

Mutations in human DNA polymerase η motif II alter bypass of DNA lesions

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Human DNA polymerase η (hPol η) is one of the newly identified Y-family of DNA polymerases. These polymerases synthesize past template lesions that are postulated to block replication fork progression. hPol η accurately bypasses UV-associated *cis-syn* cyclobutane thymine dimers *in vitro* and contributes to normal resistance to sunlight-induced skin cancer. We describe here mutational analysis of motif II, a highly conserved sequence, recently reported to reside in the fingers domain and to form part of the active site in Y-family DNA polymerases. We used a yeast-based complementation system to isolate biologically active mutants created by random sequence mutagenesis, synthesized the mutant proteins *in vitro* and assessed their ability to bypass thymine dimers. The mutability of motif II in 210 active mutants has parallels with natural evolution and identifies Tyr52 and Ala54 as prime candidates for involvement in catalytic activity or bypass. We describe the ability of hPol η S62G, a mutant polymerase with enhanced activity, to bypass five other site-specific lesions. Our results may serve as a prototype for studying other members of the Y-family DNA polymerases.

Keywords: bypass polymerases/DNA replication/Pol η /Rad30A/Y-family

Introduction

DNA in cells is damaged by numerous endogenous and exogenous agents. The majority of altered nucleotides in DNA are removed by DNA repair processes that restore the original DNA sequence. However, some of the damage may escape repair and many of the unrepaired lesions block DNA synthesis by replicative DNA polymerases. As a result, the replication fork stalls and the cell must then employ additional mechanisms to complete replication in the presence of the unrepaired lesion (Lindahl and Wood, 1999).

In the last two years, a new family of DNA polymerases has been identified and are referred to as the Y-family, translesion synthesis (TLS) or bypass DNA polymerases (Bridges, 1999; Johnson *et al.*, 1999c; Goodman and Tippin, 2000; Livneh, 2001; McDonald *et al.*, 2001; Ohmori *et al.*, 2001; Wang, 2001). These polymerases traverse blocking lesions in template DNA *in vitro* and it is

hypothesized that they function *in vivo* to facilitate replication across damaged or missing bases. The Y-family of DNA polymerases includes *Escherichia coli* Pol IV (*dinB*; Wagner *et al.*, 1999) and Pol V (*umuC*; Reuven *et al.*, 1999; Tang *et al.*, 1999) that have been reported to be required for the major mechanism of damage-inducible mutagenesis. The yeast *Saccharomyces cerevisiae* contains two Y-family DNA polymerases: Rev1, a DNA-dependent dCMP transferase (Nelson *et al.*, 1996) and DNA polymerase η , encoded by the *RAD30* gene (McDonald *et al.*, 1997; Johnson *et al.*, 1999b). Homologs of these bypass polymerases have been identified in higher eukaryotes. The mammalian translesion polymerases are similar to those of *S.cerevisiae* but include at least two other bypass polymerases that are similar to polymerase η . These are DNA polymerase κ , encoded by *hDINB1* (Gerlach *et al.*, 2001) and DNA polymerase ι , encoded by *hRAD30B* (McDonald *et al.*, 2001).

Human DNA polymerase η (hPol η), encoded by *hRAD30A*, is inactive in the cancer-prone genetic disorder xeroderma pigmentosum variant syndrome (XPV; Johnson *et al.*, 1999a; Masutani *et al.*, 1999b). XPV patients are 1000-times more susceptible to sunlight-induced skin cancer than are normal individuals (Clever, 1999). In culture, XPV cells are hypersensitive to the mutagenic effects of UV irradiation (Maher *et al.*, 1976). Purified hPol η synthesizes past *cis-syn* thymine–thymine (T–T) dimers *in vitro* with efficiency comparable with that observed for undamaged DNA, inserting primarily complementary adenines opposite the lesions (Johnson *et al.*, 2000; Masutani *et al.*, 2000). The phenotype of XPV cells and patients can be explained by the diminished accuracy of T–T dimer bypass caused by lack of hPol η .

In addition to T–T dimers, hPol η has been shown to replicate across a wide spectrum of damage with efficiency and accuracy that depends on the lesion and sequence context (Haracska *et al.*, 2000a,b, 2001; Masutani *et al.*, 2000; Vaisman *et al.*, 2000; Zhang *et al.*, 2000; Levine *et al.*, 2001; Yu *et al.*, 2001). The enzyme preferentially inserts complementary nucleotides opposite some of these lesions and non-complementary nucleotides opposite others. hPol η , like the other translesion synthesis polymerases, exhibits exceptionally low fidelity when replicating undamaged DNA (Matsuda *et al.*, 2000). The low fidelity of these enzymes suggested they might have functions in addition to bypass of DNA damage. Recently, evidence has been presented that shows hPol η and other translesion synthesis polymerases are involved in somatic hypermutation of the immunoglobulin variable genes (Rogozin *et al.*, 2001; Zeng *et al.*, 2001).

The primary sequences of Y-family polymerases bear no discernible homology to those of other DNA polymerases. However, the recently solved crystal structures

reveal a modified right hand-shaped enzyme, similar to the structures of the other DNA polymerases (Ling *et al.*, 2001; Trincão *et al.*, 2001; Zhou *et al.*, 2001). The polymerase domain of hPol η resides in the 513 N-terminal amino acids of the 713 amino acid polypeptide (Masutani *et al.*, 1999a; Trincão *et al.*, 2001), while the 70 C-terminal residues are required for nuclear localization (Kannouche *et al.*, 2001; Kondratick *et al.*, 2001). The N-terminal region of hPol η shares extensive sequence homology with the N-terminal portion of other related translesion polymerases, including five conserved motifs designated I–V (Kulaeva *et al.*, 1996; Johnson *et al.*, 1999c) or A, B, D, E and F (Masutani *et al.*, 1999b).

We present here mutational analysis of the conserved motif II/region B, revealed in the three-dimensional structures of Y-family DNA polymerases to reside in the fingers domain and to form part of the active site. We chose to substitute random nucleotides for all of the codons corresponding to 22 amino acids within motif II. We utilized a yeast-based functional complementation system to isolate active mutants and, by DNA sequencing of >200 such mutants, established a pattern of amino acid substitution that has parallels with evolutionary conservation and identifies residues that are likely to be involved in catalysis. We synthesized 95 of the active mutant proteins *in vitro* and, by using a primer extension assay, found accord between *in vivo* selection in our complementation system and the ability of the polymerase variants to bypass T–T dimers *in vitro*. One of the mutants, hPol η S62G, was found to exhibit higher activity than wild-type hPol η on undamaged DNA and on templates containing diverse lesions. Because motif II is highly conserved, our findings have implications for other bypass polymerases across the evolutionary spectrum.

Results

hPol η complements the UV sensitivity of S.cerevisiae rad30rad52

As a first step toward isolating and characterizing active hPol η mutants, we established *in vivo* and *in vitro* systems for detecting the function of plasmid-encoded enzymes. To select for mutants that are active *in vivo*, we used a complementation assay based on the UV sensitivity of the *S.cerevisiae rad30rad52* double mutant. Deletion of the yeast hPol η homolog Rad30p and the recombination enzyme Rad52p greatly reduces UV survival (McDonald *et al.*, 1997). As shown in Figure 1A, expression of wild-type hPol η in *rad30rad52* cells enhances UV resistance as much as 100-fold at high doses, compensating for loss of endogenous Pol η and increasing survival to that observed for the *rad52* single mutant. This enhancement in survival provided the basis for selecting active mutants from plasmid libraries harboring hPol η variants.

To assess the ability of the plasmid-encoded polymerase to bypass DNA lesions, we produced the enzyme *in vitro* and measured DNA synthesis on oligonucleotide template-primers containing site-specific adducts. A plasmid carrying the wild-type hPol η cDNA, pYEX-hPol η , served as a template for PCR amplification with an upstream primer containing the bacteriophage T7 promoter sequence. The amplified hPol η cDNA, under control of the T7 promoter, was thereafter used as a template for

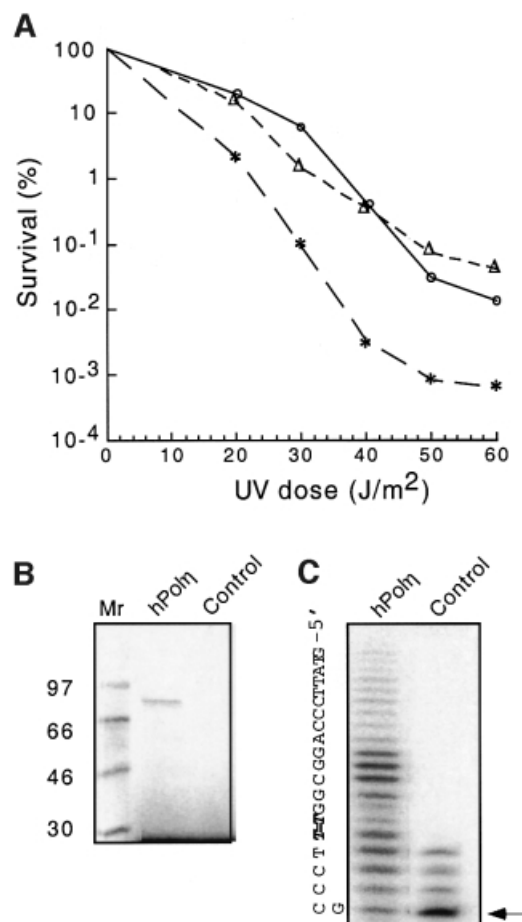


Fig. 1. Plasmid-encoded wild-type human DNA polymerase η confers UV resistance and catalyzes bypass of *cis-syn* cyclobutane thymine dimers. (A) hPol η enhances UV resistance of the *S.cerevisiae rad30rad52* double mutant that lacks both host Pol η (Rad30p) and (Rad52p). Expression of hPol η in *rad30rad52* cells increases survival to that observed with *rad52* cells. *, *rad30rad52*; O, *rad52*; Δ , *rad30rad52* transformed with pYEX-hPol η . (B) SDS-polyacrylamide gel analysis of the [³⁵S]methionine-labeled products produced by *in vitro* transcription and translation with either pYEX-hPol η or pYEX-4T (control). Molecular weight markers are shown in the left lane. (C) *In vitro* bypass of a site-specific *cis-syn* T–T dimer in a primer extension assay. The template lesion (indicated by T=T) was four nucleotides downstream of the 3' primer terminus (indicated by an arrow).

in vitro transcription and translation in the presence of ³⁵S-labeled methionine. As shown in Figure 1B, SDS gel electrophoresis of the ³⁵S-labeled translation product revealed a single major band migrating as expected for hPol η (78 kDa), whereas no labeled products were observed in reactions containing the control plasmid. The ability of *in vitro* synthesized hPol η to catalyze DNA polymerization and translesion synthesis was confirmed in primer extension assays. As shown in Figure 1C, the enzyme incorporated nucleotides onto a template-primer containing a *cis-syn* cyclobutane T–T dimer, a major lesion produced by UV damage to DNA. Extension opposite and past the dimer increased with both time of incubation and enzyme concentration (data not shown). The synthesis seen in the control reaction appears to be catalyzed by DNA polymerase activity intrinsic to the reticulocyte lysate used for transcription and translation.

The extent of synthesis by the control was uniform among different lysates and in no case was significant bypass observed.

Characterization of translesion synthesis by *in vitro* synthesized and by purified hPol η

Wild-type hPol η has been shown to bypass a variety of DNA lesions, although differing DNA sequence contexts often complicate assessment of the relative efficiencies of bypass of different lesions. Prior to mutant characterization, we established the relative efficiencies of bypass of five adducts by *in vitro* synthesized wild-type hPol η , using a single common nucleotide sequence context containing different lesions at the same site. The enzyme bypassed 8-oxo-guanine (8-oxo-G), the major oxygen free radical-induced adduct, with an efficiency (80%) approaching that observed for the template having no lesion (Figure 2A). In our particular sequence context, hPol η bypassed the methylating agent-induced lesions O^4 -methylthymine

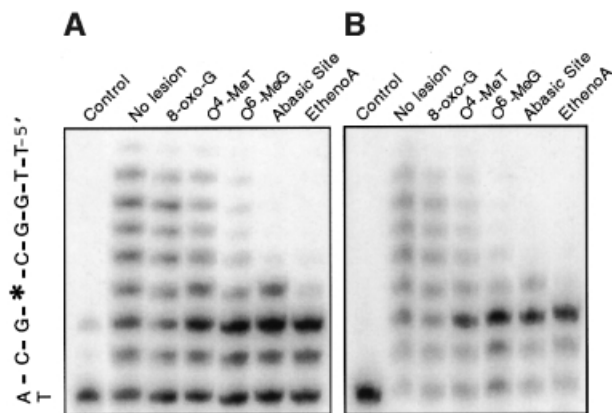


Fig. 2. Translesion synthesis by *in vitro* synthesized and purified hPol η on templates containing site-specific lesions in the same sequence context. Bypass across six different lesions within the same template sequence was measured in primer extension assays containing hPol η synthesized *in vitro* (A) or hPol η polymerase domain purified from *E. coli* (B). The template contained either no lesion, 8-oxo-G, O^4 -MeT, O^6 -MeG, an abasic site or ethenoA. The sequence of the single-stranded template is indicated with an asterisk marking the position of the lesion. Controls, both without expression vector, were reticulocyte lysate in (A) and purified cell extract in (B).

(O^4 -MeT) and O^6 -methylguanine (O^6 -MeG) with lower efficiencies i.e. 55 and 20%, respectively, relative to the unmodified template. With an abasic site, hPol η incorporated a single nucleotide opposite the lesion, but failed to significantly extend the incorporated nucleotide. No significant incorporation was observed opposite 1, N^6 -ethenoadenine (ethenoA), a lesion associated with DNA damage by a metabolite(s) of the human carcinogen vinyl chloride. To demonstrate that this pattern of bypass is intrinsic to hPol η itself, we expressed the polymerase domain of the enzyme in *E. coli* (Masutani *et al.*, 1999b; Trincao *et al.*, 2001) and measured the activity of the purified protein on the same template primers. As shown in Figure 2B, bypass by the purified enzyme was very similar, if not identical, to that exhibited by the *in vitro* synthesized polymerase, indicating that the observed pattern of translesion synthesis is characteristic of hPol η . As reported by others for different sequence contexts (Masutani *et al.*, 1999b; Johnson *et al.*, 2000), we observed that hPol η copied a template containing a T-T dimer with efficiency comparable with that found for the same template without the lesion (Figure 1C and data not shown).

Creation of a library of hPol η motif II mutants

Motif II is highly conserved in natural evolution within the translesion synthesis DNA polymerases, suggesting that it may be important for the biological functions of these enzymes. Figure 3 shows the sequence of 22 amino acids in motif II, 11 of which are invariant, or nearly so, among Pol η s across the evolutionary spectrum and among the Y-family DNA polymerases. As an initial approach to ascertaining the mutability of these motif II residues in hPol η and their roles in nucleotide incorporation and lesion bypass, we created a library of mutants containing amino acid replacements at all 22 positions. We substituted an oligonucleotide containing 7.5% random substitutions at each nucleotide encoding the amino acids Tyr52 through Pro73. The mutant library was calculated to contain five nucleotide substitutions per clone (Suzuki *et al.*, 1996) and to encode approximately four amino acid substitutions per clone. The pYEX-hPol η -LibB plasmids were transformed into *E. coli* to create the hPol η motif II random library encoding $\sim 5 \times 10^5$ mutants.

hPol η	52	Y	E	A	R	A	F	G	V	T	R	S	M	W	A	D	D	A	K	K	L	C	P
mPol η	52	Y	E	A	R	A	F	G	V	T	R	N	M	W	A	D	D	A	K	K	L	C	P
dPol η	61	Y	A	A	R	A	K	G	V	T	R	H	M	R	G	O	E	A	K	D	L	C	P
cePol η	49	Y	E	A	R	P	F	G	V	K	R	G	M	T	V	A	E	A	K	L	K	C	P
atPol η	57	Y	E	A	R	K	C	G	V	K	R	S	M	R	G	D	E	A	K	A	A	C	P
spPol η	67	Y	A	A	R	A	A	N	I	S	R	H	E	T	V	T	E	A	K	K	K	C	P
scPol η	64	Y	A	A	R	K	Y	G	I	S	R	M	D	T	I	Q	E	A	L	K	K	C	S
hPol ϵ	68	Y	E	A	R	K	L	G	V	K	K	L	M	N	V	R	D	A	K	E	K	C	P
hREV1	512	Y	E	A	R	Q	L	G	I	K	N	G	M	F	G	H	A	K	Q	L	C	P	
hPol κ	141	Y	H	A	R	R	F	G	V	R	A	A	M	P	G	F	I	A	K	R	L	C	P
Consensus		Y	E/A	A	R			G	V/I		R		M				D/E	A	K		K/L	C	P
hPol η aa		52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73

Fig. 3. Alignment of motif II primary sequences. Amino acid sequence homology of motif II in human Pol η , Pol η from other species and related human DNA polymerases, were identified using the Blast search engine (NCBI). Conserved residues are indicated in bold type in shaded boxes. The sequences displayed are hPol η , mPol η (mouse), dPol η (*Drosophila melanogaster*), cePol η (*Caenorhabditis elegans*), atPol η (*Arabidopsis thaliana*), spPol η (*Schizosaccharomyces pombe*), scPol η (*S. cerevisiae*), hPol ϵ (human), hREV1 (human) and hPol κ (human). The numbers on the left indicate the position of the N-terminal motif II residue in the protein.

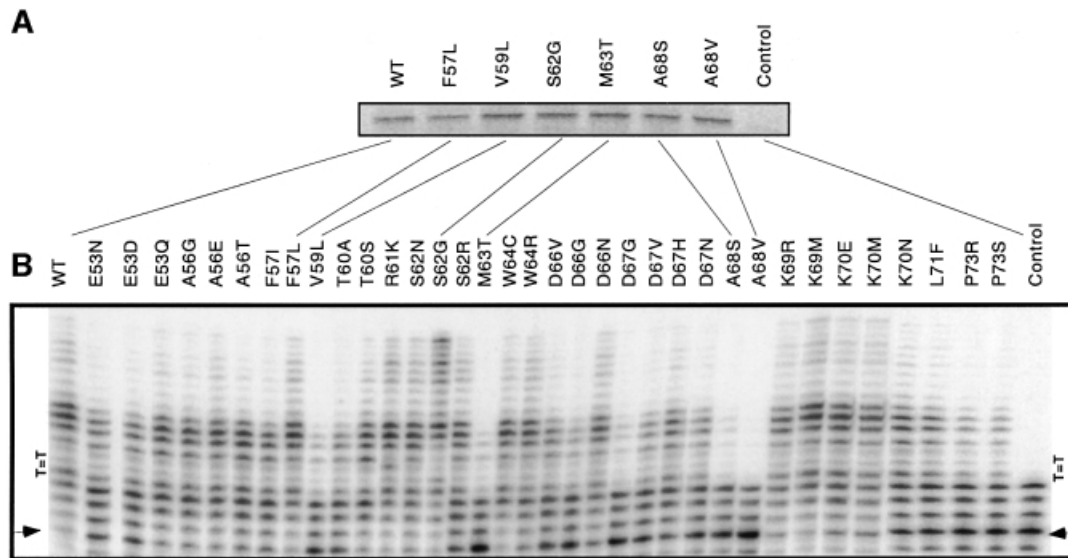


Fig. 6. Bypass of thymine dimers by hPol η motif II single mutants. (A) Mutant hPol η proteins harboring single amino acid substitutions were synthesized in a high-throughput transcription and translation system in the presence of [35 S]methionine. Amino acid substitutions in the mutants are indicated along with their position in the protein (e.g. E53A indicates a Glu to Ala substitution at position 53). (B) Bypass of a T–T dimer by the *in vitro* synthesized mutants was measured in primer extension assays. The position of the lesion (T=T) was four nucleotides downstream from the 3' primer terminus (arrow), as indicated here and in the template sequence in Figure 1.

in 31 clones in the active mutant library, 80% of which contained silent mutations at the nucleotide level.

Amino acid substitutions in selected, active motif II mutants

The predicted amino acid substitutions in 32 of the unselected mutants appeared to be located at random across motif II residues (Figure 5D). In contrast, amino acid substitutions in the 210 selected active hPol η mutants showed a specific distribution (Figures 5A–C). We found that, despite its evolutionary conservation (Figure 3), the primary sequence of motif II is highly plastic, tolerating replacements at all but two of the 22 positions. At some positions, as many as seven different amino acids could replace the wild-type residue (e.g. Asp66). Remarkably, some of the mutants contained as many as four amino acid substitutions (e.g. V59I, S62G, L71F and C72Y) and yet retained activity comparable with that of wild-type hPol η (data not shown).

No amino acid replacements were found at Tyr52 and Ala54. These two amino acids are strictly conserved in natural evolution (Figure 3) and, together with the lack of replacements in our active mutants, we infer from this preservation that both of these residues may be critical for UV resistance. In addition to Tyr52 and Ala54, four residues were not replaced among the single mutants (Figure 5A). Of these four, Arg55, Gly58 and Cys72 are highly conserved in natural evolution (Figure 3). Only one replacement was observed for each of these amino acids (i.e. R55L, G58R and C72Y) among multiple-substituted mutants (Figures 5A–C). We infer from the lack of single mutants at Arg55, Gly58 and Cys72 and the paucity of replacements in multiply substituted variants that the naturally occurring side chains at these positions may also be important in conferring UV survival. Compensation for

loss of such wild-type residues mediated by co-selected replacements is possible in multiple-substituted variants.

***In vitro* synthesis of hPol η mutant proteins and bypass of cis-syn thymine dimers**

To examine DNA polymerization and translesion synthesis by the selected active hPol η mutants, we established a system for high-throughput *in vitro* transcription and translation in a 96-well format. Plasmids encoding 95 mutants served as a template for PCR amplification. The amplified cDNAs were then used for coupled *in vitro* transcription and translation in the presence of [35 S]methionine. For each mutant, a single major band with intensity comparable with that of the wild-type enzyme was observed in SDS gels (Figure 6A and data not shown). The equivalent amounts of protein synthesized allowed us to compare relative levels of catalytic activity and bypass frequency within the 95 mutants.

Wild-type hPol η efficiently copies past T–T dimers (Figure 1A; Masutani *et al.*, 1999b; Johnson *et al.*, 2000). We used the *in vitro* synthesized hPol η proteins in a primer extension assay to examine the ability of mutant enzymes to copy an oligonucleotide template containing a site-specific T–T dimer, as illustrated for the wild-type enzyme in Figure 1C. Most of the mutants that were able to rescue UV sensitivity in our complementation system bypassed the dimer to an extent of at least 20% of that observed for wild-type hPol η (Figure 6B). A few of the mutants, including A68S and A68V, although able to complement the UV sensitivity of *rad30rad52* host cells, showed a severe reduction in bypass. Importantly, the control vector pYEX-hPol η dum (rightmost lane in Figure 6B) failed to support any detectable bypass. Thus, there is a concordance between the ability of the hPol η mutants to confer UV resistance *in vivo* and to bypass thymine dimers *in vitro* and we therefore infer that our

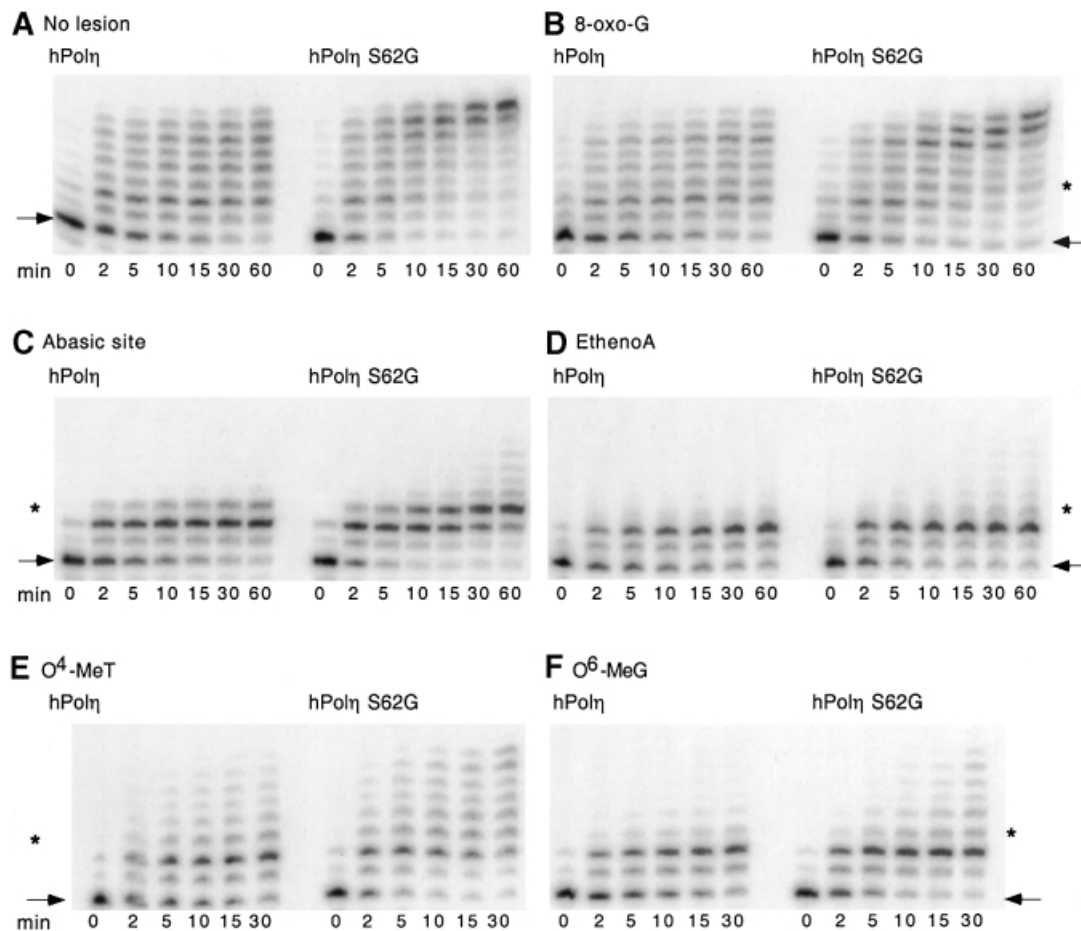


Fig. 7. Translesion synthesis by *in vitro* synthesized wild-type and S62G hPol η proteins on an unmodified template and templates containing site-specific lesions. Bypass of five lesions in the same DNA sequence context was compared in primer extension assays. The template contained either no lesion, 8-oxo-G, O^4 -MeT, O^6 -MeG, an abasic site or ethenoA. The position of the lesion (*) was three nucleotides downstream of the 3' primer terminus (arrow). The assays, containing 2 μ l of a 1:20 dilution of *in vitro* synthesized wild-type or mutant hPol η , were terminated at different times, as indicated, by addition of formamide. The elongated products observed at time 0 probably reflect limited incorporation at 4°C.

complementation assay selects for mutants that can catalyze translesion synthesis. Moreover, we observed a tight correlation between the efficiency in bypassing T–T dimers (Figure 6) and polymerization activity on unmodified DNA (data not shown).

hPol η S62G is a more active polymerase than wild-type hPol η

In preliminary studies with undamaged templates, we found that several hPol η mutant proteins catalyzed nucleotide incorporation at rates similar to or greater than that of the wild-type enzyme. One of these mutants contained a single amino acid substitution, Ser62 to Gly. Six different multiple-substituted mutants that contained S62G along with other replacements also showed relatively high activity (Figures 5A–C and data not shown).

To reconfirm that the enhanced synthesis observed for S62G hPol η represents an authentic increase in catalytic activity, we compared the initial rates of nucleotide incorporation in assays containing equal amounts of 35 S-labeled enzyme [determined by trichloroacetic acid (TCA) precipitation of the respective lysates used for transcription-translation]. Importantly, both of the freshly prepared

lysates showed a single major 35 S-labeled band in SDS gels, as illustrated for different preparations in Figures 1 and 6. Based on a linear increase in incorporation on the unmodified template primer during the initial 5 min of reaction, we calculated that the specific activity of the S62G mutant is 3-times that of the wild-type enzyme (data not shown).

As shown in Figure 6, the S62G polymerase copied past T–T dimers to a greater extent than the wild-type enzyme. The ability of the S62G polymerase to copy unmodified DNA and DNA containing other site-specific lesions is illustrated in Figure 7. As shown in Figure 7A, the S62G polymerase accumulated longer copies of the unmodified template than the wild-type enzyme at every time point up to 1 h. The S62G mutant also exhibited more efficient bypass of lesions generated by oxygen damage (8-oxo-G, Figure 7B) and alkylating agents (O^4 -MeT and O^6 -MeG; Figure 7E and F); the mutant likewise displayed slightly enhanced synthesis opposite and past abasic sites (Figure 7C) and ethenoA adducts (Figure 7D), though bypass was still very inefficient. These results illustrate the importance of catalytic efficiency in bypassing different DNA lesions.

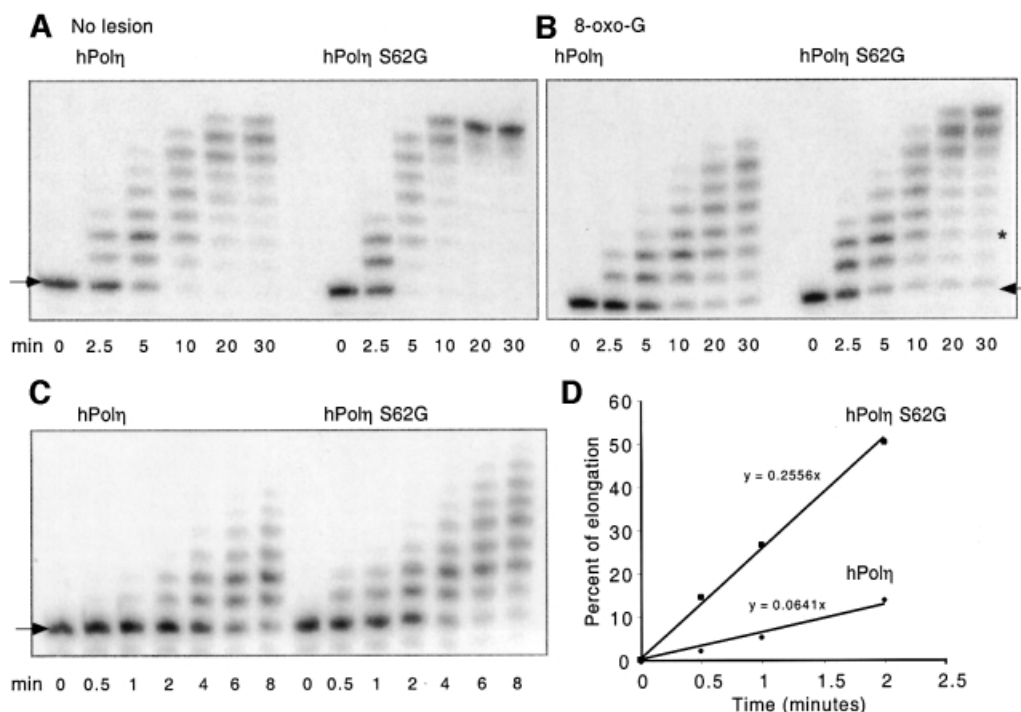


Fig. 8. Translesion synthesis by purified wild-type and S62G hPol η proteins on an unmodified template and a template containing 8-oxo-G. Primer extension and bypass of a site-specific 8-oxo-G lesion were analyzed in assays containing 0.1 pg/ μ l (2 nM) of purified wild-type or S62G polymerase domain. The time course of incorporation over 30 min is shown on (A) unmodified and (B) 8-oxo-G-containing template primers. The template lesion (*) was three nucleotides downstream of the 3' primer terminus (arrow). Initial rates of incorporation in assays containing a 2-fold higher concentration of undamaged template primer (C) were quantitated by phosphoimage analysis (D).

To confirm the enhanced activity and bypass ability of the S62G polymerase, we analyzed the mutant and wild-type polymerase domains purified from *E. coli*. As shown in Figure 8A and B, in the presence of equal amounts of the proteins, the S62G mutant exhibited greater activity than the wild-type enzyme on both the undamaged and 8-oxo-G-containing template primers. In an additional experiment (Figure 8C and D), we examined initial rates of incorporation in reactions containing an excess of unmodified template primer. Based on a linear increase in incorporation during the initial 2 min of reaction, we calculated that the specific activity of the S62G mutant is 4-times that of the wild-type enzyme. These results are very similar to those obtained with the *in vitro* synthesized enzymes (see above), corroborating the enhanced incorporation observed for the S62G polymerase. We conclude that the mutant S62G polymerase is a more catalytically active enzyme than wild-type hPol and that it has a higher capacity to bypass DNA lesions.

Discussion

Replication of nucleic acids is essential to the maintenance of living organisms. Until recently, five different families of DNA polymerases were known (Steitz, 1999). Although these polymerases have diverse amino acid sequences and diverse functions in DNA replication and repair, all appear to possess a similar overall architecture and a common catalytic mechanism (Hubscher *et al.*, 2000). The shared three-dimensional structure is conveniently compared to a human right hand, possessing 'thumb', 'palm' and 'finger' domains.

In the last two years, a new sixth family of DNA polymerases has been identified, the Y-family of TLS or bypass DNA polymerases (Bridges, 1999; Johnson *et al.*, 1999c; Goodman and Tippin, 2000; Livneh, 2001; McDonald *et al.*, 2001; Ohmori *et al.*, 2001; Wang, 2001). Members of this growing family of polymerases are capable of bypassing DNA lesions that are believed to block replication fork progression. Sequence alignment among members of the bypass DNA polymerases has revealed five conserved regions at the N-terminus of the proteins (Kulaeva *et al.*, 1996; Johnson *et al.*, 1999c; Masutani *et al.*, 1999b). Interestingly, there is no recognizable sequence homology with the polymerase domains common to other known DNA polymerases. Newly published crystal structures of Y-family DNA polymerases reveal an overall architecture similar to that of other DNA polymerases. The structures are familiar in resembling a human right hand, with 'thumb', 'palm' and 'finger' domains, although certain modifications appear to distinguish the Y-family from previously characterized DNA polymerases (Ling *et al.*, 2001; Trincao *et al.*, 2001; Zhou *et al.*, 2001).

In this work we chose to characterize human DNA polymerase η as a prototype for the other members of the Y-family DNA polymerase family. hPol η polymerizes across the UV-induced *cis-syn* T-T dimer with high efficiency and relatively high accuracy, inserting primarily adenines opposite the lesion. The ability to bypass these adducts and to incorporate the appropriate nucleotides is presumably the result of a flexible active site that allows polymerization across bulky helix-distorting lesions.

To gain insight into the function of specific amino acids in the activity of hPol η , we used random mutagenesis to study the conserved sequence designated motif II (Johnson *et al.*, 1999c). We had hypothesized, based on its conservation and its position between motifs I and III in the linear sequence, that motif II is directly involved in catalysis. The position of motif II in the 'fingers' domain in the three-dimensional structures of hPol η (Trincao *et al.*, 2001) and other Y family DNA polymerases (Ling *et al.*, 2001; Zhou *et al.*, 2001) corroborates this hypothesis.

Motif II contains the consensus sequence YxARxxGVx-RxxxxxDAKxxCP (Figure 3). DNA sequencing of hPol η mutants, which confer UV resistance and maintain polymerase activity, revealed that motif II tolerates a wide range of amino acid substitutions (Figure 5). However, no functional mutants were isolated that had replacements at the conserved residues Tyr52 or Ala54 (Figure 5). These amino acids are presumably critical for polymerase or bypass activity and are likely to have a direct function in catalysis by hPol η and other bypass polymerases; they reside in helix D in hPol η , which appears to be involved in interaction with the incoming dNTP (Trincao *et al.*, 2001). The essentiality of Tyr52 and Ala54 for activity and/or bypass, implied by the lack of replacements in this study, needs to be confirmed by saturation mutagenesis and re-selection.

Three other residues were also conserved, judging from the lack of single mutants at positions Arg55, Gly58 and Cys72. The involvement of these amino acids in catalysis and bypass is a significant possibility, since compensatory substitutions in multiple-substituted mutants can counteract the loss of critical wild-type residues (Figure 5A and B). Only conservative substitutions were observed at Val59, implying that hydrophobicity at this position may have functional significance. The substitutability of wild-type residues at these four positions can also be definitively addressed by saturation mutagenesis.

Examination of replication and translesion synthesis activity *in vitro* by 95 mutant proteins that are active *in vivo* revealed a positive correlation between their polymerization activity on unmodified DNA (data not shown) and their efficiency in bypassing T-T dimers. This finding highlights the importance of catalytic activity for the bypass capacity of hPol η and indicates that mutations in motif II affect these two functions in parallel. The correlation of activities extends to other template lesions we have examined (Figure 6 and data not shown). In analyzing the ability of the hPol η mutant S62G to incorporate nucleotides on an undamaged oligonucleotide template and to synthesize past diverse types of damage in the same sequence context, we found that the mutant polymerase exhibited greater than wild-type activity on the lesion-free template as well as on all modified templates. Moreover, the mutant enzyme maintained the same relative efficiency of lesion bypass as the wild-type enzyme (no lesion >8-oxo-G >O⁴-MeT >O⁶-MeG >abasic site >ethenoA). These findings again underscore the importance of catalytic efficiency in bypassing different DNA modifications and illustrate the parallel effects of mutations in motif II on bypass of diverse lesions.

The finding that inactivation and deletion of Pol η decreases UV survival of human (Cordonnier and Fuchs, 1999; Yamada *et al.*, 2000) and yeast cells (McDonald

et al., 1997; Roush *et al.*, 1998) provides strong evidence that the enzyme is involved in bypass of UV damage *in vivo*. It is assumed that the survival deficit reflects direct involvement of the catalytic activity of Pol η in translesion synthesis and this assumption is supported by the ability of wild-type Pol η to bypass UV dimers *in vitro* (Washington *et al.*, 2000). Our finding that hPol η mutants, which complement UV sensitivity in yeast, invariably exhibit bypass activity *in vitro* provides independent evidence that the ability of this enzyme to catalyze translesion synthesis is indeed responsible for conferring UV resistance in cells. This conclusion is reinforced by the isolation of mutants (e.g. S62G) that are more active polymerases than wild-type hPol η and exhibit increased bypass ability. Nonetheless, bypass of alterations in DNA is likely to be a complex process involving multiple proteins, including those required for the restart of DNA replication. It is possible that deletion and inactivation of Pol η disrupts protein-protein interactions that are essential for translesion synthesis *in vivo*.

In conclusion, our results show that a yeast-based complementation system can provide libraries of hPol η variants with altered properties. By examining variant enzymes selected in this system, we were able to demonstrate the importance of motif II for hPol η bypass activity and to provide the first experimental evidence indicating an important role for Tyr52 and Ala54 in maintaining the catalytic activity of the enzyme. Comparable systems for positive genetic selection of variants of other human bypass polymerases are presently lacking. Given the high degree of evolutionary conservation among the bypass DNA polymerases, the ability to readily isolate hPol η mutants foretells a prominent role for hPol η as an informative model for this important new family of polymerases.

Materials and methods

Cloning of the hPol η cDNA

hPol η cDNA was produced by RT-PCR amplification (Access RT-PCR, Promega) of mRNA isolated from HeLa cells using a direct mRNA mini kit (Oligotex, Qiagen). The 2.2 kb DNA product was cloned into the PCR 2.1-TOPO vector (Invitrogen) and subcloned using *Bam*HI and *Eco*RI into the yeast shuttle vector pYEX-4T (Clontech) to yield pYEX-hPol η . This construct allowed expression of hPol η fused to an N-terminal glutathione S-transferase (GST) peptide. The predicted fusion protein sequence was verified by DNA sequencing.

Construction of the random hPol η mutant library

Site-directed mutagenesis was performed on pYEX-hPol η , introducing the silent mutations T85C, G87C, A88C to create a *Bsp*EI site and A228G, G231A to create an *Nhe*I site, yielding pYEX-hPol η (*Bsp*EI-*Nhe*I); the numbers refer to the hPol η open reading frame. To avoid contamination with incompletely cut vectors when preparing the random library, a non-functional stuffer vector, pYEX-hPol η dum, was constructed by replacing the *Bsp*EI-*Nhe*I 142 bp fragment of hPol η with an oligonucleotide fragment (5'-TCCGGATCGGATCACGTG-3', 5'-GCTAGCACGTGATCGCGAT-3'). This 'dummy' vector harbors a nonsense codon corresponding to amino acid 36 of hPol η , to generate a truncated product.

The hPol η random library was constructed by annealing two single-stranded DNA oligonucleotides: oligo 1 was a 75-mer corresponding to the sense nucleotides 79-153 and contained a *Bsp*EI site for cloning (5'-CCTCATCTCCGGAATAAACCTTGTGCAGTTGTACAGTACA-AATCATGGAAGGGTGGTGAATAATTGCAGTGAGT-3'); oligo 2 was a 100-mer corresponding to the anti-sense strand nucleotides 236-137 and contained an *Nhe*I site (5'-TGTGCTAGCAGAAGATCTGGACATAACTTCTTAGCATCATCTGCCACATACTTCTA-

GTGACTCCAAATGCACGAGCTTCATAACTCACTGCAATTA-TTC-3'). The nucleotides in bold type in oligo 2 were designed to contain 92.5% wild-type nucleotides at each position and 2.5% each of the other three nucleotides. The 17 bp complementary regions of hybridization are underlined. Oligo 1 and oligo 2 were annealed at their non-random complementary regions and the partial duplexes were extended by mixing 100 pmol of each primer in 50 μ l of PCR mix [$1\times$ Taq buffer, 2.5 mM $MgCl_2$, 0.5 mM of all four dNTPs and 5 μ l of Taq (Promega)]. The reaction was performed by heating to 95°C for 5 min, cooling to 42°C for 2 min, followed by 60 min at 72°C. The resulting DNA was digested with *BspEI* and *NheI* and purified by phenol chloroform and ethanol precipitation. The purified DNA was inserted into pYEX-hPol η in place of the stuffer fragment. Plasmids containing the random library were transformed into *E. coli* XL10-Gold (Stratagene) and the number of transformed cells was determined by plating an aliquot onto LB agar plates containing 100 μ g/ml of carbenicillin. The remainder of the library was amplified by growing the transformed *E. coli* XL10-Gold in 3 liters of LB medium containing 100 μ g/ml of carbenicillin for 16 h at 37°C. The random library pYEX-hPol η -LibB plasmids were isolated by maxiprep (Wizard plus maxiprep, Promega).

Genetic selection for active mutants

The yeast strain M59 carrying disruptions of *RAD30* and *RAD52* (*MAT α* *ade2-1 can1-100 his3-11,15 leu2-3 112 trp1-1 ura3-1 rad30::HIS3 rad52-8::TRP1rad30rad52-8*; McDonald *et al.*, 1997) was generously provided by Drs John McDonald and Roger Woodgate. Cells were transformed with pYEX-hPol η -LibB plasmids by using a lithium acetate procedure (Burke *et al.*, 2000) and grown to an OD₆₀₀ of 2 at 30°C in synthetic complete medium lacking tryptophan, histidine and uracil (Burke *et al.*, 2000). The cells were spread (100 μ l/plate) on agar plates containing the same medium and exposed to 40 J/m² of UV-C radiation (maximum λ 254 nm). The plates were incubated at 37°C in the dark for 72 h and surviving colonies were picked for further analysis.

DNA sequence analysis of motif II

pYEX-Pol η -LibB plasmids carrying the mutant hPol η cDNA were isolated from yeast, retransformed into *E. coli* XL1-Blue (Stratagene) and purified (Wizard SV 96 miniprep kit; Promega). Following purification the 142 bp fragment, *BspEI*-*NheI* was sequenced using the primer 5'-AGCGTCGACGGCTGACCTTGTAGCTTTTGTAGTCTC-3'.

High-throughput in vitro synthesis of hPol η mutant proteins

Mutant plasmids were used as templates for PCR amplification in 96-well plates using a hPol η upstream primer that contained the T7 phage promoter sequence (5'-TTATCGAAATTAATACGACTCACTATAG-GGAGACCCAAGCTTGGTACCGAGCTCGGATCCAAAATGGCTA-CTGGACAGGATC-3') and a hPol η downstream primer that contained the sequence for a hexahistidine tag (5'-CGGAATTCCTAATGGTGA-TGGTGATGATGAGCGCCGCATGTGTTAATGGCTTAAAAAAT-GATTCC-3'). Five microliters of the amplified product (2240 bp), containing hPol η cDNA under control of the T7 promoter, served as a template for *in vitro* transcription and translation using 20 μ l/well of 'TNT T7 Quick for PCR DNA' kit (Promega) in a 96-well plate. Protein products labeled with [³⁵S]methionine were run on SDS gels to establish expression levels. For activity assays, the *in vitro* translated proteins were diluted 1:20 in dilution buffer (buffer D; 25 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 1 mM dithiothreitol, 0.05% NP-40 and 25% glycerol).

DNA polymerase assays

DNA polymerase activity was measured in primer extension assays. Templates were hybridized with 5'-end ³²P-labeled primer and 5 nM of template primer was incubated at 37°C for 15 min (or as indicated) in 10 μ l reaction mixtures containing 2 μ l of a 1:20 dilution of *in vitro* synthesized enzyme or 0.1 μ g/ μ l (2 nM) of purified enzyme, 125 μ M each of the four dNTPs in 40 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 60 mM KCl, 10 mM DTT, 250 μ g bovine serum albumin (BSA) and 2.5% glycerol. Reactions were terminated by the addition of 2.5 μ l of formamide solution; the products were analyzed by electrophoresis through 14% denaturing polyacrylamide gels and quantified by phosphorimager analysis. The oligomers used in assays for T-T dimer bypass were: template 5'-GTATTCAGGCGG<TT>TCCCATCCAAGTACTAA-CCAGGCCCGACCCTGCTTGGCTTCCGAGATCAGA-3' (DMGC3, generously provided by Dr Michael J.Smerdon) and primer 5'-³²P-TCTGATCTCGGAAGCCAAGCGGCTCGGCTGGTTAGTACTTGGATG-3'. The templates used for bypass of other lesions (8-oxo-G, O⁶-MeG, O⁴-MeT, abasic site, ethenoA and no lesion) were synthesized by the Midland certified reagent company and contained one of the above

adducts in the N position: 5'-TTGGC(N)GCAGAATATTGCTAGCGG-GAATTCGGCGCG-3'. The primer was 5'-³²P-CGCGCCGAATTC-CGCTAGCAATATTCT-3'.

Cloning of cDNAs for the hPol η polymerase domain

cDNAs corresponding to the polymerase domains of wild type and S62G hPol η (amino acids 1–513) were produced by PCR amplification and cloning of the first 1539 nucleotides of the human Pol η cDNA from pYEX-hPol η and pYEX-hPol η S62G into the expression vector pET-15B (Novagen) to yield pET-15B -hPol η 513N and pET-15B -hPol η S62G 513N, respectively. These constructs allow expression of the polymerase domain fused to an N-terminal hexahistidine sequence.

Expression and purification of the hPol η polymerase domain

E. coli BL21(DE3)pLysS (Novagen) cells containing pET-15B-hPol η 513N, pET-15B -hPol η S62G 513N or no plasmid as a control, were grown at 37°C to an OD₆₀₀ of 0.6, induced with 1 mM IPTG and grown in LB broth containing 5% ethanol for another 12 h at 25°C. Cells were harvested by centrifugation, resuspended and incubated for 20 min in 'BugBuster' (Novagen) extraction solution containing 10 U/ml Benzonase (Novagen). The clarified extract was then dialyzed against buffer D. The equilibrated extract was loaded onto a DEAE-cellulose column equilibrated with buffer D and the column was washed with buffer D, followed by buffer D containing 100 mM NaCl. Bound proteins were eluted with buffer D containing 250 mM NaCl, dialyzed against buffer N (20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 20% glycerol) containing 5 mM imidazole and loaded onto a His-Bind Ni²⁺ column (Novagen) equilibrated with buffer N. The column was washed with buffer N and then with buffer N containing 50 mM imidazole. Bound proteins were eluted in step-wise manner with buffer N containing 80 mM, 120 mM or 200 mM imidazole. His-tagged hPol η 513N and hPol η S62G 513N were resolved by SDS-PAGE and visualized by Coomassie Blue staining and by western blotting with anti-hPol η antibody (sc-5936; Santa Cruz Biotechnology). The majority of the Coomassie Blue-stained material migrated with the molecular weight expected for the 519-residue hexahistidine-tagged hPol η polymerase domain (~52 kDa) and corresponded to the only immunopositive material in the gel. The concentration of the hPol η polymerase domain used in assays was estimated by densitometric quantitation of Coomassie Blue-stained protein observed in an SDS-polyacrylamide gel with BSA as a standard.

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References

- Bridges, B.A. (1999) DNA repair: polymerases for passing lesions. *Curr. Biol.*, **9**, R475–R477.
- Burke, D., Dawson, D. and Stearns, T. (2000) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Cleaver, J.E. (1999) Stopping DNA replication in its tracks. *Science*, **285**, 212–213.
- Cordonnier, A.M. and Fuchs, R.P. (1999) Replication of damaged DNA: molecular defect in xeroderma pigmentosum variant cells. *Mutat. Res.*, **435**, 111–119.
- Gerlach, V.L., Feaver, W.J., Fischhaber, P.L. and Friedberg, E.C. (2001) Purification and characterization of polk, a DNA polymerase encoded by the human DINB1 gene. *J. Biol. Chem.*, **276**, 92–98.
- Goodman, M.F. and Tippin, B. (2000) The expanding polymerase universe. *Nature Rev. Mol. Cell Biol.*, **1**, 101–109.
- Haracska, L., Prakash, S. and Prakash, L. (2000a) Replication past O(6)-methylguanine by yeast and human DNA polymerase η . *Mol. Cell Biol.*, **20**, 8001–8007.
- Haracska, L., Yu, S.L., Johnson, R.E., Prakash, L. and Prakash, S. (2000b)

- Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase η . *Nature Genet.*, **25**, 458–461.
- Haracska,L., Washington,M.T., Prakash,S. and Prakash,L. (2001) Inefficient bypass of an abasic site by DNA polymerase η . *J. Biol. Chem.*, **276**, 6861–6866.
- Hubscher,U., Nasheuer,H.P. and Syvaaja,J.E. (2000) Eukaryotic DNA polymerases, a growing family. *Trends Biochem. Sci.*, **25**, 143–147.
- Johnson,R.E., Kondratik,C.M., Prakash,S. and Prakash,L. (1999a) hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science*, **285**, 263–265.
- Johnson,R.E., Prakash,S. and Prakash,L. (1999b) Requirement of DNA polymerase activity of yeast Rad30 protein for its biological function. *J. Biol. Chem.*, **274**, 15975–15977.
- Johnson,R.E., Washington,M.T., Prakash,S. and Prakash,L. (1999c) Bridging the gap: a family of novel DNA polymerases that replicate faulty DNA. *Proc. Natl Acad. Sci. USA*, **96**, 12224–12226.
- Johnson,R.E., Washington,M.T., Prakash,S. and Prakash,L. (2000) Fidelity of human DNA polymerase η . *J. Biol. Chem.*, **275**, 7447–7450.
- Kannouche,P., Broughton,B.C., Volker,M., Hanaoka,F., Mullenders,L.H. and Lehmann,A.R. (2001) Domain structure, localization and function of DNA polymerase η , defective in xeroderma pigmentosum variant cells. *Genes Dev.*, **15**, 158–172.
- Kondratik,C.M., Washington,M.T., Prakash,S. and Prakash,L. (2001) Acidic residues critical for the activity and biological function of yeast DNA polymerase η . *Mol. Cell Biol.*, **21**, 2018–2025.
- Kulaeva,O.I., Koonin,E.V., McDonald,J.P., Randall,S.K., Rabinovich,N., Connaughton,J.F., Levine,A.S. and Woodgate,R. (1996) Identification of a DinB/UmuC homolog in the archeon *Sulfolobus solfataricus*. *Mutat. Res.*, **357**, 245–253.
- Levine,R.L., Miller,H., Grollman,A., Ohashi,E., Ohmori,H., Masutani,C., Hanaoka,F. and Moriya,M. (2001) Translesion DNA synthesis catalyzed by human Pol η and Pol κ across 1,N6-Ethenodeoxyadenosine. *J. Biol. Chem.*, **276**, 18717–18721.
- Lindahl,T. and Wood,R.D. (1999) Quality control by DNA repair. *Science*, **286**, 1897–1905.
- Ling,H., Boudsocq,F., Woodgate,R. and Yang,W. (2001) Crystal structure of a Y-family DNA polymerase in action. A mechanism for error-prone and lesion-bypass replication. *Cell*, **107**, 91–102.
- Livneh,Z. (2001) DNA damage control by novel DNA polymerases: translesion replication and mutagenesis. *J. Biol. Chem.*, **276**, 25639–25642.
- Maher,V.M., Ouellette,L.M., Curren,R.D. and McCormick,J.J. (1976) Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells. *Nature*, **261**, 593–595.
- Masutani,C., Araki,M., Yamada,A., Kusumoto,R., Nogimori,T., Maekawa,T., Iwai,S. and Hanaoka,F. (1999a) Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. *EMBO J.*, **18**, 3491–3501.
- Masutani,C. *et al.* (1999b) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η . *Nature*, **399**, 700–704.
- Masutani,C., Kusumoto,R., Iwai,S. and Hanaoka,F. (2000) Mechanisms of accurate translesion synthesis by human DNA polymerase η . *EMBO J.*, **19**, 3100–3109.
- Matsuda,T., Bebenek,K., Masutani,C., Hanaoka,F. and Kunkel,T.A. (2000) Low fidelity DNA synthesis by human DNA polymerase- η . *Nature*, **404**, 1011–1013.
- McDonald,J.P., Levine,A.S. and Woodgate,R. (1997) The *Saccharomyces cerevisiae* RAD30 gene, a homologue of *Escherichia coli* DinB and UmuC, is DNA damage inducible and functions in a novel error-free post-replication repair mechanism. *Genetics*, **147**, 1557–1568.
- McDonald,J.P., Tissier,A., Frank,E.G., Iwai,S., Hanaoka,F. and Woodgate,R. (2001) DNA polymerase τ and related RAD30-like enzymes. *Philos. Trans R. Soc. Lond. B Biol. Sci.*, **356**, 53–60.
- Nelson,J.R., Lawrence,C.W. and Hinkle,D.C. (1996) Deoxycytidyl transferase activity of yeast REV1 protein. *Nature*, **382**, 729–731.
- Ohmori,H. *et al.* (2001) The Y-family of DNA polymerases. *Mol. Cell*, **8**, 7–8.
- Reuven,N.B., Arad,G., Maor-Shoshani,A. and Livneh,Z. (1999) The mutagenesis protein UmuC is a DNA polymerase activated by UmuD, RecA and SSB and is specialized for translesion replication. *J. Biol. Chem.*, **274**, 31763–31766.
- Rogozin,I.B., Pavlov,Y.I., Bebenek,K., Matsuda,T. and Kunkel,T.A. (2001) Somatic mutation hotspots correlate with DNA polymerase η error spectrum. *Nature Immunol.*, **2**, 530–536.
- Roush,A.A., Suarez,M., Friedberg,E.C., Radman,M. and Siede,W. (1998) Deletion of the *Saccharomyces cerevisiae* gene RAD30 encoding an *Escherichia coli* DinB homolog confers UV radiation sensitivity and altered mutability. *Mol. Gen. Genet.*, **257**, 686–692.
- Steitz,T.A. (1999) DNA polymerases: structural diversity and common mechanisms. *J. Biol. Chem.*, **274**, 17395–17398.
- Suzuki,M., Christians,F.C., Kim,B., Skandalis,A., Black,M.E. and Loeb,L.A. (1996) Tolerance of different proteins for amino acid diversity. *Mol. Div.*, **212**, 111–118.
- Tang,M., Shen,X., Frank,E.G., O'Donnell,M., Woodgate,R. and Goodman,M.F. (1999) UmuD₂C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc. Natl Acad. Sci. USA*, **96**, 8919–8924.
- Trincao,J., Johnson,R.E., Escalante,C.R., Prakash,S., Prakash,L. and Aggarwal,A.K. (2001) Structure of the catalytic core of *S.cerevisiae* DNA polymerase η : implications for translesion DNA synthesis. *Mol. Cell*, **8**, 417–426.
- Vaisman,A., Masutani,C., Hanaoka,F. and Chaney,S.G. (2000) Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase η . *Biochemistry*, **39**, 4575–4580.
- Wagner,J., Gruz,P., Kim,S.-R., Yamada,M., Matsui,K., Fuchs,R.P.P. and Nohmi,T. (1999) The *dinB* gene encodes a novel *Escherichia coli* DNA polymerase (DNA pol IV) involved in mutagenesis. *Mol. Cell*, **4**, 281–286.
- Wang,Z. (2001) Translesion synthesis by the UmuC family of DNA polymerases. *Mutat. Res.*, **486**, 59–70.
- Washington,M.T., Johnson,R.E., Prakash,S. and Prakash,L. (2000) Accuracy of thymine-thymine dimer bypass by *Saccharomyces cerevisiae* DNA polymerase η . *Proc. Natl Acad. Sci. USA*, **97**, 3094–3099.
- Yamada,A., Masutani,C., Iwai,S. and Hanaoka,F. (2000) Complementation of defective translesion synthesis and UV light sensitivity in xeroderma pigmentosum variant cells by human and mouse DNA polymerase η . *Nucleic Acids Res.*, **28**, 2473–2480.
- Yu,S.L., Johnson,R.E., Prakash,S. and Prakash,L. (2001) Requirement of DNA polymerase η for error-free bypass of UV-induced CC and TC photoproducts. *Mol. Cell Biol.*, **21**, 185–188.
- Zeng,X., Winter,D.B., Kasmer,C., Kraemer,K.H., Lehmann,A.R. and Gearhart,P.J. (2001) DNA polymerase η is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. *Nature Immunol.*, **2**, 537–541.
- Zhang,Y., Yuan,F., Wu,X., Rechkoblit,O., Taylor,J.S., Geacintov,N.E. and Wang,Z. (2000) Error-prone lesion bypass by human DNA polymerase η . *Nucleic Acids Res.*, **28**, 4717–4724.
- Zhou,B.L., Pata,J.D. and Steitz,T.A. (2001) Crystal structure of a DinB lesion bypass DNA polymerase catalytic fragment reveals a classic polymerase catalytic domain. *Mol. Cell*, **8**, 427–437.

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