

DNA polymerase ζ cooperates with polymerases κ and ι in translesion DNA synthesis across pyrimidine photodimers in cells from XPV patients

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Human cells tolerate UV-induced cyclobutane pyrimidine dimers (CPD) by translesion DNA synthesis (TLS), carried out by DNA polymerase η , the *POLH* gene product. A deficiency in DNA polymerase η due to germ-line mutations in *POLH* causes the hereditary disease xeroderma pigmentosum variant (XPV), which is characterized by sunlight sensitivity and extreme predisposition to sunlight-induced skin cancer. XPV cells are UV hypermutable due to the activity of mutagenic TLS across CPD, which explains the cancer predisposition of the patients. However, the identity of the backup polymerase that carries out this mutagenic TLS was unclear. Here, we show that DNA polymerase ζ cooperates with DNA polymerases κ and ι to carry out error-prone TLS across a TT CPD. Moreover, DNA polymerases ζ and κ , but not ι , protect XPV cells against UV cytotoxicity, independently of nucleotide excision repair. This presents an extreme example of benefit-risk balance in the activity of TLS polymerases, which provide protection against UV cytotoxicity at the cost of increased mutagenic load.

carcinogenesis | DNA repair | lesion bypass | replication | ultraviolet

TLS is a fundamental mechanism for tolerating DNA damage that has escaped repair, carried out by specialized low-fidelity DNA polymerases, which synthesize across a wide variety of DNA lesions (1). At least 5 TLS DNA polymerases are present in mammals, four of which, DNA polymerases η , ι , κ , and REV1, belong to the Y superfamily. The fifth TLS polymerase is pol ζ , which belongs to the B family, and is the only TLS polymerase known to be essential in mammals (2–5). TLS polymerases exhibit a certain degree of specificity for their substrate DNA lesions, and their activity is tightly regulated (6–9). The most well characterized TLS polymerase is pol η , which is specialized to bypass cyclobutane pyrimidine dimers (CPD) in a relatively error-free manner. The biological significance of pol η is illustrated by the hereditary disease xeroderma pigmentosum variant (XPV), in which germ-line mutations in the *POLH* gene, encoding pol η , cause an extreme 1000-fold increased predisposition to sunlight-induced skin cancer (10, 11). Cells from XPV patients exhibit a slightly increased UV sensitivity, and a dramatic UV hypermutability (12), which is responsible for their extreme cancer predisposition. The UV hypermutability is explained by the activity of a back-up DNA polymerase that performs TLS across CPD with lower efficiency and higher error-frequency. Although there is evidence that pol ι is involved in TLS across CPD in XPV cells (13–15), additional polymerases may be involved, and the picture is far from being complete. This is an important issue because these polymerases are likely to be driving sunlight-induced skin carcinogenesis in XPV patients.

Here, we show that 3 TLS polymerases, pol ζ , pol κ , and pol ι , are involved in TLS across CPD in XPV cells. Moreover, pol ζ and pol κ , but not pol ι , also provide protection against UV cytotoxicity, independently of nucleotide excision repair (NER).

Results

Pol ζ , pol κ , and pol ι Are Involved in TLS Across a Site-Specific TT CPD in Human XPV Cells. To identify the polymerase responsible for TLS across CPD in XPV cells we used a shuttle gapped-plasmid

TLS assay with XPV cells in which the expression of defined DNA polymerases was knocked-down using siRNA. The assay involves transfection of cultured cells with a plasmid carrying a gap opposite a site-specific TT CPD (GP-TT-CPD), along with a control, gapped plasmid, without the CPD (Fig. 1A). After allowing for TLS to occur in the cells, the plasmids are extracted under alkaline conditions, introduced into a TLS-defective *E. coli recA* strain, and plated in parallel on LB-kanamycin (for repaired GP-TT-CPD) and LB-chloramphenicol (for repaired control plasmid). The ratio of kan^R/cm^R *E. coli* colonies is a measure of the efficiency of TLS in the mammalian cells, and sequence analysis of plasmids extracted from individual colonies provides data on DNA sequence changes during TLS. The assay was found to be very useful to study TLS in mammalian cells (8, 16, 17), perhaps because it is a good model system of postreplication gaps (18). Using this system we have shown that TLS across a TT CPD is an order of magnitude more mutagenic in XPV cells compared with normal cells (19), consistent with the hypermutability of XPV cells (12), and the relatively accurate TLS across CPD by purified pol η (10, 20).

Plasmid GP-TT-CPD was used to transfect a cell line from an XPV patient, in which the expression of *POLH*, encoding pol η , *POLK*, encoding pol κ , and/or *REV3L*, encoding the catalytic subunit of pol ζ were knocked-down using siRNA. As shown in Fig. S1, each siRNA, effectively and specifically, knocked-down the expression of its target polymerase. When the efficiency of TLS was assayed, knocking-down *POLH* or *POLK* expression had a marginal effect compared with the treatment of cells with control siRNA (Fig. 1B and Table S1). In contrast, knocking down the expression of *REV3L* caused a significant 74% decrease in TLS. Considering the possibility that pol ι and pol κ may back-up each other, we examined TLS under conditions in which the expression of both *POLH* and *POLK* was knocked-down. Under these conditions TLS across the TT CPD decreased by 65%, similar to the effect of *REV3L* alone (Fig. 1B and Table S1). Knocking-down *REV3L* expression in combination with *POLK*, or *POLH* and *POLK*, had an effect similar to knocking down *REV3L* alone (Fig. 1B). Thus, on one hand most TLS events across TT CPD require pol κ or pol ι , but on the other hand most TLS events require pol ζ . This suggests that in XPV cells pol ζ cooperates with pol κ and/or with pol ι in 2-polymerase TLS reactions across a TT CPD.

Pol ζ , Pol κ , and Pol ι Are Responsible for Mutagenic TLS Across TT CPD in XPV Cells. To determine the extent of mutagenic TLS under conditions in which the expression of certain DNA polymerases

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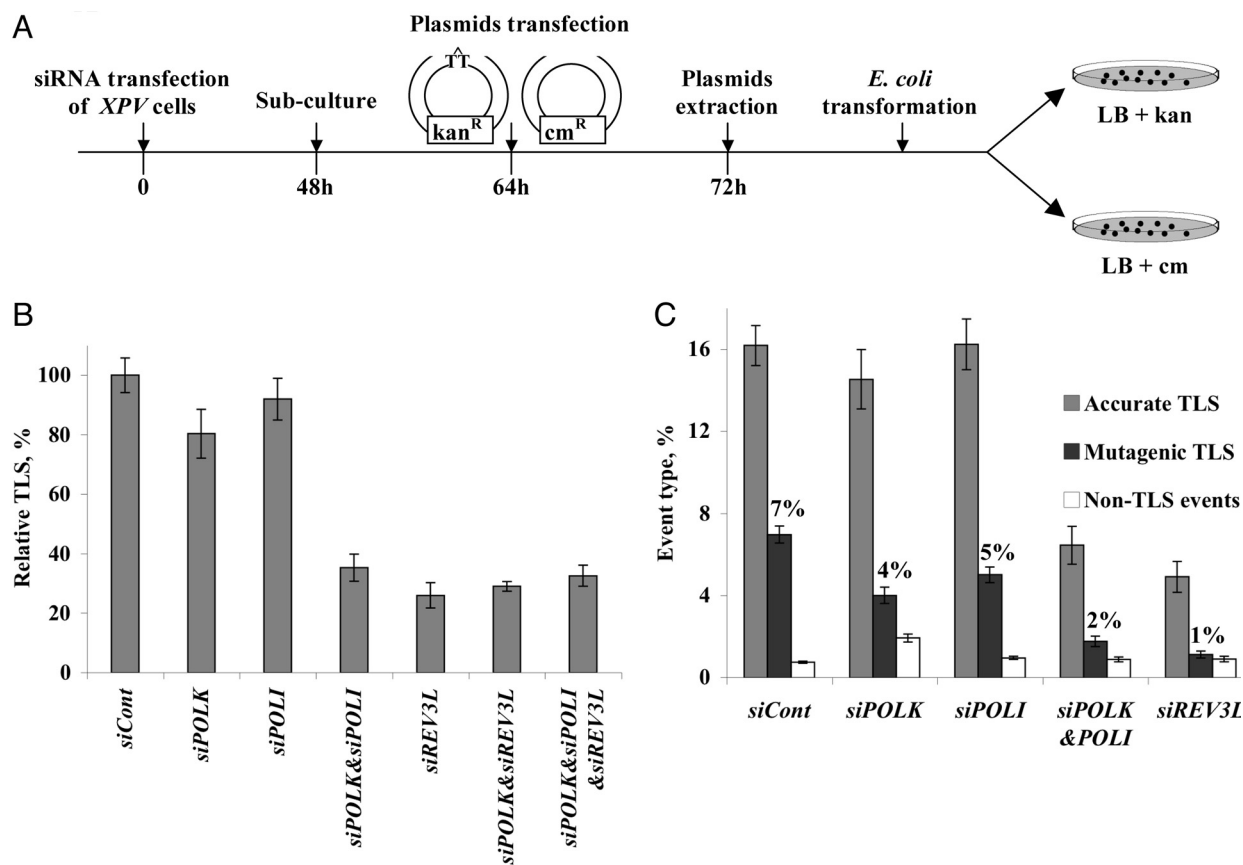


Fig. 1. TLS across a TT CPD in *XPV* cells pretreated with siRNA against specific TLS polymerases. (A) Outline of the gapped plasmid TLS assay. See text for details. (B) Relative TLS extent across TT CPD lesions in *XPV* cells pretreated with siRNA against TLS polymerases. Each column represents the average of 6–10 measurements. See details in [Table S1](#) and in *Materials and Methods*. (C) Extent of accurate and mutagenic TLS in *XPV* cells pretreated with siRNA against TLS polymerases. Sequences were classified as accurate TLS (insertion of AA opposite the TT CPD), mutagenic TLS (nucleotides other than AA inserted opposite the TT CPD, or mutations at the nucleotides flanking the TT CPD), and non-TLS events (large insertions and deletions). The extent of each event type was obtained by multiplying the extent of plasmid repair ([Table S1](#)) by the fraction of that event obtained from the DNA sequence analysis ([Table S2](#)), as presented in [Table S3](#).

was knocked-down, one needs to multiply the efficiency of gap repair by the fraction of mutagenic TLS obtained from DNA sequence analysis of plasmid isolates. To that end we used the extents of TLS from [Fig. 1B](#) and [Table S1](#), and the DNA sequence information presented in [Table S2](#). In cells treated with control siRNA, the extent of TLS across the TT CPD was 23%, composed of 7% mutagenic TLS, and 16% accurate TLS ([Fig. 1C](#) and [Tables S2 and S3](#)). In *XPV* cells in which the expression of *REV3L* was knocked down, the extent of mutagenic TLS across TT CPD dramatically decreased by 84% ([Fig. 1C](#) and [Tables S2 and S3](#)). Similarly, knocking-down the expression of both *POLK* and *POLI* decreased the extent of mutagenic TLS by 76%. Knocking-down the expression of *POLK* or *POLI* alone had only a marginal effect on the extent of mutagenic TLS ([Fig. 1C](#) and [Tables S2 and S3](#)). Generally similar effects were observed also for accurate TLS across TT CPD ([Fig. 1C](#) and [Table S2 and S3](#)). Thus, $pol\kappa$, $pol\iota$, and $pol\zeta$ are involved in both mutagenic and accurate TLS across TT CPD in *XPV* cells.

The mutational signature in *XPV* cells treated with control siRNA showed that the majority of mutations were targeted to the TT CPD (71%, 20/28 events; [Table S2](#)), mostly to the 3' T of the CPD (80%, 16/20 events) ([Table S2](#)), and a significant fraction was semitargeted to the most proximal nucleotides flanking the TT CPD (29%, 8/28 events) ([Table S2](#)). One tandem double mutation opposite the CPD was also observed. In contrast to the mutational load (extent of mutagenic TLS), the mutational signature, which is consistent with previous results

(19), did not significantly change when the expression of *POLK*, *POLI*, *REV3L* or both *POLI* and *POLK* was knocked-down ([Table S2](#); see *Discussion*).

Pol ζ and pol κ , but Not pol ι , Protect *XPV* Cells Against UV Cytotoxicity.

The *in vivo* involvement of $pol\kappa$ in TLS across a TT CPD was somewhat unexpected, because $pol\kappa$ was reported to be blocked by this lesion (21). To examine whether $pol\kappa$, and the other polymerases have a similar function in human chromosomes we analyzed the UV sensitivity of *XPV* cells in which the expression of these polymerases was knocked-down. This was done by assaying ATP level, which rapidly decreases in cells undergoing apoptosis or necrosis ([Fig. 2A](#)). As shown in [Fig. 2B](#), knocking-down the expression of *POLH* had no effect on UV sensitivity of the *XPV* cells, as expected, because these cells are $pol\eta$ -deficient anyway ([Fig. 2B](#)). Similarly, knocking-down the expression of *POLI* had no effect on UV sensitivity, which was different from several (but not all) previous reports (13–15, 22). In contrast, knocking-down the expression of *POLK* increased UV sensitivity up to 3.5–5-fold, and *REV3L* by up to 33- to 39-fold ([Fig. 2B](#)). Knocking-down the expression of both *POLK* and *POLI* had an effect similar to knocking down *POLK* alone ([Fig. 2B](#)). Measuring UV sensitivity by 5-bromo-2'-deoxyuracil (BrdU) incorporation gave similar results ([Fig. S2A](#)). Thus, $pol\zeta$ and $pol\kappa$, but not $pol\iota$, protect *XPV* cells against UV cytotoxicity. Generally consistent, although milder, effects were observed in MRC5 $pol\eta$ -proficient human cells ([Fig. 2C](#)). Notably, knock-

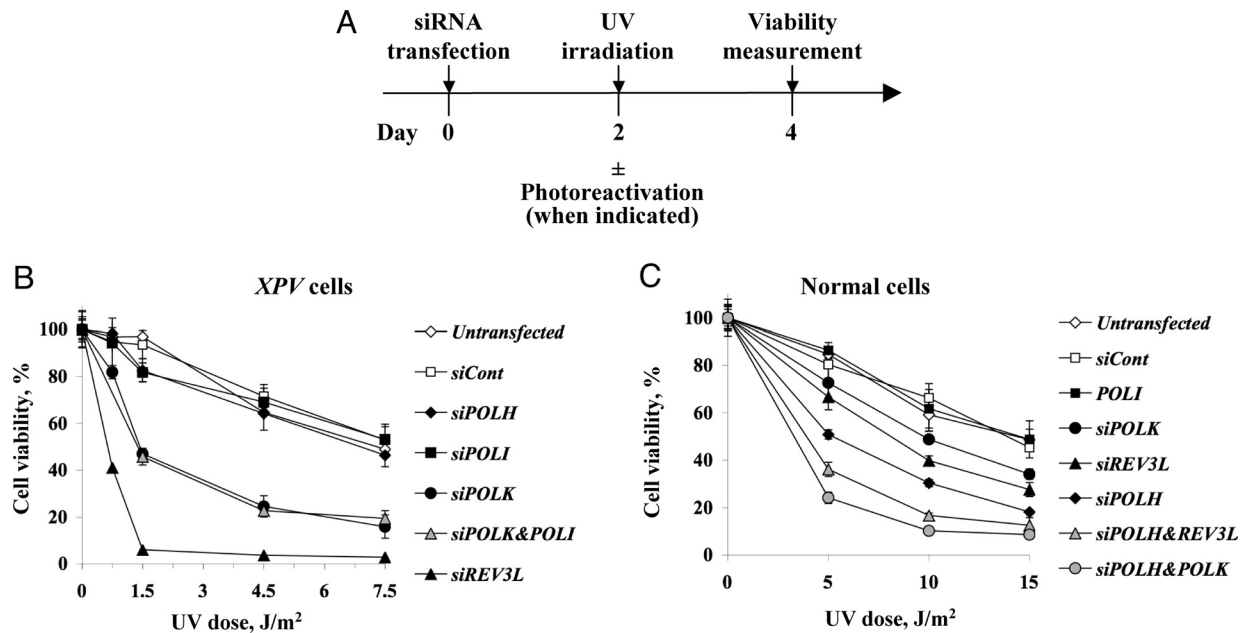


Fig. 2. UV sensitivity of XPV and normal cells pretreated with siRNA against TLS polymerases. (A) Outline of the experimental scheme. (B and C) XPV cells (B) or normal MRC5 human cells (C) were transfected with siRNA, and UV irradiated after 48 h. For MRC5 cells, 1 mM caffeine was added immediately following UV irradiation. Viability was determined 48 h after UV irradiation by measuring cellular ATP as described in *Material and Methods*.

ing-down the expression of *POLK* on top of knocking-down the expression of *POLH* caused significantly increased UV sensitivity compared with knocking down *POLH* alone, and a similar effect was observed by knocking down the expression of *REV3L* along with *POLH* (Fig. 2C).

Protection Against UV Cytotoxicity Provided by *polζ* and *polκ* Occurs in the Absence of Nucleotide Excision Repair. XPV cells are proficient in nucleotide excision repair (NER), and therefore the effects on UV sensitivity observed when the expression of TLS polymerases was knocked-down could be mediated via NER. This possibility is supported by the report that *polκ* is involved in NER of UV lesions in human cells (23). To examine this possibility we repeated the UV sensitivity experiments with a cell line from an *XPA* patient, totally defective in NER. As shown in Fig. 3A, a control siRNA and siRNA against *XPA* had no effect on UV sensitivity of the *XPA* cell line, as expected. Like in the case of XPV cells, siRNA against *POLI* had no effect on UV sensitivity. However, knocking-down the expression of *POLH* or *POLK* caused an up to 2-fold decrease in UV sensitivity (Fig. 3A). Remarkably, knocking-down the expression of *REV3L* caused a strong 4- to 5-fold increase in UV sensitivity compared with cells treated with a control siRNA (Fig. 3A). A similarly strong 5- to 6-fold increase in UV sensitivity was observed upon knocking-down the expression of both *POLK* and *POLH* (Fig. 3A). Knocking-down the expression of *POLI*, in addition to *POLH* and *POLK*, had no additional effect. Similar results were obtained using 3 different siRNA sequences for each *POLK*, *POLH* and *REV3L*, minimizing the possibility of off-target effects (Fig. S3B). In addition, similar results were obtained using incorporation of BrdU to assay UV sensitivity (Fig. S2B). Surprised by the importance of *polκ* in protecting *XPA* cells against UV cytotoxicity, we repeated the experiments using the colony forming ability assay. UV sensitivity of *XPA* cells in this assay was higher, because cells had been assayed 2 weeks after irradiation, when many more cells complete the UV-induced death process (24). However, also in this assay knocking-down the expression of either *POLH* or *POLK* yielded similar UV sensitization, with a bigger effect when both together were

knocked-down (Fig. 3B). Thus, the function of *polκ* and *polζ* in protection against UV cytotoxicity is due to their involvement in DNA damage tolerance, not NER, consistent with their involvement in TLS across the TT CPD in the gapped plasmid assay.

Protection Against UV Cytotoxicity Provided by *polκ* Occurs at CPD.

To examine whether *polκ* exerts its protective effect against CPD, we used an *XPA* cell line expressing the *Potorous tridactylus* CPD photolyase, which specifically repairs CPD by photoreactivation. As shown in Fig. 3C, in the absence of photoreactivation, knocking-down *POLH*, *POLK*, or *REV3L* expression decreased cell viability upon UV irradiation, as in the parental *XPA* cells (Fig. 3A). We then repeated the experiment, except that the cells were photoreactivated by illumination with visible light immediately after the UV irradiation (Fig. 3C), a treatment that had eliminated 85% of the CPD (Fig. S4). Under such photoreactivation conditions, knocking-down *POLH* expression had no effect on UV sensitivity, consistent with the action of *polη* on CPD (Fig. 3C). Similarly, knocking-down *POLK* expression had no effect on UV sensitivity after photoreactivation, indicating that the protection provided by *polκ* is indeed against CPD (Fig. 3C). In contrast, knocking-down *REV3L* expression still caused a strong increase in UV sensitivity under the same photoreactivation conditions, consistent with its role in TLS across 6–4 PP, in addition to CPD.

Discussion

Given the critical role that mutations play in carcinogenesis, an attractive model is that the high error frequency of TLS across CPD in XPV patients is an important cause in their extreme cancer predisposition. The results presented in this study suggest that such a mutagenic bypass involves no less than 3 TLS polymerases, which are needed to back-up the absence of *polη*. The epistasis siRNA analysis presented above suggests that *polζ* has a critical role in TLS across a TT CPD, and that it cooperates with *polκ* and *polι*, which can back-up each other. This suggests that in XPV cells CPD are bypassed via 2-polymerase mechanisms. Based on the ability of purified *polι* to insert nucleotides opposite a TT CPD (25), and the activity of the *S. cerevisiae* *polζ*

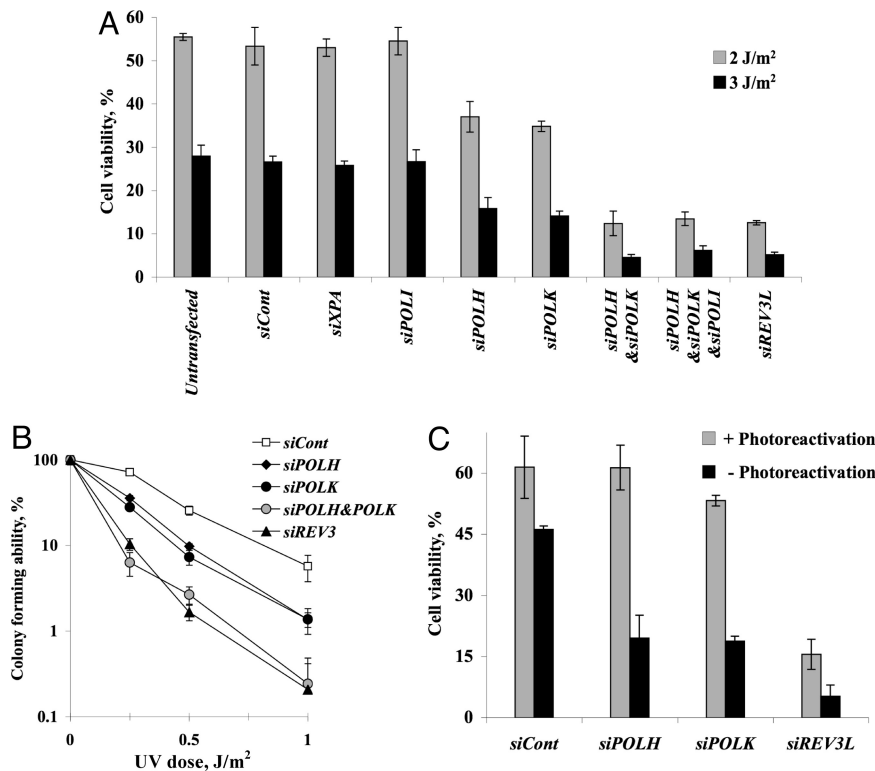


Fig. 3. UV sensitivity of XPA cells pretreated with siRNA against TLS polymerases. Cells were transfected with siRNA, and UV irradiated after 48 h at the indicated doses. Viability was determined 48 h after UV irradiation by measuring cellular ATP (A) or 12 days after UV irradiation by measuring colony forming ability (B). (C) XPA cells stably expressing CPD photolyase were UV irradiated at 3 J m^{-2} and immediately illuminated with visible light to photoreactivate CPD lesions. Cell viability was determined as in A. See *Materials and Methods* for details.

as a general mismatch extender (26), a plausible model is that pol ι or pol κ perform insertion opposite the CPD, and pol ζ performs the extension (Fig. 4). Although purified human pol κ was reported to have extender properties similar to those of the

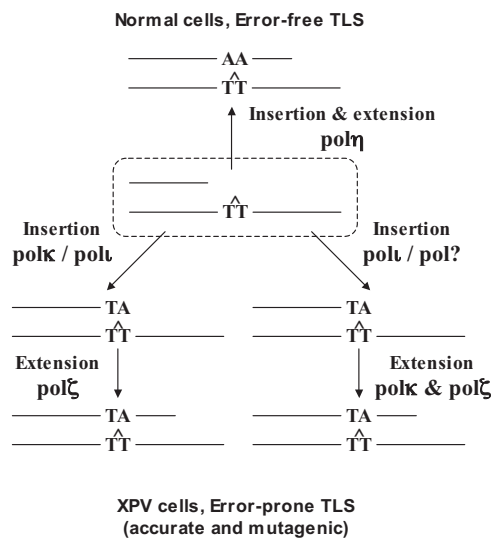


Fig. 4. Model describing TLS across CPD in human cells. In human normal cells pol η carries out efficient and relatively accurate TLS across CPD. In human XPV cells, TLS across CPD is performed by a back-up system in 2-polymerase reactions, in which pol κ or pol ι perform insertion opposite the CPD, whereas pol ζ performs the extension step. This pathway is less efficient and more mutagenic than the pol η -dependent pathway. A possible 3-polymerase mechanism is also presented. See text for details.

yeast pol ζ (27), our *in vivo* results indicate that it was unable to effectively bypass a CPD without the help of pol ζ . More complex mechanisms are also possible. For example, pol ι and perhaps another polymerase may each perform the insertion step, whereas extension, which is generally more difficult than mis-insertion, may require both pol κ and pol ζ , which is consistent with their extender properties (Fig. 4). Alternatively, insertion opposite the first and the second pyrimidines of a CPD may involve different polymerases, including perhaps pol ζ . These last 2 possibilities reflect 3-polymerase mechanisms of TLS.

Recently, we showed that TLS across 2 additional DNA lesions in human cells involves 2-polymerase mechanisms: cisplatin-GG, a major intrastrand adduct formed in DNA by the chemotherapeutic drug cisplatin, and benzo[a]pyrene-G, a major adduct formed by tobacco smoke. TLS across these adducts involves pol η or pol κ cooperating with pol ζ in combinations that determine error-prone or error-free bypass (17). Taken together with the results presented here, it appears that 2-polymerase mechanisms comprise an important TLS strategy, supporting the original model proposed by the Prakash group (26). The important role of pol ζ in this process is consistent with the situation in *S. cerevisiae* (28), and with its essential role during mouse embryonic development (5, 17). It is also consistent with the increased UV sensitivity of normal, XPV and XPA cells in which the expression of REV3L was knocked-down, as shown in this study, and in other cell types in previous studies (15, 29–31). There are also earlier studies, which reported no effect of REV3L on UV sensitivity; this discrepancy might be due to differences in cell lines and methodologies (e.g., antisense RNA versus siRNA) (32–34).

Knocking-down the expression of POLI (confirmed at the mRNA and protein levels; Fig. S1) had no effect on UV sensitivity, which is different from several (but not all) previous

reports (13–15, 22). As far as mutagenesis is concerned, we saw essentially no effect on mutagenic TLS across a TT CPD when *POLI* was knocked-down. Previous studies reported conflicting results of either decreased, altered or unchanged UV mutagenesis in cells deficient in pol ι (13–15, 35). Like in the case of pol ζ , this may reflect differences in methodologies or cell types.

The involvement of pol κ in TLS across CPD was somewhat surprising, because purified pol κ was reported to be blocked by TT CPD (21). Most likely accessory factors present in the cell assist pol κ in carrying out insertion opposite CPD. Such situations are not uncommon, e.g., the inability of the purified *E. coli* polV to carry out TLS in vitro in the absence of RecA (36, 37). Importantly, our data on the involvement of pol κ in TLS may explain, at least in part, the puzzling UV sensitivity exhibited by mouse embryonic fibroblasts lacking pol κ (38).

The study in ref. 23 reports that pol κ functions in NER of UV lesions in human cells. Clearly the protective effects of pol κ against UV cytotoxicity described above do not occur via NER, because it was observed also in NER-deficient human *XPA* cells. Thus, although our results do not exclude the possibility that pol κ may function in NER under certain conditions, it is clear that its major protective effect against UV cytotoxicity is mediated via tolerance of DNA damage rather than NER, consistent with the role of pol κ in TLS across TT CPD as described above.

The extents of mutagenic TLS decreased 4-fold when expression of *POLK* and *POLI* combined was knocked-down, and 7-fold when *REV3L* was knocked-down, indicating their involvement in mutagenic TLS (Fig. 1C; Table S3). Interestingly, the mutagenic signature under the various conditions was similar (Table S2). This may be dictated by the structure of the CPD in DNA, which maintains the base pairing region of the pyrimidines, and causes only a minor distortion in DNA (39, 40). Alternatively, because TLS under these conditions is very low (Fig. 2), it may reflect residual TLS by the knocked-down polymerases.

Although in the TLS gapped plasmid assay pol κ and pol ι backed-up each other in TLS across a TT CPD, pol κ , but not pol ι , was important for protecting the cells against UV cytotoxicity. In considering this difference it should be noted that, although the gapped plasmid assay specifically measures TLS, the cell UV sensitivity assay involves both DNA damage tolerance, and cell death. It is thus possible that pol κ but not pol ι can act at sites of potentially lethal CPD, such as in overlapping postreplication daughter gaps, or near double-strand breaks (41, 42), where a deficiency in tolerance may cause cell death. In addition, pol κ may be involved in DNA damage tolerance via homology-dependent repair (homologous recombination or template switch recombination), mechanisms known to operate in *E. coli* and *S. cerevisiae*, but poorly understood in mammalian cells (1). In this context, it is noteworthy that pol η was reported to be involved also in homologous recombination (43, 44), and

that pol κ from *T. cruzi* can perform DNA synthesis in a recombination intermediate (42).

The UV hypermutability of cells from *XPV* patients is believed to play a major role in their extremely high predisposition to sunlight-induced skin cancer (1). The results presented here show that backup TLS DNA polymerases protect *XPV* cells against UV cytotoxicity, but at the cost of increased mutagenesis due to error-prone TLS across CPD, representing an extreme example of benefit-risk balance in responses to DNA damage in human cells.

Materials and Methods

Cell Cultures. SV40-transformed normal (MRC5), *XPA* (XP12RO), and *XPV* (XP30RO) human fibroblasts were gifts from A. R. Lehmann (University of Sussex, Brighton, U.K.). Cells were cultured in MEM Eagle (Sigma) supplemented with 2 mM l-glutamine (GIBCO/BRL), 100 units/mL of penicillin, 100 μ g/mL of streptomycin (Biological Industries), and 15% FBS (HyClone). SV40-transformed *XPA* human fibroblasts (XP12RO) stably expressing a CPD photolyase gene (*CPDphr*) derived from the rat kangaroo *P. tridactylis* are described in ref. 24. Cells were cultured in DMEM (GIBCO/BRL) supplemented with 100 units/mL of penicillin, 100 μ g/mL of streptomycin, and 10% FBS. The cells were incubated at 37 °C in a 5% CO₂ atmosphere, and periodically examined for Mycoplasma contaminations by EZ-PCR test kit (Biological Industries).

Knocking Down the Expression of TLS DNA Polymerase Genes. The expression of specific DNA polymerase genes in normal, *XPV*, or *XPA* cells was knocked-down by transfection with polymerase-specific siRNAs. The extent of knock-down was estimated by RT-PCR using polymerase-specific probes, and by immunoblot analysis for pol η and pol ι , for which good antibodies are available. The experimental details are described in *SI Materials and Methods*.

Construction of Gapped DNA Substrates. DNA oligonucleotides without a lesion were supplied by Sigma-Genosys. The construction of the control gapped plasmid is described in ref. 45. A gapped plasmid with a site-specific TT CPD in the sequence context: 5'-GCAAGTTGGAG-3' (the CPD is underlined) was constructed as described in ref. 19.

TLS Assay in Cultured Human Cells. The TLS assay is described in refs. 8, 16, and 19. The details of the experiments performed in the current study are presented in *SI Materials and Methods*.

UV Sensitivity and Photoreactivation Assays. Viability of UV-irradiated cells was determined using the CellTiter-Glo luminescent cell viability assay (Promega), measuring the amount of cellular ATP present, which signals the presence of metabolically active cells. Cell viability was determined also by incorporation of 5-bromo-2-deoxyuridine (BrdU), and by colony forming ability. The protocols for these assays, and the photoreactivation of *XPA* cells stably expressing CPD photolyase, are described in *SI Materials and Methods*.

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- Friedberg EC, et al. (2006) DNA Repair and Mutagenesis (ASM Press, Washington DC), 2nd edition.
- Livneh Z (2001) DNA damage control by novel DNA polymerases: Translesion replication and mutagenesis. *J Biol Chem* 276:25639–25642.
- Prakash S, Johnson RE, Prakash L (2005) Eukaryotic translesion synthesis DNA polymerases: Specificity of structure and function. *Annu Rev Biochem* 74:317–353.
- Yang W, Woodgate R (2007) What a difference a decade makes: Insights into translesion synthesis. *Proc Natl Acad Sci USA* 104:15591–15598.
- Gan GN, Wittschieben JP, Wittschieben BO, Wood RD (2008) DNA polymerase ζ (pol ζ) in higher eukaryotes. *Cell Research* 18:174–183.
- Hoegge C, Pfander B, Moldovan GL, Pyrowolkakis G, Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419:135–141.
- 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.
- Avkin S, et al. (2006) p53 and p21 regulate error-prone DNA repair to yield a lower mutation load. *Mol Cell* 22:407–413.
- Soria G, Podhajcer O, Gottifredi V (2006) P21Cip1/WAF1 downregulation is required for efficient PCNA ubiquitination after UV irradiation. *Oncogene* 25:2829–2838.
- Masutani C, et al. (1999) The *XPV* (xeroderma pigmentosum variant) gene encodes human DNA polymerase η . *Nature* 399:700–704.
- Johnson RE, Kondratik CM, Prakash S, Prakash L (1999) hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* 285:263–265.
- Maher VM, Ouellette LM, Curren RD, McCormick JJ (1976) Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells. *Nature* 261:593–595.
- Dumstorf CA, et al. (2006) Participation of mouse DNA polymerase ι in strand-biased mutagenic bypass of UV photoproducts and suppression of skin cancer. *Proc Natl Acad Sci USA* 103:18083–18088.
- Wang Y, et al. (2007) Evidence that in xeroderma pigmentosum variant cells, which lack DNA polymerase η , DNA polymerase ι causes the very high frequency and unique spectrum of UV-induced mutations. *Cancer Res* 67:3018–3026.
- Gueranger Q, et al. (2008) Role of human DNA polymerases η , ι , and ζ in UV resistance and UV-induced mutagenesis. *DNA Repair* 7:1551–1562.

16. Avkin S, et al. (2004) Quantitative analysis of translesion DNA synthesis across a benzo[a]pyrene-guanine adduct in mammalian cells. The Role of DNA polymerase κ . *J Biol Chem* 279:53298–53305.
17. Shachar S, et al. (2009) Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. *EMBO J* 28:383–393.
18. Lehmann AR, Fuchs RP (2006) Gaps and forks in DNA Replication: Rediscovering old models. *DNA Repair* 5:1595–1598.
19. Hendel A, Ziv O, Gueranger Q, Geacintov N, Livneh Z (2008) Reduced fidelity and increased efficiency of translesion DNA synthesis across a TT cyclobutane pyrimidine dimer, but not a TT 6–4 photoproduct, in human cells lacking DNA polymerase η . *DNA Repair* 7:1636–1646.
20. Johnson RE, Prakash S, Prakash L (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol η . *Science* 283:1001–1004.
21. Ohashi E, et al. (2000) Error-prone bypass of certain DNA lesions by the human DNA polymerase κ . *Genes Dev* 14:1589–1594.
22. Petta TB, et al. (2008) Human DNA polymerase ι protects cells against oxidative stress. *EMBO J* 27:2883–2895.
23. Ogi T, Lehmann AR (2006) The Y-family DNA polymerase κ (pol κ) functions in mammalian nucleotide-excision repair. *Nat Cell Biol* 8:640–642.
24. 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.
25. Vaisman A, et al. (2003) Sequence context-dependent replication of DNA templates containing UV-induced lesions by human DNA polymerase ι . *DNA Repair* 2:991–1006.
26. Johnson RE, Washington MT, Haracska L, Prakash S, Prakash L (2000) Eukaryotic polymerases ι and ζ act sequentially to bypass DNA lesions. *Nature* 406:1015–1019.
27. Washington MT, Johnson RE, Prakash L, Prakash S (2002) Human *DINB1*-encoded DNA polymerase κ is a promiscuous extender of mispaired primer termini. *Proc Natl Acad Sci USA* 99:1910–1914.
28. Gibbs PE, McDonald J, Woodgate R, Lawrence CW (2005) The relative roles in vivo of *Saccharomyces cerevisiae* Pol η , Pol ζ , Rev1 protein and Pol32 in the bypass and mutation induction of an abasic site, T-T (6–4) photoadduct and T-T *cis-syn* cyclobutyl dimer. *Genetics* 169:575–582.
29. Wittschieben JP, Reshmi SC, Gollin SM, Wood RD (2006) Loss of DNA polymerase zeta causes chromosomal instability in mammalian cells. *Cancer Res* 66:134–142.
30. Zander L, Bemark M (2004) Immortalized mouse cell lines that lack a functional Rev3 gene are hypersensitive to UV irradiation and cisplatin treatment. *DNA Repair (Amsterdam)* 3:743–752.
31. Sonoda E, et al. (2003) Multiple roles of Rev3, the catalytic subunit of pol ζ in maintaining genome stability in vertebrates. *EMBO J* 22:3188–3197.
32. Gibbs PE, McGregor WG, Maher VM, Nisson P, Lawrence CW (1998) A human homolog of the *Saccharomyces cerevisiae* *REV3* gene, which encodes the catalytic subunit of DNA polymerase zeta. *Proc Natl Acad Sci USA* 95:6876–6880.
33. Li Z, et al. (2002) hREV3 is essential for error-prone translesion synthesis past UV or benzopyrene diol epoxide-induced DNA lesions in human fibroblasts. *Mutat Res* 510:71–80.
34. Diaz M, et al. (2003) Decreased Frequency and Highly Aberrant Spectrum of Ultraviolet-Induced Mutations in the *hprt* Gene of Mouse Fibroblasts Expressing Antisense RNA to DNA Polymerase ζ . *Mol Cancer Res* 1:836–847.
35. Choi JH, Besaratinia A, Lee DH, Leeb CS, Pfeifer GP (2006) The role of DNA polymerase ι in UV mutational spectra. *Mutat Res* 599:58–65.
36. Tang M, et al. (1999) UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc Natl Acad Sci USA* 96:8919–8924.
37. Reuven NB, Arad G, Maor-Shoshani A, Livneh Z (1999) The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA and SSB, and specialized for translesion replication. *J Biol Chem* 274:31763–31766.
38. Ogi T, Shinkai Y, Tanaka K, Ohmori H (2002) Pol κ protects mammalian cells against the lethal and mutagenic effects of benzopyrene. *Proc Natl Acad Sci USA* 99:15548–15553.
39. Ling H, Boudsocq F, Plosky BS, Woodgate R, Yang W (2003) Replication of a *cis-syn* thymine dimer at atomic resolution. *Nature* 424:1083–1087.
40. Li Y, et al. (2004) Nucleotide insertion opposite a *cis-syn* thymine dimer by a replicative DNA polymerase from bacteriophage T7. *Nat Struct Mol Biol* 11:784–790.
41. Garinis GA, et al. (2005) Transcriptome analysis reveals cyclobutane pyrimidine dimers as a major source of UV-induced DNA breaks. *EMBO J* 24:3952–3962.
42. Rajao MA, et al. (2008) DNA polymerase κ from *Trypanosoma cruzi* localizes to the mitochondria, bypasses 8-oxoguanine, and performs DNA synthesis in a recombination intermediate. *Mol Microbiol* In press.
43. Kawamoto T, et al. (2005) Dual roles for DNA polymerase η in homologous DNA recombination and translesion DNA synthesis. *Mol Cell* 20:793–799.
44. McIlwraith MJ, et al. (2005) Human DNA polymerase η promotes DNA synthesis from strand invasion intermediates of homologous recombination. *Mol Cell* 20:783–792.
45. Tomer G, Livneh Z (1999) Analysis of unassisted translesion replication by the DNA polymerase III holoenzyme. *Biochemistry* 38:5948–5958.