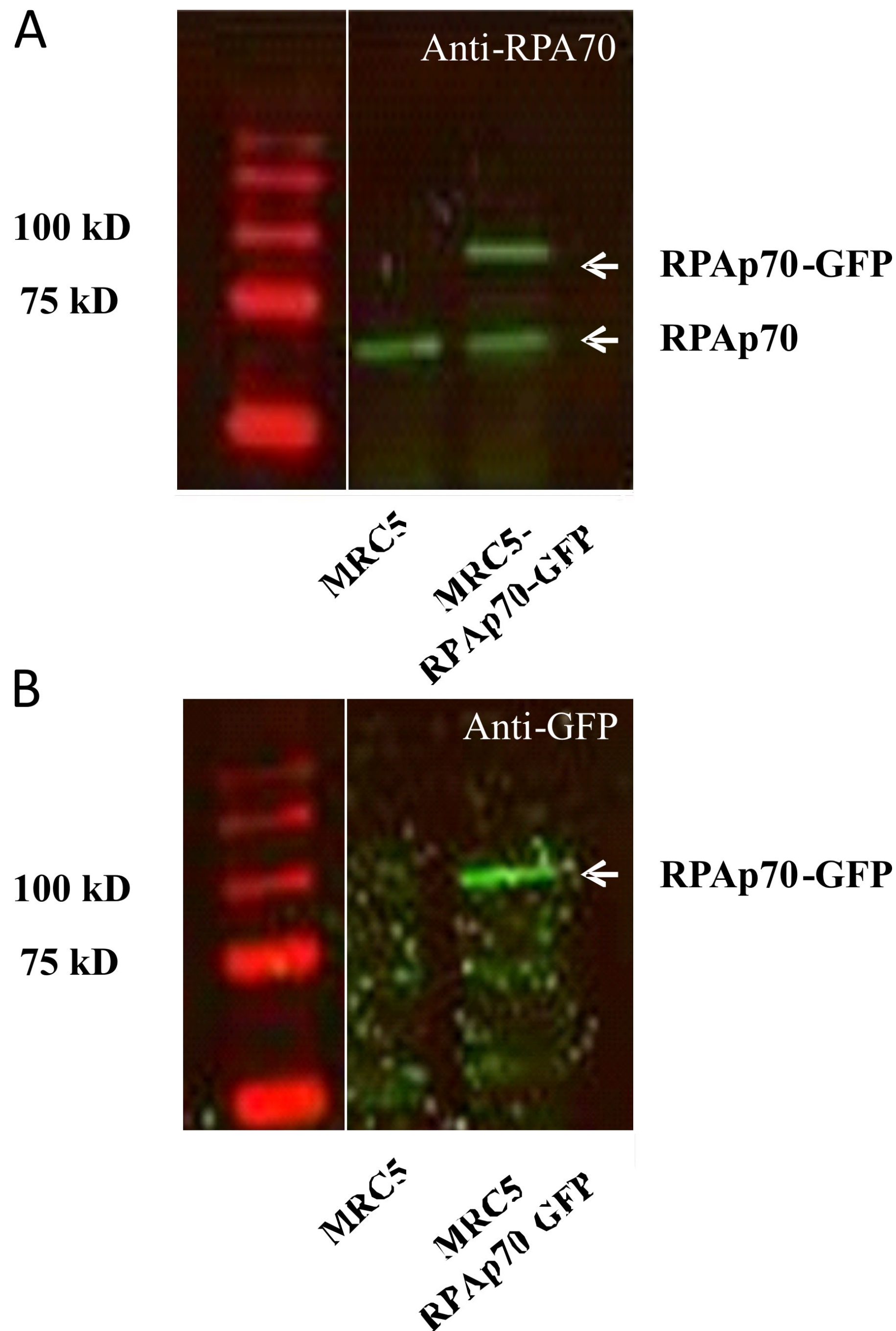

Figure S2. **Immunoblot analysis of RPA70-EGFP cells.** (A and B) Immunoblot probed with monoclonal anti-RPA antibodies (A) and monoclonal anti-GFP antibodies (B) of whole cell extracts from untransfected MRC5 cells and MRC5 cells stably expressing EGFP-tagged RPA70. The molecular mass of protein markers is indicated in kilodaltons. (A) EGFP-tagged RPA70 migrates slower than endogenous RPA (arrows), as detected by anti-RPA antibodies. This allows for a direct comparison between the expression level of RPA70-GFP relative to the levels of endogenous RPA, which shows that endogenous RPA70 and RPA70-EGFP are expressed at a 1:1 ratio. (B) Anti-EGFP antibodies specifically detect RPA70-EGFP at the size expected for the full-length fusion protein. Detection was performed using an infrared imaging system.

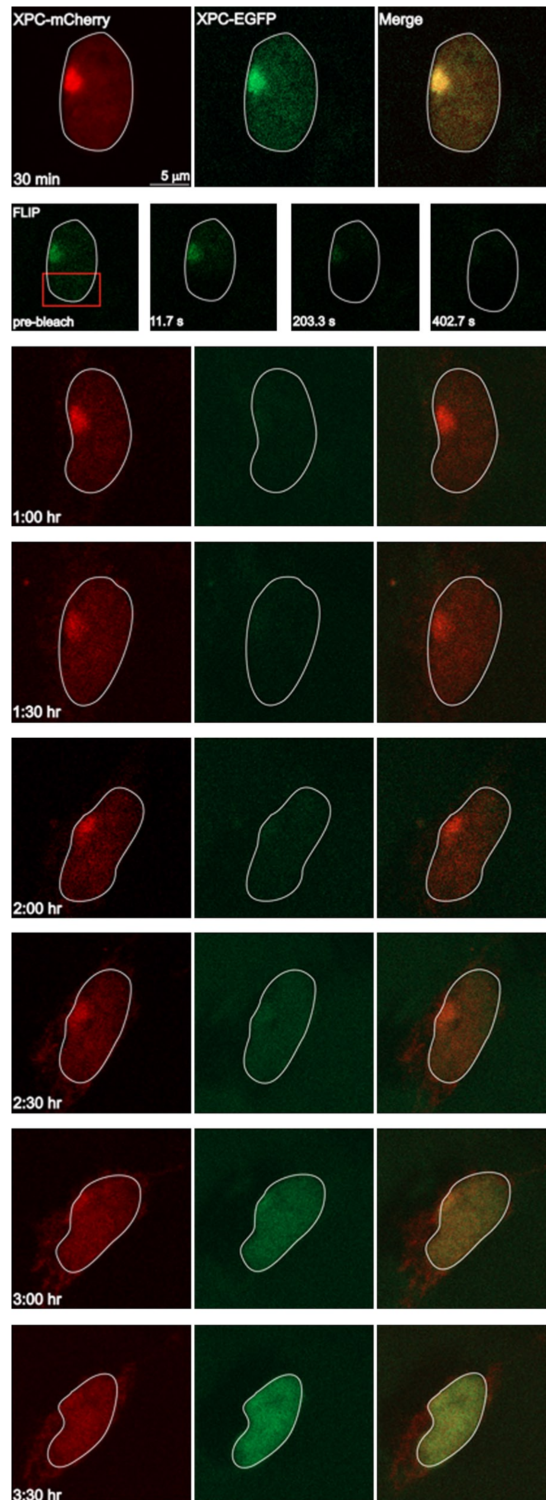

Figure S3. **Cells analyzed by FLIP remain repair competent.** XP4PA cells stably expressing XPC-GFP and transiently expressing XPC-mCherry were locally irradiated (5 µm; 100 J.m^{-2}). 30 min after local UV irradiation, cells were analyzed by FLIP as described in Fig. 3 and Material and methods. The FLIP experiment resulted in the loss of the XPC-GFP signal (as a result of bleaching) but not of the XPC-mCherry signal. The same cells that were analyzed by FLIP were subsequently monitored for several hours after UV irradiation until binding of XPC mCherry could no longer be detected, indicating successful repair of 6-4 PPs. Note that the signal in the green channel gradually increases several hours after FLIP as a result of synthesis of new XPC-GFP. This experiment was performed three times with similar results. Red rectangle indicates the area of photobleaching.

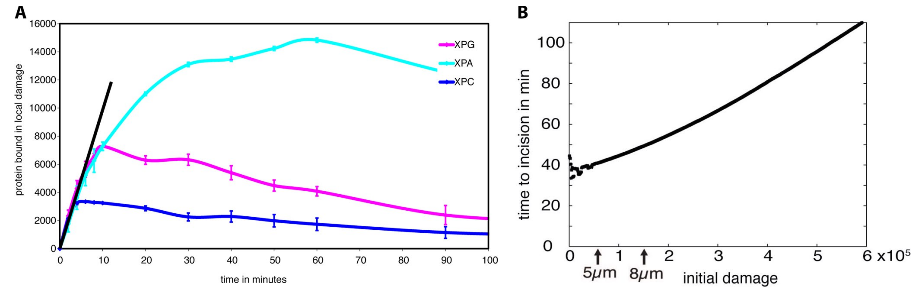

Figure S4. **NER rate.** (A) Cells expressing XPC-EGFP, XPG-EGFP, and EGFP-XPA were locally irradiated (5-μm pores; 100 $J.m^{-2}$) as described in Materials and methods. The binding curve shows the first 100 min of the binding curve shown in Fig. 2 B. The assembly rates of these NER proteins can be estimated from the initial slope of the binding curves. The tangent shows an immobilization of ∼3.6%, ∼1.1%, and ∼0.5% of the total protein pool per minute for XPC, XPG, and XPA, respectively. Given that the concentrations of XPC (about three 10^4 molecules/cell), XPG (about eight 10^4 molecules/cell), and XPA (about two 10^5 molecules/cell) are different (Araújo et al., 2001), this tangent translates to assembly rates of ∼900 molecules/min or ∼15 molecules/s for XPC, XPG, and XPA. (B) Computation of the mean time to incision in the model versus the initial amount of DNA damage (6-4 PPs). The arrows indicate the amounts of damage estimated for the experimental setup with UV-permeable pores of 5- and 8-μm diameter.

Table S1. **Model assumptions**

Repair intermediate	Binding proteins	Catalyzed process required proteins	Remarks	References
Damaged DNA with helical distortion	XPC, TFIIH (3 states)	Partial unwinding (reaction α) XPC and TFIIH	Initiation by binding of XPC and subsequent recruitment of TFIIH	Evans et al., 1997; Rademakers et al., 2003; Riedl et al., 2003; Volker et al., 2001; Yokoi et al., 2000
Partially unwound DNA	XPC, TFIIH, XPG, XPA, ERCC1/XPF, RPA (48 states)	Full unwinding (reaction α') XPC, TFIIH, XPG, XPA, RPA	ERCC1/XPF only binds to repair complexes that contain XPA; if the DNA becomes devoid of any protein, it will reanneal to repair intermediate I (reaction ε_1)	Evans et al., 1997; Mu et al., 1997; Volker et al., 2001
Fully unwound DNA	XPC, TFIIH, XPG, XPA, XPF-ERCC1, RPA (48 states)	Dual incision (reaction β) TFIIH, XPG, XPA, ERCC1-XPF, RPA	If the DNA becomes devoid of any protein, it will reanneal (reaction ε_2); dual incision requires the endonucleases XPG and ERCC1-XPF and is stimulated by TFIIH, XPA, RPA, and possibly XPC	Evans et al., 1997; Winkler et al., 2001; O'Donovan et al., 1994; de Laat et al., 1998; Sijbers et al., 1996
Incised DNA	XPC, TFIIH, XPG, XPA, ERCC1/XPF, RPA, PCNA (96 states)	Repair synthesis (reaction γ) XPA, RPA, PCNA	PCNA binds to the free 3′-OH group generated by the ERCC1-XPF incision. DNA polymerase is also required (not measured)	Evans et al., 1997; Winkler et al., 2001
Resynthesized DNA	XPA, RPA, PCNA (9 states)	Rechromatinization (reaction δ) RPA, PCNA	Accumulation and FLIP data imply that XPA binds to repaired DNA, whereas the preincision proteins do not (Figs. 2 and 3)	Moser et al., 2007; Shivji et al., 1995; This study
Rechromatinized DNA	RPA, PCNA (4 states)	ND	RPA and PCNA associate with repair intermediate VI, as levels of bound EGFP-PCNA and EGFP-RPA are high up to at least 4 h after UV irradiation, whereas other repair proteins are no longer bound	Riedl et al., 2003; This study

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