

Human DNA polymerase iota protects cells against oxidative stress

Tirzah Braz Petta¹, Satoshi Nakajima²,
Anastasia Zlatanou¹, Emmanuelle
Despras¹, Sophie Couve-Privat³,
Alexander Ishchenko³, Alain Sarasin¹,
Akira Yasui² and Patricia Kannouche^{1,*}

¹Centre National de la Recherche Scientifique (CNRS) FRE2939, Unit of genetic stability and oncogenesis, Institut Gustave Roussy, Université Paris-Sud, Villejuif, France, ²Department of Molecular Genetics, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan and ³Centre National de la Recherche Scientifique (CNRS) UMR8126, Unit of molecular interactions and cancer, Institut Gustave Roussy, Université Paris-Sud, Villejuif, France

Human DNA polymerase iota (*poli*) is a unique member of the Y-family of specialised polymerases that displays a 5′deoxyribose phosphate (dRP) lyase activity. Although *poli* is well conserved in higher eukaryotes, its role in mammalian cells remains unclear. To investigate the biological importance of *poli* in human cells, we generated fibroblasts stably downregulating *poli* (MRC5-*poli*^{KD}) and examined their response to several types of DNA-damaging agents. We show that cell lines downregulating *poli* exhibit hypersensitivity to DNA damage induced by hydrogen peroxide (H₂O₂) or menadione but not to ethyl-methane sulphonate (EMS), UVC or UVA. Interestingly, extracts from cells downregulating *poli* show reduced base excision repair (BER) activity. In addition, *poli* binds to chromatin after treatment of cells with H₂O₂ and interacts with the BER factor XRCC1. Finally, green fluorescent protein-tagged *poli* accumulates at the sites of oxidative DNA damage in living cells. This recruitment is partially mediated by its dRP lyase domain and ubiquitin-binding domains. These data reveal a novel role of human *poli* in protecting cells from oxidative damage.

The EMBO Journal (2008) 27, 2883–2895. doi:10.1038/emboj.2008.210; Published online 16 October 2008

Subject Categories: genome stability & dynamics

Keywords: BER; DNA polymerase; oxidative DNA damage; Y-family polymerase

Introduction

The genome is continuously exposed to damaging agents, both endogenous, resulting from hydrolysis and oxidation during normal cell metabolism, and exogenous such as UV light, ionising radiation and a wide variety of chemical

carcinogens. Among several mechanisms employed to protect genetic integrity from the detrimental effects of genotoxic compounds, base excision repair (BER) is a crucial process that eliminates many types of base damage (Lindahl, 1982). On the basis of current models, mammalian BER is divided into at least two subpathways that are designated ‘single nucleotide BER’ (SN BER) and ‘long-patch BER’ (LP BER). In both subpathways, repair is initiated by damage-specific DNA glycosylases that specifically recognise and remove the damaged base by cleavage of the bond between the base and the deoxyribose sugar of the DNA strands. Strand incision can be carried out by the same glycosylases (bifunctional glycosylases) or by an AP endonuclease. The resulting gaps are filled either with one nucleotide (SN BER) or with 2–10 nucleotides (LP BER) followed by a ligation step. For the SN BER, the DNA polymerase β (*polβ*) contributes to both gap-filling and removal of the 5′deoxyribose phosphate (dRP) (Srivastava *et al*, 1998; Almeida and Sobol, 2007). Mouse embryonic fibroblasts (MEFs) deficient in *polβ* have demonstrated the requirement of *polβ* mainly in the repair of alkylated lesions. In contrast, a defect in *polβ* has very little impact on cell proliferation after exposure of cells to oxidative DNA damage suggesting that other polymerases might have an important function in this BER pathway (Sobol *et al*, 1996; Fortini *et al*, 2000; Horton *et al*, 2002, 2005; Polosina *et al*, 2004; Yoshimura *et al*, 2006).

poli is a member of the recently discovered Y-family of DNA polymerases (Ohmori *et al*, 2001), which have been best characterised *in vitro* for their lesion-bypassing properties (reviewed in Goodman, 2002). Human *poli* is a highly distributive low-fidelity enzyme lacking an intrinsic exonuclease proofreading activity. Strikingly, *poli* incorporates dGMP opposite T 3–10 times more frequently than the correct nucleotide dAMP (Johnson *et al*, 2000; Tissier *et al*, 2000; Zhang *et al*, 2000). In addition to its polymerisation activity, *poli* also displays a dRP lyase activity (Bebenek *et al*, 2001). In reactions reconstituted with uracil-DNA glycosylase, AP endonuclease and a DNA ligase, *poli* can use both its dRP lyase and polymerase activities to repair G·U and A·U pairs on the DNA. Moreover, *poli* is able to complement *in vitro* the single-nucleotide BER deficiency of a DNA *polβ*-null cell extract (Bebenek *et al*, 2001; Prasad *et al*, 2003). Taken together, these features suggested a putative role for *poli* in BER in human cells.

In an effort to further clarify the role of *poli* in BER *in vivo*, we generated human fibroblasts stably downregulating *poli* (hereafter abbreviated as MRC5-*poli*^{KD}) and found that these cells exhibit hypersensitivity to oxidative stress. This sensitivity is correlated with an accumulation of MRC5-*poli*^{KD} cells in G1 phase. Interestingly, extracts from cells downregulating *poli* show reduced BER activity. Furthermore, we observed that *poli* is recruited to the chromatin after H₂O₂ treatment and is associated with XRCC1, a scaffold protein involved in single-strand DNA break (SSB) repair. In addition, *poli* is recruited to sites of oxidised chromatin in living cells.

*Corresponding author. Centre National de la Recherche Scientifique (CNRS) FRE2939, Institut Gustave Roussy, Université Paris-Sud, PR2, Villejuif 94805 Cedex, France. Tel.: +33 1 4211 4030; Fax: +33 1 4211 5008; E-mail: kannouche@igr.fr

Received: 28 April 2008; accepted: 15 September 2008; published online: 16 October 2008

Altogether, these data suggest that polt is implicated in particular forms of BER in human cells.

Results

Generation of human fibroblasts downregulating polt protein

As no human cell line lacking polt has been identified, we generated human fibroblasts stably downregulating polt protein. The MRC5-SV cell line was transfected with the vector pSUPER.puro harbouring an shRNA directed against *POLI* mRNA. In parallel, a scrambled sequence was also employed as a control. Isolation of stable clones after puromycin selection and protein quantification by western blotting revealed that the level of polt was greatly decreased (up to 90%) in different MRC5-polt^{KD} clones when compared with clones harbouring a nonspecific sequence (MRC5-NT) (Figure 1A). In this study, we used two clones displaying a drastic reduction of polt level (clone nos. 1 and 5) and one clone with an intermediate level of polt protein (clone no. 4).

The growth properties of MRC5-polt^{KD} cells were monitored by measuring growth curves. We observed a small delay in the growth of cells downregulating polt when compared with MRC5-NT cells (Figure 1B). Likewise, cell cycle analysis showed that MRC5-polt^{KD} remained longer in G1 phase than did the control cells (Figure 1C, top panel). This accumulation in G1 was correlated with the extent of downregulation of polt as clone nos. 1 and 5 displayed a greater percentage of cells in G1 than clone no. 4 (Figure 1C, bottom panel). Hence, these results indicated that human cells expressing only 5–10% of polt protein are able to grow almost normally, except a slight accumulation of cells in G1 phase.

Downregulation of polt provokes an increase in sensitivity to killing by H₂O₂, but not to an alkylating agent or UVC

To study the biological function of human polt, we exposed MRC5-polt^{KD} cells, to different DNA-damaging agents. MRC5-polt^{KD} cells showed no significant increase in sensitivity

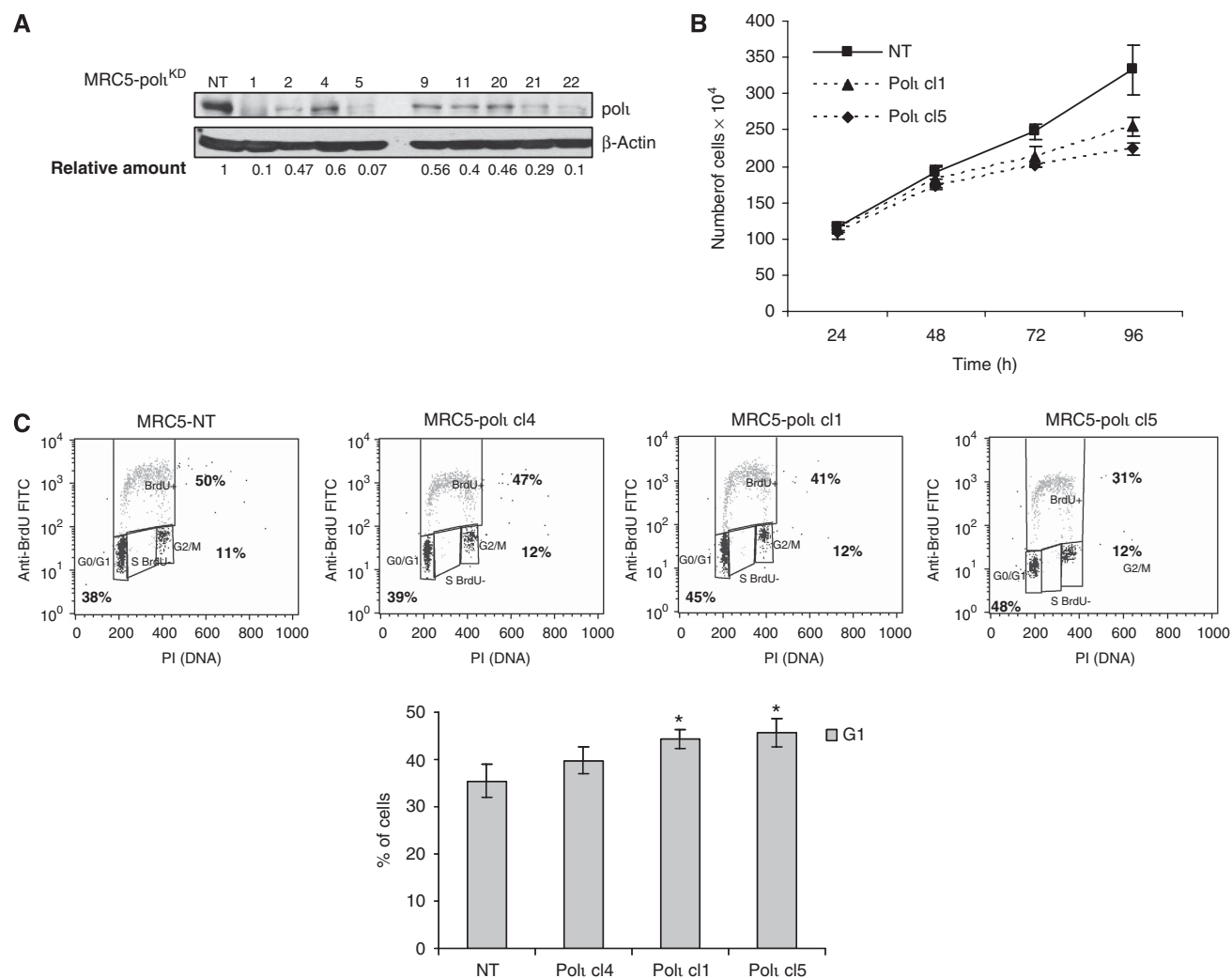


Figure 1 Characterisation of cell lines stably downregulating polt (MRC5-polt^{KD}). (A) Western blotting analysis of MRC5-polt^{KD} clones (numbered above). Relative amount was calculated as a ratio of polt/NT after normalisation with β -actin amount. (B) Growth kinetics over 4 days of two MRC5-polt^{KD} clones (nos. 1 and 5) and control cells (MRC5-NT). (C) Cell cycle analysis of MRC5-polt^{KD} and MRC5-NT cell lines. The top panel shows a representative cell cycle distribution of the indicated cell line as measured by BrdU incorporation in flow cytometric analysis. The percentage of cells in all phases (G1/S/G2–M) is indicated. In the bottom panel, the histograms report the percentages of cells in G1 phase. The reported values are the means \pm s.d. of nine independent experiments (* $P < 0.05$).

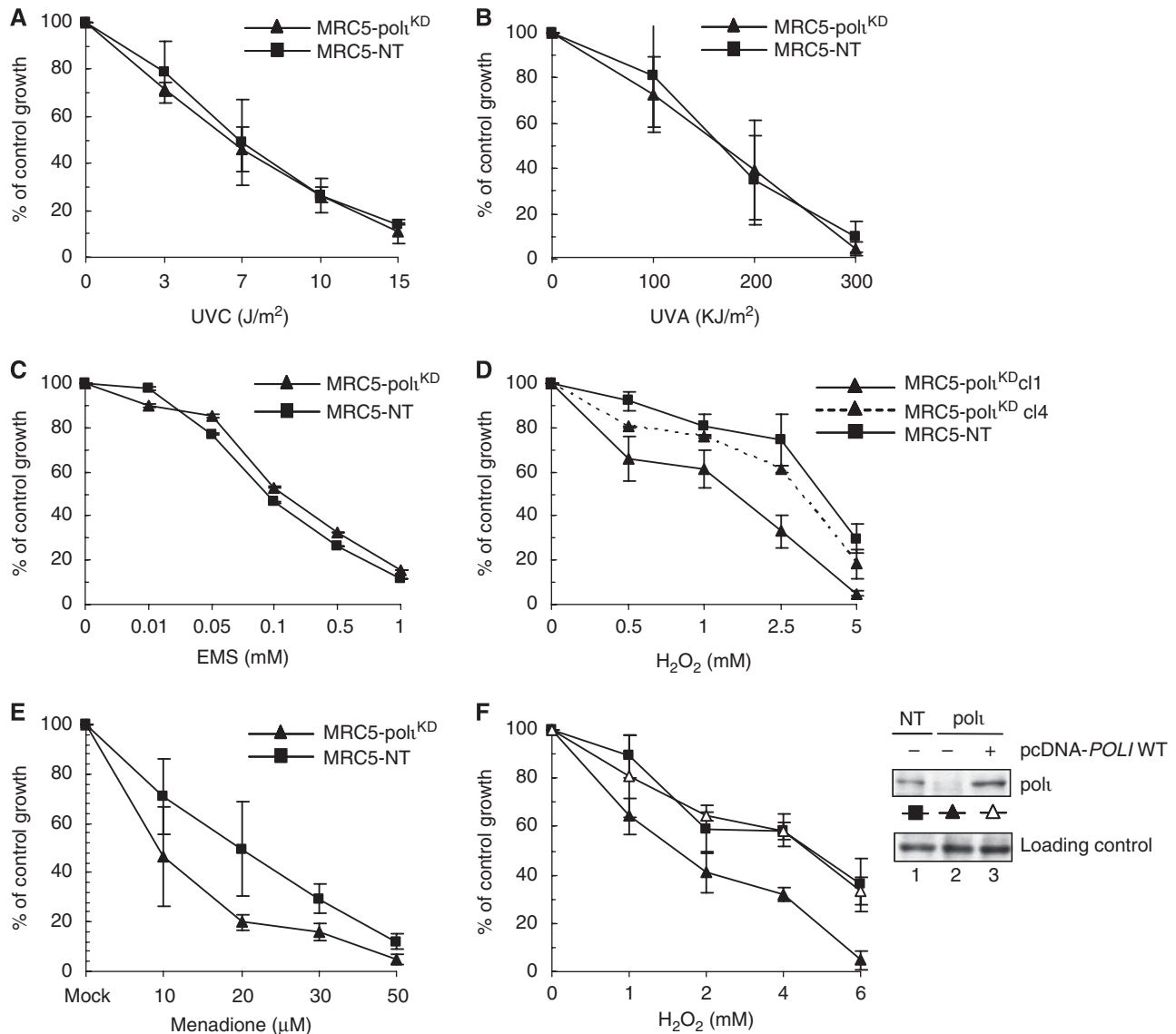


Figure 2 Sensitivities of MRC5-polt^{KD} cells to killing by various DNA-damaging agents. For each survival experiment, cells were exposed to increasing doses of the indicated genotoxic agent and counted 72 h later. Percentage of control growth was plotted for each data point, representing the mean of three independent experiments. (A) Cells were exposed to UVC, (B) UVA and (C) EMS for 1 h at 37°C in serum-free medium. (D) Cells were exposed to H₂O₂ for 10 min at 4°C. (E) Cells were exposed to menadione for 1 h at 37°C. (F) On the left panel, MRC5-polt^{KD} cells were transfected with either an empty vector (closed triangle) or a vector expressing polt (open triangle). After 24 h, cells were exposed to H₂O₂ as described above. On the right panel, western blots of extracts of MRC5-polt^{KD} and MRC5-NT cells containing an empty vector or polt expression vector, which were separated by SDS-PAGE and blotted using anti-polt antibody, are shown.

either to UVC and UVA irradiations or to ethylmethane sulphate (EMS) treatment (Figure 2A–C). In great contrast, MRC5-polt^{KD} cells were found to be hypersensitive to hydrogen peroxide (H₂O₂), which causes mainly oxidation of DNA bases and SSBs (Figure 2D), and to menadione (Figure 2E), a redox-cycling quinone that generates intracellular ROS (Watanabe *et al*, 2004). Moreover, these cells were moderately sensitive to low doses of potassium bromate (data not shown), an agent that allows a more specific introduction of 8-oxo-G in the genome (Ballmaier and Epe, 1995). To rule out the possibility that this sensitivity could be a side effect of the stable shRNA procedure, we transiently complemented the MRC5-polt^{KD} cells with *POL1* cDNA bearing silent mutations to avoid the recognition of the resulting mRNA by shRNA polt (Figure 2F). We observed that the sensitivity of complemen-

ted MRC5-polt^{KD} cells to H₂O₂ was rescued to the level of the control cell line. This indicates that the sensitivity of the knockdown cell line to H₂O₂ treatment is due to the low level of polt protein.

Because polβ^{-/-} MEFs are only slightly sensitive to H₂O₂ exposure (Sobol *et al*, 1996; Fortini *et al*, 2000; Horton *et al*, 2002, 2005; Polosina *et al*, 2004; Yoshimura *et al*, 2006), we investigated whether human cells expressing low levels of both polt and polβ show a stronger sensitivity to oxidative DNA damage. We therefore reduced the endogenous levels of polβ up to 80% using siRNA duplexes in MRC5-polt^{KD} and MRC5-NT cells (Figure 3A) and we then monitored cell survival after H₂O₂ treatment. We did not observe any difference in the sensitivity of MRC5-polt^{KD}/siRNA polβ when compared with MRC5-polt^{KD}/siRNA NT (Figure 3B).

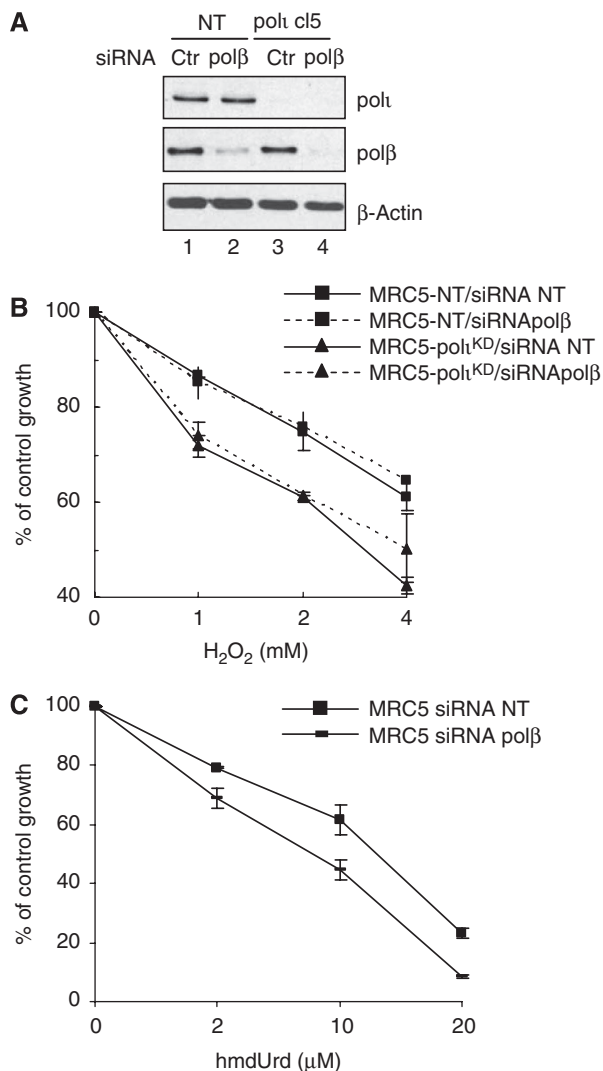


Figure 3 Human cells downregulating polβ are not sensitive to oxidative damaging agents. (A) MRC5-pol^{tKD} and MRC5-NT cells were transfected with polβ-specific siRNAs (lanes 2 and 4) or scrambled siRNAs (lanes 1 and 3). After 72 h, cells were harvested and extracts were separated by SDS-PAGE, and incubated with anti-polt and anti-polβ antibodies. β-Actin detection was used as a loading control. (B) MRC5-pol^{tKD} and MRC5-NT cells were transfected with a pool of polβ-specific siRNAs 24 h before treatment with increasing doses of H₂O₂ for 10 min at 4°C. Cells were counted 72 h later. Percentage of control growth was plotted for each data point, representing the mean of three independent experiments. (C) MRC5 cells were transfected with polβ-specific siRNA. After 48 h, they were exposed to hmdUrd for 24 h and surviving cells were counted 72 h later and plotted as described above.

Furthermore, the MRC5-NT cells transiently downregulating polβ were not sensitive to H₂O₂. However, we could observe a moderate sensitivity of MRC5-NT/polβ^{KD} cells to the thymidine analogue 5-hydroxymethyl-2'-deoxyuridine (hmdUrd), demonstrating that the reduction in the levels of polβ we obtained by siRNA had a biological effect (Figure 3C). Our latest observation is in agreement with the reported study of Horton *et al* (2005) who used polβ^{-/-} MEFs. Altogether, these data suggest that some types of oxidation-induced DNA damages are repaired in a polβ-independent and polt-dependent manner in human cells.

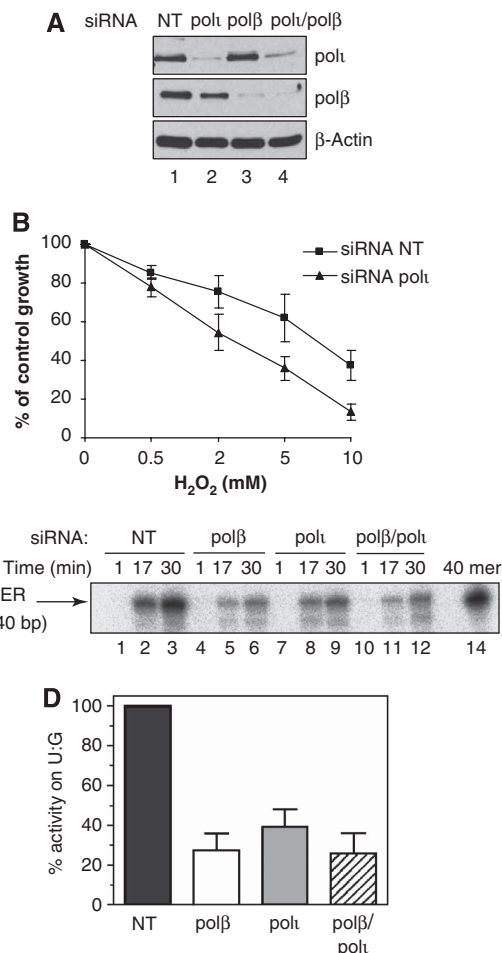


Figure 4 Cells downregulating polt show an impaired uracil-initiated base excision DNA repair synthesis in human cell-free extracts. (A) MRC5 cells were transfected with scrambled siRNAs (lane 1) or polt-specific siRNAs (lanes 2 and 4) or with polβ-specific siRNAs (lanes 3 and 4). After 72 h, cells were harvested and extracts were separated by SDS-PAGE. Immunoblots were incubated with anti-polt or anti-polβ antibodies. β-Actin detection was used as a loading control. (B) MRC5 cells were transfected with a pool of polt-specific siRNAs 24 h before treatment with increasing doses of H₂O₂ for 10 min at 4°C. Cells were counted 72 h later. Percentage of control growth was plotted for each data point, representing the mean of three independent experiments. (C) Denaturing gel of G·U repair products after incubation with siRNA-transfected MRC5 whole-cell extracts: 50 nM of G·U was incubated with 10 μg extract at 37°C, and aliquots were withdrawn at different time intervals and analysed on a 20% denaturing PAGE. Formation of 40 mer repair product was quantified by phosphorimaging. (D) The average initial rate of BER DNA repair synthesis in whole-cell extracts. For comparison, the DNA repair synthesis in extract from cells transfected with scrambled siRNA (NT) was assumed as 100%. Error bars show the s.d. of the mean of three independent experiments.

BER activity is reduced in extracts from cells downregulating polt

To examine whether polt can participate in SN-patch BER pathway, we reconstituted the repair of G·U oligonucleotide duplex in the presence of radioactively labelled dCTP and whole-cell extracts of MRC5 depleted for specialised DNA polymerases (Biade *et al*, 1998). To compare the BER activity in cell extracts having the same genetic background, we prepared extracts from MRC5 fibroblasts transiently transfected with siRNA specific to polβ, polt or both polβ/polt and not from the stable clone MRC5-pol^{tKD}. We used non-targeted

siRNA (NT) as a negative control. Downregulation of the DNA polymerases was verified by western blot (Figure 4A). Likewise, the H₂O₂ sensitivity of MRC5 transiently transfected with siRNA against pol η was confirmed (Figure 4B). As shown in Figure 4C, after 30 min incubation of G·U oligonucleotide duplex, a full-length 40 mer product was generated with high efficiency in control NT-MRC5 cell extract (lane 3). This indicates that uracil-DNA glycosylase excises U base, AP endonuclease cleaves resulting abasic site and generates single-strand nick with 5' deoxyribose phosphate residue. A DNA repair polymerase then removes dRP residue and incorporates ³²P-labelled dCMP nucleotide. Finally DNA ligase completes the restoration of the full-length 40 mer G·C duplex. In agreement with previous observations (Yoshimura *et al*, 2006), the pol β -depleted cell extract showed dramatic (up to 70%) decrease in the formation of fully repaired 40 mer duplex (Figure 4C, lanes 5 and 6, and Figure 4D) as compared with that of wild-type cell extract (Figure 4C, lanes 2 and 3, and Figure 4D). Interestingly, the pol η -deficient cell extract also showed a strong reduction in the generation of a 40-mer product (Figure 4C, lanes 8 and 9, and Figure 4D) similar to that of the pol β -deficient extract (Figure 4C, lanes 5 and 6), suggesting that the DNA polymerase pol η is involved in the BER pathway. However, depletion of both pol β and pol η in MRC5 cell extracts did not decrease further formation of a 40-mer product (Figure 4D), suggesting (i) that the residual amount of pol η and/or pol β is responsible for the remaining BER activity or (ii) the presence of third BER-specific DNA repair polymerase.

pol η -deficient cells display a delay in G1 phase following exposure to H₂O₂

To better characterise the impact of pol η downregulation on the response to oxidative DNA damage, we analysed the cell cycle profile of MRC5-pol η ^{KD} and MRC5-NT cells following H₂O₂ treatment. We, therefore, employed two different approaches to measure the progression of cells. In the first set of experiments, MRC5-pol η ^{KD} and MRC5-NT cells were exposed to H₂O₂, and were then pulse-labelled with BrdU just before harvesting at the indicated time points. In MRC5-NT cells, 36 h after treatment the number of cells in G1 phase decreased from 35 to 15%, whereas S-phase content remained unchanged (Figure 5A and B). In pol η ^{KD} cells, the number of cells in G1 was moderately affected post-treatment. However, 36 h later it re-increased, whereas S-phase cell number decreased (Figure 5A). At this time point, both cell lines displayed a slight accumulation in G2/M phase. This shows that pol η ^{KD} cells accumulate in G1 phase at later times after H₂O₂ and have difficulties in entering into a new S phase.

We asked whether this delay was a consequence of the fraction of cells exposed to H₂O₂ during DNA replication. To specifically monitor the progression of cells treated during the S phase, we pulse-labelled asynchronous cells with BrdU just before exposure or not to H₂O₂ (Figure 5C, left panel, the big box corresponds to cells treated in S phase). The cells were then grown in fresh medium for 8–48 h before analysis by flow cytometry. As they progressed through the cell cycle, BrdU-positive cells were gated in G1/early S-, S-, late S/G2-phase compartments according to their total DNA content (Figure 5C, right panel). We observed that both cell lines needed 24 h to progress through S phase after treatment (Figure 5D, left panel), whereas S phase lasted approximately

10–12 h in mock-treated cells (data not shown). This shows that H₂O₂ causes a S-phase delay in both cell lines. Interestingly, when we examined the progression of BrdU-positive cells through the G1 phase, we found that the percentage of MRC5-pol η ^{KD} cells was higher than control cells, especially for the late time points (Figure 5D, right panel and Figure 5E). Altogether, these results suggested that the absence of pol η affects the progression of cells through G1/S phase, following exposure to an oxidising agent. It raises the possibility that pol η might operate in a 'backup' mechanism to repair oxidising DNA lesions that were either bypassed or not repaired at all during the S and G2/M phases, and were, as such, found present in the G1 phase of the next cell cycle.

pol η is recruited to sites of DNA damage in human cells

So far, our results suggested a role of pol η in the repair of oxidative DNA damage. We next investigated the localisation pattern of green fluorescent protein (GFP)-tagged pol η in living cells after inducing DNA damage in a restricted area of the nucleus by laser (Lan *et al*, 2005). We therefore overexpressed a full-length GFP-pol η in HeLa cells and irradiated the cell nucleus with a 405 nm laser light. As shown in Figure 6A, left panel, pol η accumulation was observed immediately after irradiation with 500 scans, which produces SSBs, base damage and double-strand breaks (Lan *et al*, 2005). As GFP-pol η does not accumulate at the sites irradiated with lower laser dose, which mainly produces SSBs, possible substrates for GFP-pol η may be either base damage or/and DNA double-strand breaks. However, the accumulation of pol η was further enhanced by the presence of a photosensitiser (8-methoxypsoralen (8-MOP)) (Figure 6A, right panel) that increases accumulation of DNA glycosylases after irradiation (Orimo *et al*, 2006; Prasad *et al*, 2007), suggesting that pol η is recruited to sites of base damage in living cells.

After UVC irradiation, the accumulation of pol η into replication foci is largely dependent on pol η (Kannouche *et al*, 2003). We aimed to ask whether pol η could relocalise at the sites of laser-induced DNA damage in the absence of pol η . XP-variant cells (XP2SASV) were transfected with pGFP-pol η and were then irradiated. We observed an accumulation of GFP-pol η at the irradiated sites (Figure 6A, right panel), suggesting that the recruitment of pol η at the sites of oxidative DNA damage does not require the presence of pol η .

The recruitment of pol η to sites of DNA damage in human cells requires the NLD and UBM domains

To identify the domains of pol η required for this relocalisation, we examined the localisation pattern of different GFP-pol η mutants containing gross deletions and/or point mutation after irradiation. The primary sequence of pol η (715 amino acids) contains no clear NLS. However, it has been reported that the sequences necessary for nuclear localisation are in the region of amino acids 219–451 (named NLD for nuclear localisation domain) (Kannouche *et al*, 2003). In this region (aa 228–252), Bebenek *et al* (2001) identified a helix-hairpin-helix (HhH) motif similar to that found in the NH2-terminal domain of pol β , which catalyses excision of a 5'dRP group from DNA. Recently, two ubiquitin-binding motifs (UBM1 and UBM2) were identified in the C terminus of pol η at positions 498–526 and 679–707 in the human

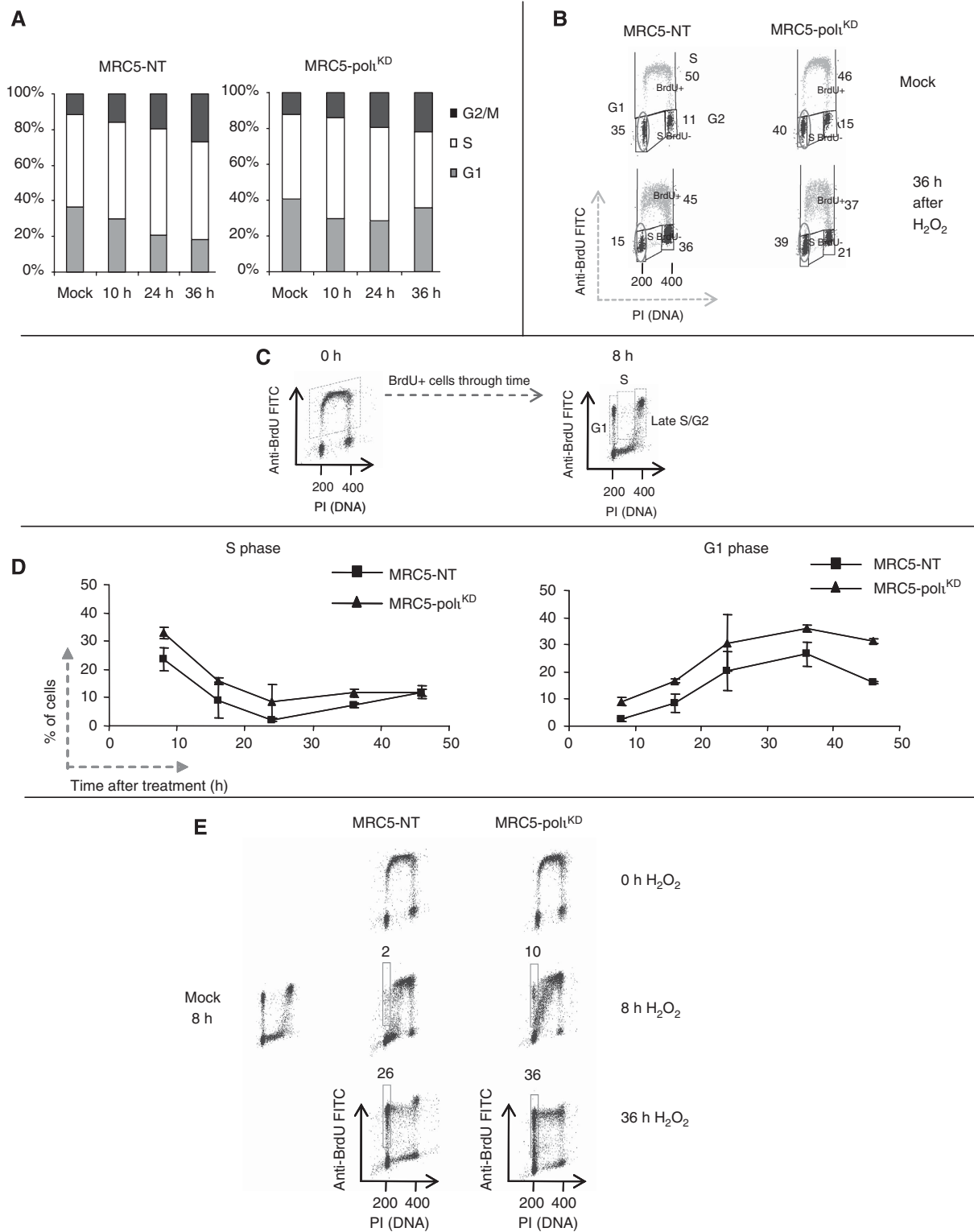


Figure 5 MRC5-pol^{KD} cells are delayed in G1 phase after H₂O₂ treatment. (A) MRC5-pol^{KD} and MRC5-NT cells were exposed to 2.5 mM H₂O₂ for 10 min at 4°C and pulse-labelled with BrdU for 20 min before harvesting at indicated time points. The percentage of cells in all phases (G1/S/G2–M) is indicated in the histogram (mean of three independent experiments). (B) Cells were treated as described above and representative cell cycle distribution of the indicated cells as measured by BrdU incorporation (vertical axis, log scale) and DNA content (horizontal axis, linear scale) in flow cytometry analysis is shown. The top panels show the cell cycle distributions in mock-treated cells and bottom panels show cell cycle distribution 36 h after H₂O₂ treatment. The percentage of cells in all phases (G1/S/G2–M) is indicated. (C, D) Cells were pulse-labelled with 10 μM BrdU for 10 min prior to H₂O₂ treatment (10 min at 4°C). (C) Progression of BrdU-positive cells through time in the cell cycle was assessed by flow cytometry analysis. Representative distribution of mock-treated MRC5-NT is shown at T = 0 and 8 h. BrdU-positive cells were gated as G1/early S, S and lateS/G2 cells. (D) MRC5-pol^{KD} and MRC5-NT cells were treated as described above then the curves report the percentages of BrdU + cells in S phase (left) or in G1 phase (right) at the indicated time points. The reported values are the mean of at least two independent experiments. (E) Representative cell cycle distribution of the indicated cells as measured by BrdU incorporation in flow cytometry analysis is shown. In red squares, the amount of BrdU + cells in G1 phase is highlighted. A full-colour version of this figure is available at *The EMBO Journal* Online.

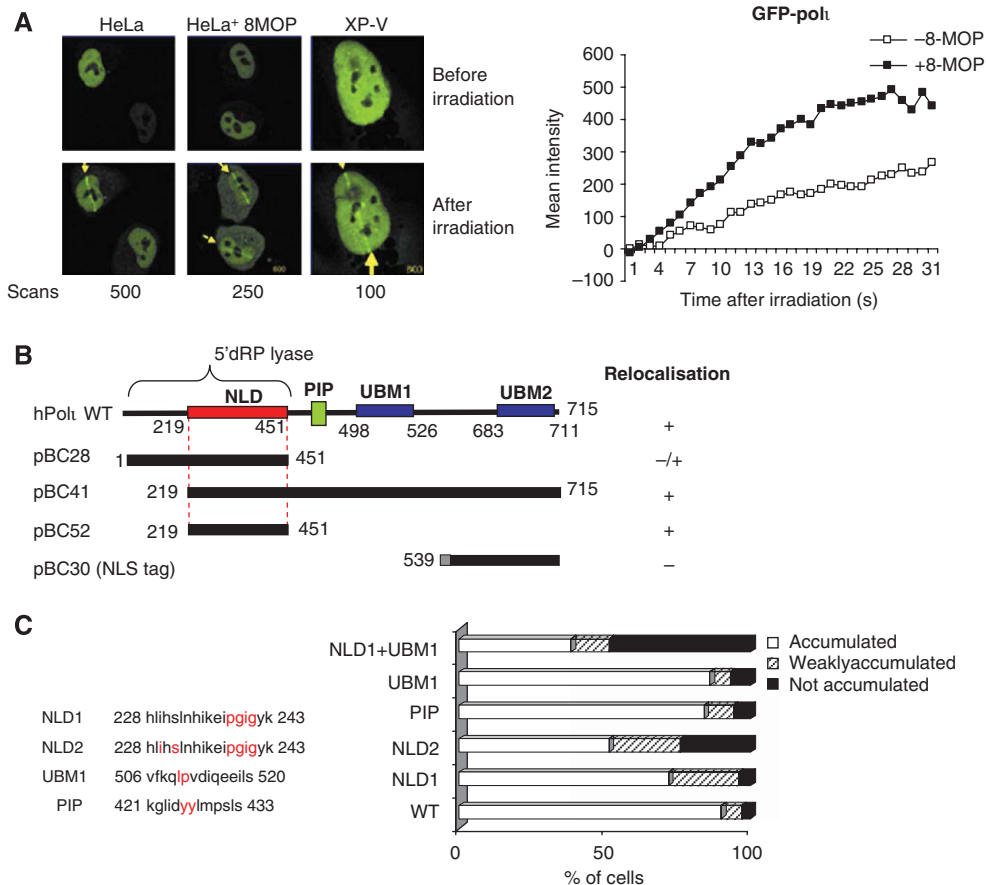


Figure 6 Accumulation of pol ι at the sites of base damage induced by UV laser irradiation. **(A)** HeLa or XP-V cells transiently expressing GFP-pol ι were irradiated with 405 nm laser light (with or without preincubation with the photosensitiser 8-MOP). GFP signals before (top) and after (bottom) irradiation are shown (left panel). Numbers of scans are indicated below. Time course for the accumulation of GFP-pol ι without (open squares) or with (open squares) 8-MOP, after irradiation with a 405 nm laser (right panel). **(B)** Schematic representation of pol ι domains: UBM, ubiquitin-binding motif; NLD, nuclear localisation domain; PIP, interaction domain with PCNA; 5'dRP lyase domain. pol ι deletion constructs were made as described earlier (Kannouche *et al*, 2003). The fragments were cloned downstream of the GFP tag and transfected into HeLa cells. The cells were irradiated with a 405 nm laser light and the accumulation of mutants at the sites of DNA damage was analysed as indicated: +: accumulation in all scanned cells; +/-: accumulation in some cells; -: no accumulation. **(C)** Point mutations were introduced in GFP-pol ι by site-directed mutagenesis. The residues that were changed to alanine are indicated in red. The first and the last amino acids of each sequence are numbered. In the right panel, HeLa cells were transfected with different GFP-pol ι mutants and incubated for 24 h. Cells were then irradiated with a 405 nm laser light and the percentage of GFP-pol ι -expressing cells in which the protein was localised at the sites of oxidative damage was determined.

sequence. These UBM domains bind ubiquitin in a direct and non-covalent manner (Bienko *et al*, 2005; Plosky *et al*, 2006) (Figure 6B).

As shown in Figure 6B, removal of the N-terminal 218 amino acids (pBC41) did not affect the relocalisation of the protein at irradiated sites. pBC28, which contains a deletion of the C terminus from amino acid 451–715, was also able to relocalise but less efficiently, suggesting that the UBM domains might have a function in the recruitment of pol ι . Surprisingly, the construct containing amino acid 219–451 (pBC52), which corresponds to the NLD was sufficient for relocalisation. In contrast, the last 80 aa of pol ι are not sufficient to allow a relocalisation of the fusion protein at irradiated sites. These results indicate that the sequences necessary for relocalisation of pol ι at the sites of oxidative DNA damage are probably within the NLD region, even though the UBMs might also have a function in these dynamics.

We then investigated more precisely the importance of these domains in the relocalisation of pol ι by making point mutations (Figure 6C). The HhH of pol ι , which is containing the NLD, is also conserved among X-family proteins such as

pol β and pol λ (Moon *et al*, 2007). In contrast, this conservation is not detected among Y-family DNA polymerases (Supplementary Figure 1A). The 3D schematic structure shows some overlapping of the residues of the HhH of pol β and pol ι , mainly with the hairpin and the second helix (Supplementary Figure 1B). However, this overlap is less obvious than the one reported for pol β and pol λ (Moon *et al*, 2007). We introduced point mutations in the helix 1 (H1) as well as in the hairpin of the pol ι sequence (Figure 6C). In addition, we constructed mutants harbouring a point mutation in the UBM1 domain. The protein with a mutation in the PCNA-interacting protein (PIP) box motif has been described earlier (Vidal *et al*, 2004). All these mutants were made as fusions with GFP protein to allow analysis of their localisation after irradiation by the laser. As expected, wild-type pol ι was able to accumulate at the sites of laser-induced DNA damage in 91% of irradiated cells (Figure 6C, right panel). Mutations in the UBM1 or PIP domain did not affect the relocalisation of the protein. In striking contrast, the NLD1-UBM1 mutant relocalised in only 38% of irradiated cells, whereas the single mutants

NLD1 and NLD2 accumulated at the sites of DNA damage in 69 and 63% of cells, respectively. We therefore conclude that the HhH motif of pol ι , together with the UBM, contribute to an efficient recruitment to sites of laser-induced DNA damage.

To validate these observations, we examined the importance of these pol ι domains in the sensibility of cells to H₂O₂. In addition, the lysine residue K72 of pol β functions as a Schiff base during the β -elimination reaction. We speculated that the Lys237 of pol ι is its orthologue (see the arrow in Supplementary Figure 1). We checked whether the Lys237 was required to complement the MRC5-pol ι ^{KD} phenotype. We then transiently complemented the MRC5-pol ι ^{KD} cells with *POLI* cDNA-bearing silent mutations and different point mutations in NLD or UBM domains (Supplementary Figure 2). We observed that the sensitivity of complemented MRC5-pol ι ^{KD} cells to H₂O₂ was rescued with most of the pol ι mutants. Interestingly, we found an incomplete complementation of MRC5-pol ι ^{KD} phenotype when we expressed the NLD1-UBM1 mutant confirming that the HhH motif, together with the UBM of pol ι , is required for its function in the response to oxidative stress.

pol ι binds to chromatin after oxidative damage

We reported earlier that pol ι accumulates together with pol η and PCNA in replication factories following UVC irradiation.

Yet whereas pol η foci were completely resistant to extraction by triton, a non-ionic detergent, pol ι was totally solubilised, suggesting that pol ι is less tightly bound to the chromatin than pol η after UVC exposure (Kannouche *et al*, 2003; Kannouche and Lehmann, 2004). To determine the subnuclear localisation of pol ι and of different BER factors after oxidative damage, MRC5 fibroblasts were treated with H₂O₂, harvested in an appropriate buffer containing triton (Kannouche *et al*, 2004). After centrifugation, the supernatant was kept (triton-soluble fraction), whereas the pellet was solubilised in a buffer containing DNase enzyme (chromatin-bound fraction). The fractions were analysed for pol ι by western blotting. We found that the amount of pol ι in the chromatin-bound fraction was increased substantially following H₂O₂ treatment (Figure 7A). Similar results were obtained after irradiation of the cells with UVA (200 KJ/m²) (Supplementary Figure 3A). Additionally, pol ι foci induced by UVA irradiation were resistant to triton extraction (Supplementary Figure 3B). We also found that the scaffold factor XRCC1 became triton-resistant under conditions of oxidative stress as previously observed in HeLa cells (Heale *et al*, 2006). Unexpectedly, pol β was found in the triton-soluble fraction after both H₂O₂ (Figure 7A) and methylmethane sulphonate (MMS) treatment (data not shown), suggesting that its interaction with chromatin during BER must be very transient. The flap endonuclease 1 (FEN1),

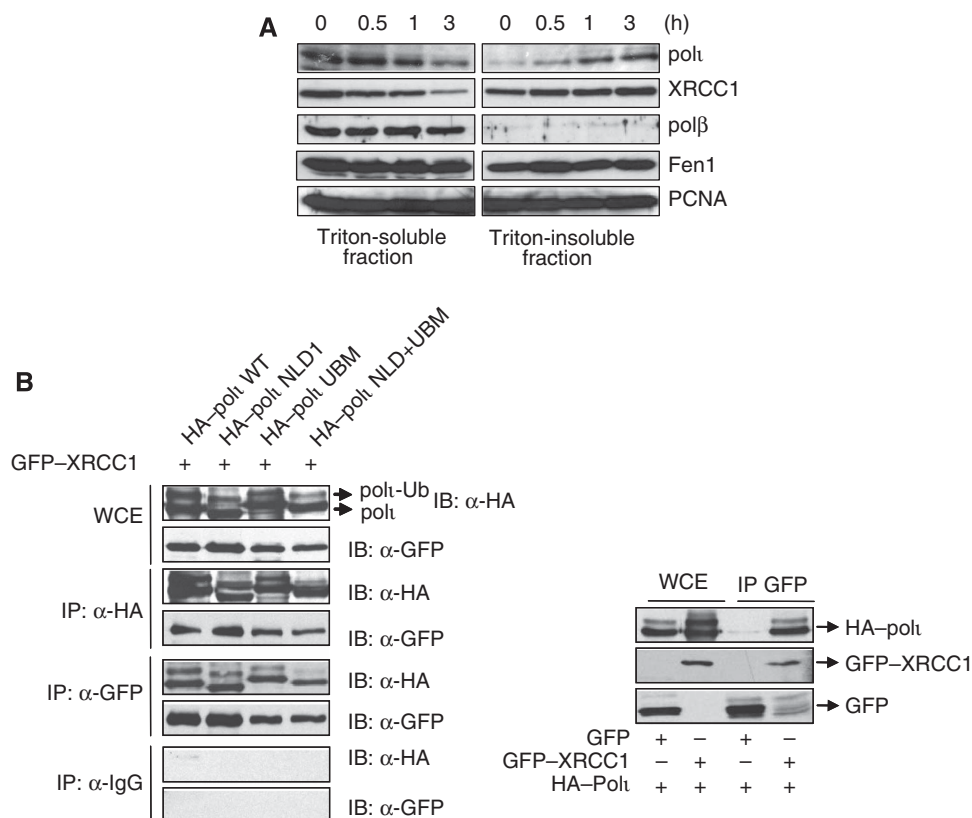


Figure 7 pol ι becomes chromatin-bound after H₂O₂ and interacts with XRCC1. **(A)** MRC5 cells were exposed to 5 mM H₂O₂ for 10 min at 4°C and incubated for the indicated times prior to harvesting and subjected to fractionation into triton-soluble and triton-insoluble fractions. Extracts were analysed by western blotting with the indicated antibodies. **(B)** HEK293 cells were co-transfected with GFP-XRCC1 and different HA-pol ι mutants as indicated. For immunoprecipitation, anti-HA, anti-GFP or pre-immune IgG antibodies were incubated with cell lysates. On the left, immunoprecipitates were analysed by immunoblotting with α -HA or α -GFP antibodies. WCE: whole-cell extracts containing 1/20 of the lysates used for immunoprecipitation. On the right, immunoprecipitation of GFP alone was used as a negative control for interaction with HA-pol ι .

which is involved in LP BER as well as in DNA replication, was partially found in the triton-insoluble fraction. These data indicate that pol ι and XRCC1 are recruited to the chromatin after H₂O₂ treatment and we assume that this recruitment reflects a biological role of pol ι following oxidative damage in human cells.

pol ι interacts with XRCC1 in human cells

After oxidative DNA damage, the BER factor XRCC1 has a major function by interacting with multiple repair partners, including PARP-1, pol β , PCNA, ligase III, APE1 and PNK (reviewed in Caldecott, 2003a). As our cellular fractionation experiments showed that both pol ι and XRCC1 became triton resistant following H₂O₂ treatment, we sought to see whether they could also interact physically *in vivo*. To check this hypothesis, both full-length and mutated pol ι proteins were tagged with N-terminal HA epitopes and were co-expressed in cells with GFP-XRCC1. Cell lysates were immunoprecipitated with anti-HA antibody. Interestingly, GFP-XRCC1 was observed in the immunoprecipitate containing the full length of pol ι or the mutants, reflecting a tight association of the two proteins *in vivo* (Figure 7B). This interaction was also confirmed by a reverse immunoprecipitation of GFP-XRCC1 with α -GFP antibodies and immunoblotting with anti-HA antibodies. These results strongly support a role for pol ι in some aspect of BER *in vivo*. However, as all pol ι mutants were able to interact with XRCC1, we concluded that the HhH and UBM1 motifs are not required for this interaction.

Discussion

Nine years after its discovery, the function of the Y-family DNA polymerase iota has remained an enigma. Mice lacking pol ι have a normal phenotype (McDonald *et al*, 2003). We have now uncovered a clear role of pol ι in protecting cells from oxidative damage.

We have demonstrated that human cells downregulating pol ι exhibit hypersensitivity to oxidative base damage induced by H₂O₂. We also found that pol ι becomes triton resistant after H₂O₂ treatment and interacts with the BER factor XRCC1. Furthermore, GFP-tagged pol ι accumulates at the sites of oxidative DNA damage in living cells. Our mutagenesis experiments suggest that two different domains of pol ι are required for this relocalisation: its dRP lyase region and its UBM1. Altogether, our results strongly suggest that pol ι can protect cells against oxidative damage.

Human pol ι is involved in the cellular response to oxidising agents

This is the first report that shows a menadione and H₂O₂ sensitivities in human fibroblasts deficient for a DNA polymerase. This sensitivity is a direct result of the reduction of functional pol ι as transfection of MRC5-pol ι ^{KD} cells with full-length POLI cDNA reversed this phenotype. So far, sensitivities to DNA-damaging agents have been observed in chicken DT40 cells and/or MEFs inactivated or mutated in POLB, POLL or POLQ genes (Horton *et al*, 2002; Braithwaite *et al*, 2005; Yoshimura *et al*, 2006; Tano *et al*, 2007).

pol β is the classical BER protein for removal of the 5'dRP moiety resulting from APE1 cleavage (reviewed in Almeida and Sobol, 2007). It has been extensively reported that MEF deficient for pol β is hypersensitive to mono-functional DNA-

alkylating agents such as MMS. In contrast, pol β ^{-/-} cells are not or are only slightly sensitive to oxidising agents that produce oxidative DNA damage, suggesting that pol β is not of primary importance in BER of oxidised DNA, or its activity can be complemented by another DNA polymerase. pol ι has been shown to be capable of removing the 5'dRP lesion subsequent to APE1 strand cleavage, but a deficiency in pol ι does not render cells sensitive to alkylation (Trivedi *et al*, 2005 and this paper). In contrast, cells with a reduced level of pol ι are sensitive to H₂O₂ and this phenotype is not synergistically increased by concomitant decrease of pol β level, suggesting that human DNA polymerase iota is important for protection of cells against cell killing due to oxidative stress. Altogether, these data indicate that, although both DNA polymerases exhibit dRP lyase activity, the two polymerases do not share the same lesion substrate. Presumably, pol ι participates in a specialised form of BER as speculated earlier by Bebenek *et al* (2001). This assumption is strengthened by the fact that extracts from cells downregulating pol ι show a reduced BER activity.

Accumulation of pol ι -deficient cells in G1 phase after H₂O₂ treatment

We showed that reduced levels of the pol ι protein have an impact on cell cycle progression before and after DNA damage. Indeed, we detected a slight accumulation of MRC5-pol ι ^{KD} cells in G1 phase in untreated cells, probably due to spontaneous DNA damage (Figure 1C). This effect is increased after exposure to H₂O₂ (Figure 5). This G1 arrest was not observed immediately after treatment but rather occurred at late time points (24–36 h post-treatment). When we analysed the cell cycle progression of cells treated with H₂O₂ in S phase (revealed with BrdU pulse labelling prior to treatment), we obtained the same FACS profile with an accumulation of MRC5-pol ι ^{KD} cells in G1 phase 24–36 h post-treatment as compared with the control cells. These data revealed that when MRC5-pol ι ^{KD} cells are exposed to H₂O₂ during S phase, replication rates are reduced in the first S phase to the same extent as in control cells (Figure 5D). However, progression into the second cell cycle causes a G1 arrest in pol ι -deficient cells, whereas the control cells can progress through the second S phase. The simplest interpretation is that when MRC5-pol ι ^{KD} cells arrive in the G1 phase, the limited quantity of pol ι leads to a reduced capacity in repairing or tolerating the remaining oxidised lesions present on the DNA inducing G1 arrest.

Nevertheless, it is noteworthy that in normal cells, pol ι is located in replication foci during S phase (Kannouche *et al*, 2003). This polymerase is able to incorporate a guanine opposite uracil and its derivatives providing a possible S-phase-dependent mechanism that can decrease the mutagenic potential of lesions formed by the deamination of cytosines (Vaisman and Woodgate, 2001). Therefore, even if the MRC5-pol ι ^{KD} cells are allowed to undergo the first round of replication, we hypothesise that the absence of pol ι in S phase after H₂O₂ exposure affects the repair and/or bypass of some oxidised DNA lesions. Thus, MRC5-pol ι ^{KD} cells will contain more mispairs and/or unrepaired lesions as compared with the control cells when cells will progress through the G1 phase inducing a G1 arrest.

pol η interacts with XRCC1 and is recruited to sites of oxidative DNA damage

We observed that pol η was bound to chromatin following H₂O₂ treatment. This recruitment was also detected after 200 kJ/m² of UVA irradiation. In contrast, pol η remained triton soluble after UVC irradiation (Kannouche and Lehmann, 2004), demonstrating a specific involvement of pol η in the response to oxidative stress. Further *in vivo* evidence for a role of pol η in some aspect of BER is provided by its interaction with XRCC1, which has been shown to have central function in the repair of breaks in DNA (Caldecott, 2003b; Lan *et al*, 2004; Almeida and Sobol, 2007; Dianov and Parsons, 2007). This indicates that XRCC1 and pol η exist in a common complex *in vivo*.

We also found that pol η was recruited to sites of oxidative DNA damage induced with high laser dose. Although the laser treatment used in this study produces oxidative base damage, SSBs and double-strand DNA breaks (DSBs), we assume that pol η is localised to sites of oxidative base damage as we observed that the accumulation of this polymerase was enhanced in the presence of 8-MOP, a photosensitiser that enhances the accumulation of human OGG1 and NTH1 after laser irradiation (Orimo *et al*, 2006; Prasad *et al*, 2007). Moreover, we excluded the possibility that pol η was implicated in double-strand break repair as cells downregulating pol η were not sensitive to neocarzinostatin (data not shown), a drug that induces high ratio of DSBs (Ohtsuki and Ishida, 1975; Favaudon, 1982).

We showed that the NLD regions of pol η and its UBM1 domain were required for an efficient localisation of the protein to sites of oxidative DNA damage. Likewise, these domains are required to rescue the H₂O₂ sensibility of MRC5-pol η ^{KD} cells. In contrast, they appeared to be independent of the XRCC1–pol η interaction, as all proteins mutated in NLD and/or UBM domains are still able to bind to XRCC1. It is possible that these domains of pol η might target it to complexes assembled at the sites of damage. The UBM1 of pol η binds ubiquitin in a direct and non-covalent manner (Bienko *et al*, 2005; Plosky *et al*, 2006). Therefore, pol η could bind to any number of proteins that are specifically ubiquitinated. It has been shown that pol η is able to bind monoubiquitinated PCNA through its PIP and UBM1 domains (Vidal *et al*, 2004; Bienko *et al*, 2005; Plosky *et al*, 2006). In our experiments, it seems unlikely that the localisation of pol η at the sites of oxidative damage was dependent on PCNA as (1) pol η mutated in the PIP domain is still localised to irradiated sites and (2) PCNA accumulates at SSBs, whereas pol η relocalises to oxidative base damage (Lan *et al*, 2004; Hashiguchi *et al*, 2007). Interestingly, we also observed that the recruitment of pol η at the sites of oxidative base damage was independent of pol η , in contrast to its recruitment into replication factories during S phase (Kannouche *et al*, 2003). Thus, after UVC pol η interacts with pol η and PCNA into replication factories, whereas after oxidative DNA damage, pol η is probably assembled into a new complex involving ubiquitinated proteins and XRCC1.

Are our data consistent with other features of pol η ?

Several lines of evidence are consistent with our finding that pol η is involved in the response to oxidative DNA damage:

- At the biochemical level, pol η displays a 5'dRP activity. In reactions reconstituted *in vitro* with uracil-DNA glycosylase, AP endonuclease and DNA ligase, pol η can use its dRP lyase and polymerase activities to repair G·U and A·U pairs in DNA. Moreover, pol η is partially able to complement *in vitro* single-nucleotide BER deficiency of a DNA pol β null-cell extract (Bebenek *et al*, 2001; Prasad *et al*, 2003), suggesting that pol η and pol β have at least some degree of mechanistic overlap.
- At the gene expression level, in response to hypoxia/reoxygenation, hsPOLI transcript level accumulates in a hypoxia-inducible factor 1 (HIF1)-dependent way. This induction is not observed for the other Y-family polymerases (Ito *et al*, 2006). As reoxygenation produces reactive oxygen species (ROS) that generate DNA damage (Hammond *et al*, 2003a,b), this upregulation of POLI strongly suggests that pol η protein is required to repair and/or bypass the ROS-induced DNA damage. In addition, it has been shown that POLI is overexpressed in a wide range of tumour types (Albertella *et al*, 2005). This observation could be explained by the fact that within a three-dimensional microenvironment, tumour cells can be exposed to fluctuating levels of cycling hypoxia (acute hypoxia followed by reoxygenation) (Bristow and Hill, 2008).
- At the genetic level, Ohkumo *et al* (2006) have reported that UVB induces mesenchymal tumours in mice deficient for pol η . The authors concluded that UV-induced DNA lesions are primarily responsible for mesenchymal skin tumour formation in POLI-deficient mice. However, chronic UVB exposure of mice also produces ROS (Pelle *et al*, 2003; Wulff *et al*, 2008). Regarding our data, we can speculate that UVB-induced mesenchymal tumours in POLI-deficient mice are caused, to a certain extent, by ROS, which induce oxidative DNA lesions that are not properly repaired in dermis, in the absence of the pol η protein.

Taken together, our data reveal a novel role of human DNA pol η in the maintenance of the genome integrity following exposure of cells to oxidative stress. We show for the first time a phenotype of human cells downregulating pol η , and we have, thus, expanded our understanding to elucidate the biological role of this DNA polymerase. The fact that pol η exists as a complex together with XRCC1 in cells implicates of a TLS polymerase in mechanisms of repair of oxidative lesions. Recent data have reported that other Y-family polymerases are involved in non-TLS processes such as pol η in homologous recombination and pol κ in NER (Kawamoto *et al*, 2005; McIlwraith *et al*, 2005; Ogi *et al*, 2006). The next step will be to understand how pol η is targeted to use its intrinsic polymerase and dRP lyase activities only at the appropriate time and place in human cells.

Materials and methods

Transfection and plasmid constructions

Downregulation of pol η was carried out with the vector pSUPER.puro (Oligoengine) to express a short hairpin RNA (shRNA) directed against the POLI mRNA. The 21-nucleotide sequence against pol η was synthesised by Oligoengine and is located towards the C terminus of the protein (5'-GAAGGAAGCCTCATACAGTTT-3' and 3'-ACTGTATGAGGCTTCCTTCTT-5'). As a control, we used a

Table I Primers used in site-directed mutagenesis

Primer name	Sequence
NLD 240_243	5'-TATTCATAGTTTGAATCACATAAAGGAAATAGCTGCTGCTGCCTATAAAAAGTCCCAAATGTCTTGAAGCACTG-3'
NLD 230_232	5'-GTCTTATTACCTGAAAGTTGTCAACATCTTGCTCATGCTTTGAATCACATAAAGGAAATAGCTGCTGC-3'
UBM 510_511	5'-GAAGGTGTTGACCAAGAAGTCTTCAAGCGGCCCAAGTAGATATTCAAGAAGAAATCCTT-3'
Silencing 336_340	5'-CTTTAAACAGAGTATGCCAAGATGGTCTGGAAACACACACAGTGAGATTAATAATCCGTCGGTA-3'

scrambled sequence (5'-CCCCGCGCGCTTTGTAGGATT-3' and 3'-AAAGCGCGCTTTGTAGGATT-5').

The protocol used to obtain the shRNA-polt plasmid was in accordance with the manufacturer's protocol. For stable transfectants, MRC5 cells were transfected with pSUPER plasmids containing the appropriate shRNA. At 48 h after transfection, cells were incubated in a selection medium containing 0.45 µg/ml puromycin (Sigma-Aldrich). Selection was continued for 2 weeks and stable transfectants were isolated. We designated the cell lines down-regulating polt MRC5-polt^{KD} followed by the clone number. In parallel, we made cell line expressing scrambled sequence as a negative control and it was named MRC5-NT. The GFP-XRCC1 and GFP-polt plasmid have been described earlier (Kannouche *et al*, 2003; Campalans *et al*, 2005, respectively). To generate the HA-polt, the full-length cDNA for human polt was inserted into the vector phCMV2 (Gene Therapy Systems) by cloning into restriction sites *XhoI*-*Bam*HI. The sequence of polt was confirmed by DNA sequencing. Cells were transfected with the plasmids using Exgen500 (Fermentas) according to the manufacturer's protocol. For polβ siRNA transfection, we used the Dharmacon SMARTpool of four polβ-specific siRNAs. Likewise, for transient downregulation of polt, we used the Dharmacon SMARTpool of four polt-specific siRNAs. Cells were transfected with 30 nM of siRNA pool using Interferin reagent (Polyplus) according to the manufacturer's instructions, and were incubated for the indicated time points.

In vitro site-directed mutagenesis

Point mutations were introduced in different positions of GFP-polt using QuickChange II site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The sequences of the primers used are described in Table I. Mutant NLD1 was made using the primers NLD 240-243, mutant NLD2 was made using the primers NLD 240-243 and NLD 230-232, mutant UBM1 was made using the primers UBM 510-511 and mutant PIP was constructed as described earlier (Vidal *et al*, 2004). The panel of polt mutants were cloned into phCMV2 vector as described above. A silencing mutation in the wt *POL1* cDNA was introduced using primers 336-340.

Proliferation assay

MRC5-polt^{KD} or MRC5-NT cells were plated at 2×10^5 per well of a six-well plate and incubated for 24 h. Cells were then exposed to increasing doses of H₂O₂ (0-7 mM; Sigma-Aldrich) in cold PBS at 4°C for 10 min; or EMS (0.01-1 mM; Sigma-Aldrich) in a medium without FCS at 37°C for 1 h or were treated with 0-20 µM of 5-hydroxymethyl-2'-deoxyuridine (hmdUrd; Sigma-Aldrich) for 24 h or exposed to increasing doses of menadione for 1 h in complete MEM medium at 37°C (0-50 µM; Sigma-Aldrich). UVA (320-400 nm) radiation was achieved by a metal halide lamp equipped with anticaloric filter KG1. The fluency of the UVA lamp was 12.3 J/s/m² and the cells were irradiated in MEM medium without phenol red. For the UVC (254 nm) lamp, the fluency was 0.35 J/s/m² and the cells were irradiated without any medium. After each treatment, cells were rinsed twice with PBS, incubated for 72 h in complete MEM medium and counted by Trypan blue staining using a Neubauer haemocytometer or an automatic counter.

Flow cytometric analysis

To study the cell cycle distribution, cells were treated for 10 min with 2.5 mM H₂O₂ in cold PBS, at 4°C and, at indicated times, 10 µM

of BrdU (Sigma-Aldrich) was added to the dishes for 20 min prior harvesting. Alternatively, S-phase cells were pulse-labelled with 10 µM BrdU in complete medium for 10 min at 37°C. Cells were then treated as described above and re-incubated in fresh medium for the indicated time. Cells were then trypsinised, washed in PBS and fixed in 80% ice-cold ethanol overnight at -20°C. For BrdU staining, samples were incubated in 15 mM pepsin for 20 min at 37°C. The cells were pelleted and incubated with 2 N HCl and incubated for 20 min at room temperature. The pellet was washed in buffer Bu (0.1% FCS, 0.1% Tween 20, 0.1 M Hepes in PBS), and was incubated with anti-BrdU antibody diluted at 1:50 in buffer Bu (clone Bu20a; Dako) for 45 min. After centrifugation, cells were resuspended and incubated with anti-mouse-FITC antibody diluted at 1:50 in buffer Bu (Vector). For total DNA staining, cells were centrifuged and resuspended in PBS containing 25 µg/ml propidium iodide (Sigma-Aldrich) and 50 µg/µl RNAse (Sigma-Aldrich). The samples were analysed by flow cytometry on a FACScalibur (Becton Dickinson, BD Sciences) using the CellQuest Pro software (BD Sciences).

Laser light irradiation and microscopy

Fluorescence images were obtained and processed using a FV-500 confocal scanning laser microscopy system (Olympus). The microscope is coupled with microirradiation facilities to emit 405 nm laser light as described earlier (Lan *et al*, 2005). Precisely, we used the 405 nm scan laser system for irradiating the cells in the epifluorescence path of the microscope system. The power of the scan laser can be controlled by altering the scanning times (250 or 500 scans). The scan laser light of 405 nm wavelength delivers within 5 ms around 1600 nW of energy. Thus, cells were incubated on a 37°C hot plate in glass-bottomed dishes placed in chambers to prevent evaporation and then irradiated. After irradiation, images were captured at 10- to 20-s intervals for up to 5-10 min. The energy of the fluorescent light at the irradiated site was measured. The mean intensity of the track in the irradiated cell was obtained after subtracting the background intensity.

Further details of materials and methods are presented as Supplementary data.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

Acknowledgements

We thank Dr R Woodgate (NIH Bethesda, MD, USA) for providing purified antibody for polt, Dr P Radicella for the GFP-XRCC1 plasmid and Dr V Favaudon for providing the neocarzinostatin drug. We also thank AR Lehmann for the GFP-polt plasmids and for his critical comments on the paper. We are grateful to Caroline Pouvelle for assistance with cell culture and Yann Lecluse for FACS analysis. This study was supported by Association pour la Recherche sur le Cancer (ARC, no. 3573), by Fondation pour la Recherche Medicale (INE20040300464) and by Institut National du Cancer no. PL28 to PK, by Genome Network Project Japan to AY, and by CNRS GDRE182, ARC No. 4032, 3655 and EDF RB2007-02 to Murat Saparbaev. TBP was supported by an ARC fellowship and AZ was supported by Contract MRTN-Ct-2003-503618.

References

- Albertella MR, Lau A, O'Connor MJ (2005) The overexpression of specialized DNA polymerases in cancer. *DNA Repair (Amst)* **4**: 583–593
- Almeida KH, Sobol RW (2007) A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. *DNA Repair (Amst)* **6**: 695–711
- Ballmaier D, Epe B (1995) Oxidative DNA damage induced by potassium bromate under cell-free conditions and in mammalian cells. *Carcinogenesis* **16**: 335–342
- Bebenek K, Tissier A, Frank EG, McDonald JP, Prasad R, Wilson SH, Woodgate R, Kunkel TA (2001) 5'-Deoxyribose phosphate lyase activity of human DNA polymerase η *in vitro*. *Science* **291**: 2156–2159
- Biade S, Sobol RW, Wilson SH, Matsumoto Y (1998) Impairment of proliferating cell nuclear antigen-dependent apurinic/apyrimidinic site repair on linear DNA. *J Biol Chem* **273**: 898–902
- Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, Kannouche P, Wider G, Peter M, Lehmann AR, Hofmann K, Dikic I (2005) Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* **310**: 1821–1824
- Braithwaite EK, Kedar PS, Lan L, Polosina YY, Asagoshi K, Poltoratsky VP, Horton JK, Miller H, Teebor GW, Yasui A, Wilson SH (2005) DNA polymerase λ protects mouse fibroblasts against oxidative DNA damage and is recruited to sites of DNA damage/repair. *J Biol Chem* **280**: 31641–31647
- Bristow RG, Hill RP (2008) Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* **8**: 180–192
- Caldecott KW (2003a) Protein-protein interactions during mammalian DNA single-strand break repair. *Biochem Soc Trans* **31**: 247–251
- Caldecott KW (2003b) XRCC1 and DNA strand break repair. *DNA Repair (Amst)* **2**: 955–969
- Campalans A, Marsin S, Nakabeppu Y, O'Connor TR, Boiteux S, Radicella JP (2005) XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. *DNA Repair (Amst)* **4**: 826–835
- Dianov GL, Parsons JL (2007) Co-ordination of DNA single strand break repair. *DNA Repair (Amst)* **6**: 454–460
- Favaudon V (1982) On the mechanism of reductive activation in the mode of action of some anticancer drugs. *Biochimie* **64**: 457–475
- Fortini P, Pascucci B, Belisario F, Dogliotti E (2000) DNA polymerase β is required for efficient DNA strand break repair induced by methyl methanesulfonate but not by hydrogen peroxide. *Nucleic Acids Res* **28**: 3040–3046
- Goodman MF (2002) Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem* **71**: 17–50
- Hammond EM, Dorie MJ, Giaccia AJ (2003a) ATR/ATM targets are phosphorylated by ATR in response to hypoxia and ATM in response to reoxygenation. *J Biol Chem* **278**: 12207–12213
- Hammond EM, Green SL, Giaccia AJ (2003b) Comparison of hypoxia-induced replication arrest with hydroxyurea and aphidicolin-induced arrest. *Mutat Res* **532**: 205–213
- Hashiguchi K, Matsumoto Y, Yasui A (2007) Recruitment of DNA repair synthesis machinery to sites of DNA damage/repair in living human cells. *Nucleic Acids Res* **35**: 2913–2923
- Heale JT, Ball Jr AR, Schmiesing JA, Kim JS, Kong X, Zhou S, Hudson DF, Earnshaw WC, Yokomori K (2006) Condensin I interacts with the PARP-1-XRCC1 complex and functions in DNA single-strand break repair. *Mol Cell* **21**: 837–848
- Horton JK, Baker A, Berg BJ, Sobol RW, Wilson SH (2002) Involvement of DNA polymerase β in protection against the cytotoxicity of oxidative DNA damage. *DNA Repair (Amst)* **1**: 317–333
- Horton JK, Stefanick DF, Naron JM, Kedar PS, Wilson SH (2005) Poly(ADP-ribose) polymerase activity prevents signaling pathways for cell cycle arrest after DNA methylating agent exposure. *J Biol Chem* **280**: 15773–15785
- Ito A, Koshikawa N, Mochizuki S, Omura K, Takenaga K (2006) Hypoxia-inducible factor-1 mediates the expression of DNA polymerase η in human tumor cells. *Biochem Biophys Res Commun* **351**: 306–311
- Johnson RE, Washington MT, Haracska L, Prakash S, Prakash L (2000) Eukaryotic polymerases η and ζ act sequentially to bypass DNA lesions. *Nature* **406**: 1015–1019
- Kannouche P, Fernandez de Henestrosa AR, Coull B, Vidal AE, Gray C, Zicha D, Woodgate R, Lehmann AR (2003) Localization of DNA polymerases η and ι to the replication machinery is tightly co-ordinated in human cells. *EMBO J* **22**: 1223–1233
- Kannouche PL, Lehmann AR (2004) Ubiquitination of PCNA and the polymerase switch in human cells. *Cell Cycle* **3**: 1011–1013
- 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
- Kawamoto T, Araki K, Sonoda E, Yamashita YM, Harada K, Kikuchi K, Masutani C, Hanaoka F, Nozaki K, Hashimoto N, Takeda S (2005) Dual roles for DNA polymerase in homologous DNA recombination and translesion DNA synthesis. *Mol Cell* **20**: 793–799
- Lan L, Nakajima S, Komatsu K, Nussenzweig A, Shimamoto A, Oshima J, Yasui A (2005) Accumulation of Werner protein at DNA double-strand breaks in human cells. *J Cell Sci* **118**: 4153–4162
- Lan L, Nakajima S, Oohata Y, Takao M, Okano S, Masutani M, Wilson SH, Yasui A (2004) *In situ* analysis of repair processes for oxidative DNA damage in mammalian cells. *Proc Natl Acad Sci USA* **101**: 13738–13743
- Lindahl T (1982) DNA repair enzymes. *Annu Rev Biochem* **51**: 61–87
- McDonald JP, Frank EG, Plosky BS, Rogozin IB, Masutani C, Hanaoka F, Woodgate R, Gearhart PJ (2003) 129-derived strains of mice are deficient in DNA polymerase ι and have normal immunoglobulin hypermutation. *J Exp Med* **198**: 635–643
- McIlwraith MJ, Vaisman A, Liu Y, Fanning E, Woodgate R, West SC (2005) Human DNA polymerase η promotes DNA synthesis from strand invasion intermediates of homologous recombination. *Mol Cell* **20**: 783–792
- Moon AF, Garcia-Diaz M, Batra VK, Beard WA, Bebenek K, Kunkel TA, Wilson SH, Pedersen LC (2007) The X family portrait: structural insights into biological functions of X family polymerases. *DNA Repair (Amst)* **6**: 1709–1725
- Ogi T, Lehmann AR (2006) The Y-family DNA polymerase (ρ k) functions in mammalian nucleotide-excision repair. *Nat Cell Biol* **8**: 640–642
- Ohkumo T, Kondo Y, Yokoi M, Tsukamoto T, Yamada A, Sugimoto T, Kanao R, Higashi Y, Kondoh H, Tatematsu M, Masutani C, Hanaoka F (2006) UV-B radiation induces epithelial tumors in mice lacking DNA polymerase η and mesenchymal tumors in mice deficient for DNA polymerase ι . *Mol Cell Biol* **26**: 7696–7706
- Ohmori H, Friedberg EC, Fuchs RP, Goodman MF, Hanaoka F, Hinkle D, Kunkel TA, Lawrence CW, Livneh Z, Nohmi T, Prakash L, Prakash S, Todo T, Walker GC, Wang Z, Woodgate R (2001) The Y-family of DNA polymerases. *Mol Cell* **8**: 7–8
- Ohtsuki K, Ishida N (1975) Neocarzinostatin-induced breakdown of deoxyribonucleic acid in HeLa-S3 cells. *J Antibiot (Tokyo)* **28**: 143–148
- Orimo H, Tokura Y, Hino R, Kasai H (2006) Formation of 8-hydroxy-2'-deoxyguanosine in the DNA of cultured human keratinocytes by clinically used doses of narrowband and broadband ultraviolet B and psoralen plus ultraviolet A. *Cancer Sci* **97**: 99–105
- Pelle E, Huang X, Mammone T, Marenus K, Maes D, Frenkel K (2003) Ultraviolet-B-induced oxidative DNA base damage in primary normal human epidermal keratinocytes and inhibition by a hydroxyl radical scavenger. *J Invest Dermatol* **121**: 177–183
- Plosky BS, Vidal AE, Fernandez de Henestrosa AR, McLenigan MP, McDonald JP, Mead S, Woodgate R (2006) Controlling the sub-cellular localization of DNA polymerases ι and η via interactions with ubiquitin. *EMBO J* **25**: 2847–2855
- Polosina YY, Rosenquist TA, Grollman AP, Miller H (2004) 'Knock down' of DNA polymerase β by RNA interference: recapitulation of null phenotype. *DNA Repair (Amst)* **3**: 1469–1474
- Prasad R, Bebenek K, Hou E, Shock DD, Beard WA, Woodgate R, Kunkel TA, Wilson SH (2003) Localization of the deoxyribose phosphate lyase active site in human DNA polymerase ι by controlled proteolysis. *J Biol Chem* **278**: 29649–29654
- Prasad R, Liu Y, Deterding LJ, Poltoratsky VP, Kedar PS, Horton JK, Kanno S, Asagoshi K, Hou EW, Khodyreva SN, Lavrik OI, Tomer

- KB, Yasui A, Wilson SH (2007) HMGB1 is a cofactor in mammalian base excision repair. *Mol Cell* **27**: 829–841
- Sobol RW, Horton JK, Kuhn R, Gu H, Singhal RK, Prasad R, Rajewsky K, Wilson SH (1996) Requirement of mammalian DNA polymerase-beta in base-excision repair. *Nature* **379**: 183–186
- Srivastava DK, Berg BJ, Prasad R, Molina JT, Beard WA, Tomkinson AE, Wilson SH (1998) Mammalian abasic site base excision repair. Identification of the reaction sequence and rate-determining steps. *J Biol Chem* **273**: 21203–21209
- Tano K, Nakamura J, Asagoshi K, Arakawa H, Sonoda E, Braithwaite EK, Prasad R, Buerstedde JM, Takeda S, Watanabe M, Wilson SH (2007) Interplay between DNA polymerases beta and lambda in repair of oxidation DNA damage in chicken DT40 cells. *DNA Repair (Amst)* **6**: 869–875
- Tissier A, Frank EG, McDonald JP, Iwai S, Hanaoka F, Woodgate R (2000) Misinsertion and bypass of thymine–thymine dimers by human DNA polymerase iota. *EMBO J* **19**: 5259–5266
- Trivedi RN, Almeida KH, Fornasaglio JL, Schamus S, Sobol RW (2005) The role of base excision repair in the sensitivity and resistance to temozolomide-mediated cell death. *Cancer Res* **65**: 6394–6400
- Vaisman A, Woodgate R (2001) Unique misinsertion specificity of poliota may decrease the mutagenic potential of deaminated cytosines. *EMBO J* **20**: 6520–6529
- Vidal AE, Kannouche P, Podust VN, Yang W, Lehmann AR, Woodgate R (2004) Proliferating cell nuclear antigen-dependent coordination of the biological functions of human DNA polymerase iota. *J Biol Chem* **279**: 48360–48368
- Watanabe N, Dickinson DA, Liu RM, Forman HJ (2004) Quinones and glutathione metabolism. *Methods Enzymol* **378**: 319–340
- Wulff BC, Schick JS, Thomas-Ahner JM, Kusewitt DF, Yarosh DB, Oberyszyn TM (2008) Topical treatment with OGG1 enzyme affects UVB-induced skin carcinogenesis. *Photochem Photobiol* **84**: 317–321
- Yoshimura M, Kohzaki M, Nakamura J, Asagoshi K, Sonoda E, Hou E, Prasad R, Wilson SH, Tano K, Yasui A, Lan L, Seki M, Wood RD, Arakawa H, Buerstedde JM, Hocheegger H, Okada T, Hiraoka M, Takeda S (2006) Vertebrate POLQ and POLbeta cooperate in base excision repair of oxidative DNA damage. *Mol Cell* **24**: 115–125
- Zhang Y, Yuan F, Wu X, Wang Z (2000) Preferential incorporation of G opposite template T by the low-fidelity human DNA polymerase iota. *Mol Cell Biol* **20**: 7099–7108