Mutagenic and non-mutagenic bypass of DNA lesions by Drosophila DNA polymerases dpol\(\eta\) and dpol\(\iota\) *

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**Running title:** Translesion synthesis by Drosophila pol\(\eta\) and pol\(\iota\)

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SUMMARY

cDNA sequences were identified and isolated that encode *Drosophila* homologues of human Rad30A and Rad30B, called *drad30A* and *drad30B*. Here we show that C-terminal truncated forms of the *drad30A* and *drad30B* gene products, designated dpol\_C and dpol\_C, respectively, exhibit DNA polymerase activity. dpol\_C and dpol\_C efficiently bypass a *cis*-syn cyclobutane thymine-thymine dimer (TT-CPD) in a mostly error-free manner. dpol\_C shows limited ability to bypass a (6-4) photoproduct at thymine-thymine (TT-(6-4)PP) or at thymine-cytosine (TC-(6-4)PP) in an error-prone manner. dpol\_C scarcely bypasses these lesions. Thus, the fidelity of translesion synthesis (TLS) depends on the identity of the lesion and on the polymerase. The human *XPV* gene product, hpol\_\eta, bypasses TT-CPD efficiently in a mostly error-free manner but does not bypass TT-(6-4)PP, while *E. coli* DNA polymerase V (UmuD’\_C complex) bypasses both lesions, especially TT-(6-4)PP in an error-prone manner (Tang, M., Pham, P., Shen, X., Taylor, J.S., O'Donnell, M., Woodgate, R. and Goodman, M.F. (2000) *Nature* 404, 1014-1018). Both dpol\_\eta\_C and DNA polymerase V preferentially incorporate GA opposite TT-(6-4)PP. The chemical structure of the lesions and the similarity in the nucleotides incorporated suggest that structural information in the altered bases contribute to nucleotide selection during incorporation opposite these lesions by these polymerases.
INTRODUCTION

DNA is frequently damaged by environmental and endogenous genotoxic agents. Although various mechanisms exist to ensure that the majority of DNA damage is recognized and repaired and the integrity of the DNA is faithfully restored, some DNA lesions escape repair and persist in the cell. Unrepaired DNA lesions can block the progress of the replication machinery. To help resolve this problem, cells have specialized polymerases that carry out translesion DNA synthesis (TLS), which is a mechanism that permits nucleotides to be incorporated opposite lesions. After TLS bypasses the DNA damage, replication can continue with the normal replication machinery downstream of the site of the damage.

Recently, a new family of DNA polymerases was identified called the UmuC/DinB/Rev1/Rad30 superfamily (1 – 3). Several of these polymerases participate in TLS (4-11) and members of this protein family exhibit lower fidelity and processivity than replicative DNA polymerases (10, 12-15). The lower fidelity enables these polymerases to carry out TLS and the lower processivity facilitates dissociation of these enzymes after bypass of a lesion; this is important because it allows the normal replication machinery, with high fidelity and processivity, to preferentially carry out DNA synthesis distal to the lesion.

However, TLS is mutagenic when the base inserted opposite the DNA lesion is different than the base normally inserted opposite an undamaged base at that site. The chemical structure of the lesion is an important determinant for mutagenic or nonmutagenic bypass during TLS. Irradiation of DNA with UV light produces a variety of photoproducts that can cause mutations. cis-syn cyclobutane pyrimidine dimer (CPD) and (6-4) photoproducts ((6-4)PP) are the two major classes of UV-induced DNA photoproducts. In DNA irradiated with UV, CPD is the most abundant lesion and it is approximately 3-5-fold more abundant than (6-4)PP. However, the mutagenic properties of these two lesions are different, and the relative contributions of the two lesions to the
mutagenesis are not simply proportional to their relative abundance. The mutagenic specificities of CPD and (6-4)PP have been studied using well characterized site-specific UV photoproducts in DNA substrates. The mutation frequency obtained either with a site-specific TT-CPD or a TC-CPD is quite low (17 - 18), and in comparison, (6-4)PP is more mutagenic. The (6-4)PP at the 5’-TT site was highly mutagenic, and most of the mutations induced by this lesion are T to C transitions at the 3’-T (19). However, this type of mutation is not very common, because the (6-4)PP at the TT site is relatively rare. On the other hand, the TC-(6-4)PP is less mutagenic than the TT-(6-4)PP but more mutagenic than the TT-CPD. Of the mutations at this site, 80% were C to T transitions at the 3’ base (20). Since TC to TT is frequently observed in DNA exposed to UV, and (6-4)PP forms most frequently in the TC sequence, the TC-(6-4)PP could be a candidate for a strongly premutagenic lesion.

Human cells have three TLS polymerases that belong to the UmuC/DinB/Rev1/Rad30 superfamily: polη (encoded by the XPV/RAD30A gene,(21, 22)), polι (encoded by RAD30B, (10, 23)) and polκ (encoded by DINV1, (24)). In addition, the human protein hREV1 is a homologue of yeast REV1 (25). Polη, polι and polκ bypass UV-induced damage with different efficiencies. Polη bypasses TT-CPD efficiently and inserts AA opposite the lesion; however, it adds only one base opposite the 3’-T of TT-(6-4)PP (5). Polι bypasses TT-CPD and TT-(6-4)PP with low efficiency, adding one or a few bases opposite these lesions (26, 27). Polκ stops DNA synthesis and is blocked one base before TT-CPD and TT-(6-4)PP (9). Thus, in human cells none of these polymerases bypass (6-4)PP. In contrast, E. coli DNA polymerase V (UmuD’C, (28)) efficiently bypasses both lesions, inserting AA or GA opposite TT-CPD or TT-(6-4)PP, respectively (8). Thus, the chemical structure and the properties of
each TLS polymerase determine which base is inserted and the likelihood of a mutation at a specific lesion.

The goal of this work was to identify and characterize the *Drosophila* homologue(s) of the UmuC/DinB/Rev1/Rad30 superfamily. Two cDNAs were isolated that encode *Drosophila* Rad30A and Rad30B, called *drad30A* and *drad30B*. Truncated forms of dRAD30A and dRAD30B proteins, designated dpolηΔC and dpolΔC, respectively, have been purified and their properties studied, including the mechanism of UV-induced mutagenesis and the products of lesion bypass in reactions with these enzymes. The bypass templates used in this study include TC-(6-4)PP, TT-CPD or TT-(6-4)PP; a template with TC-(6-4)PP was studied because of the biological relevance this lesion. The results indicate that both polymerases bypass the TT-CPD in an error-free manner. Furthermore, dpolηΔC bypasses TT-(6-4)PP and TC-(6-4)PP in a highly error-prone manner.

**EXPERIMENTAL PROCEDURES**

*Isolation of Drosophila rad30A and rad30B cDNAs*

To identify the genes encoding the UmuC/DinB/Rev1/Rad30 superfamily proteins in *Drosophila*, we searched the database of expressed sequence tag (GenBank dbEST) for *Drosophila* cDNAs that share homology with the proteins of this family. Several *Drosophila* cDNA sequences that share significant homology with the proteins of the superfamily were identified. Three such clones, SD05329, LD09220, and GH11153, were obtained from Research Genetics (Huntsville, AL), and the entire insert of each clone was sequenced with an automated DNA sequence analyzer (Applied Biosystems PRISM310). Translation of the large continuous open reading frame (ORF) from each cDNA revealed that each translated protein shares significant similarity to Rad30A,
Rad30B, and Rev1 proteins, respectively. Thus, the clones were designated as \textit{drad30A}, \textit{drad30B}, and \textit{drev1}, respectively. The cDNAs did not contain in-frame stop codons. Thus, the 5' ends of each clone were amplified by 5' RACE (5' rapid amplification of cDNA ends). The sequence determination of the amplified 5' ends revealed the presence of the in-frame stop codon. Screening of the dbEST indicated that \textit{Drosophila} seems to lack a true DinB ortholog, which was confirmed by searching the recently completed sequence of the \textit{Drosophila} genome. The \textit{drad30A} and \textit{drad30B} cDNAs each hybridized to \textit{Drosophila} polytene chromosomes at a single site corresponding to bands 3L-79BC and 3R-84EF, respectively.

\textbf{Overproduction of the dRAD30A and dRAD30B proteins}

The expression vector used in these studies was pGEX-4T (Pharmacia Biotech). To overexpress the \textit{Drosophila} dRAD30A and dRAD30B proteins, each gene was cloned in-frame with the glutathione S-transferase gene. A chimeric \textit{GST-drad30A} gene was constructed by recloning the 3 kilobase pair (kb) \textit{EcoRI/XhoI} DNA fragment from the EST clone SD05329 in \textit{EcoRI/XhoI} digested pGEX-4T-1, thus generating plasmid pGEX-dRad30A. The Carboxyl (C-) terminal deletion mutant of the \textit{drad30A} gene was constructed by recloning the 1640 base pair (bp) \textit{EcoRI/EcoRI52I} DNA fragment, which contains the N-terminal 545 residues, in \textit{EcoRI/NotI} digested pGEX-4T-1, thus generating pGEX-dRad30AΔC. The chimeric \textit{GST-drad30B} gene was constructed as follows. \textit{EcoRI} restriction site was generated just upstream of the start codon by PCR using the primer oligonucleotides 30B5'Eco, 5'-CGGAATTCCATGGGACTTCTGATGAGC-3' and 3 kb \textit{EcoRI/XhoI} DNA fragment from the EST clone LD09220 was then cloned in-frame with GST in the \textit{EcoRI/XhoI} digested pGEX4T-1, thus generating plasmid pGEX-dRad30B. The C-terminal deletion mutant of the \textit{GST-rad30B} gene was constructed by first synthesizing the oligomer 30Bdel3’Xho, 5' - CCGGCTCGAGTGAGGACTTCTGAGACT-3'. Two PCR
primers, 30B5'Eco and 30Bdel3'Xho, were used to amplify a 1.36 kb DNA fragment, which contained the amino (N-) terminal 448 residues of the \textit{drad30B} gene. The PCR products were digested with \textit{EcoRI} and \textit{XhoI}, and the resultant 1.35 kb fragment was cloned in the \textit{EcoRI/XhoI} digested pGEX-4T-1, thus generating pGEX-dRad30BΔC.

Mutant forms of the \textit{GST-rad30A} and \textit{GST-rad30B} genes were constructed by utilizing the “Mutant-Super Express” mutagenesis kit (Takara, Japan). The adjacent residues, D126- E127 in \textit{drad30A} and D115-E116 in \textit{drad30B}, were changed to alanine using primers R30A-AA 5'-GCTTCCGTGGCAGCAGCCGTACT -3' and R30B-AA 5' -CTAGGCTCTGCTGCAAAACTTTA -3', respectively. The 400 bp \textit{SphI} fragment from pKF30A and the 800 bp \textit{EcoRI/BglII} fragment from pKF30B were sequenced to verify that the region only contained the desired mutation, and then each fragment was subcloned into the similarly digested pGEXrad30AΔC and pGEXrad30BΔC to create pGEXrad30A-DE126AA and pGEXrad30B-DE115AA, respectively.

\textit{E. coli} JM109 harboring pGEX-dRad30AΔC, pGEX-dRad30BΔC, pGEX-dRad30A-DE126AA, or pGEX-drad30B-DE115AA was grown in 1L LB medium containing 150 mg/L ampicillin at 25°C until an OD$_{600}$ of 0.9 - 1.0 was reached. Isopropyl $\beta$-D-thiogalactopyranoside (IPTG) (0.1 mM) was added to induce expression of the chimeric gene. After incubation at 25°C for 12 h, the cells were harvested by centrifugation and were stored at -80°C.

\textit{Purification of the dRAD30A and dRAD30B protein}
To purify the GST tagged dRAD30A or dRAD30B, \textit{E. coli} cells were resuspended in PBS and disrupted using a sonicator before centrifugation at 10,000 x g. 10 mM Mg-ATP and 5 mg/mL casein were added to the extract, which was then incubated at room temperature for 20 min. The extract was then passed over a 5 mL glutathione-Sepharose 4B column (Amersham Pharmacia Biotech) and then washed with 10 column volumes of
wash buffer (1M NaCl and 50 mM Tris-HCl (pH 8.0)). The GST tagged protein was eluted from the GST column in a stepwise fashion by a wash with five column volumes of elution buffer (50 mM Tris-HCl (pH 8.0), 10 mM glutathione). The GST-fusion protein in each eluate was monitored by the GST activity, according to the manufacturer’s protocol (Amersham Pharmacia Biotech), and the eluates containing GST activity were pooled. The pooled eluates were concentrated by Centriplus 50 ultrafiltration (Amicon, USA) to an approximate final volume of 1.0 mL. The concentrated eluate was applied to a HiTrap Q column (Amersham Pharmacia Biotech) equilibrated with buffer A (50 mM Tris (pH 8.0)). The column was subsequently washed with 10 column volumes of buffer A and the GST-fusion protein was eluted with a 0 - 1 M linear gradient of NaCl in buffer A. The GST activity in each fraction was measured and the positive fractions were pooled. The pooled fractions were dialyzed against buffer A and were loaded on a HiTrap-Heparin column (Amersham Pharmacia Biotech) equilibrated with buffer A. The column was washed with buffer A and the protein was eluted by a linear gradient of 0 - 0.5 M NaCl in buffer A. Glycerol was added to the GST-containing fraction (10% v/v) prior to storage at -80°C.

The eluate from the glutathione-Sepharose 4B column contained a contaminating DNA polymerase activity derived from *E. coli*, because the eluate from the column of the cell extract from *E. coli* harboring the GST vector had a weak DNA polymerase activity. To remove the contaminating DNA polymerase activity, we tested two kinds of antibodies, one specific for *E. coli* pol I and another specific for pol II. The addition of the antibody for pol I to the eluate, but not the antibody for pol II, eliminated the DNA polymerase activity of the eluate from *E. coli* expressing GST, indicative that the contaminating DNA polymerase is *E. coli* pol I. The pol I antibody also eliminated the DNA polymerase activity in the eluate of the cell extract from *E. coli* harboring the mutant plasmids, pGEXrad30A-DE126AA and pGEXrad30B-DE115AA. Thus, the pol I
antibody was added to the purified protein in all experiments in this paper. However, after addition of pol I antibody a slight 3'-exonuclease activity was still remaining.

DNA polymerase assays
The 30mer oligomer (non-damaged TT) with the sequence 5’-CTCGTCAGCATCCTTCATCATACAGTCAGTG-3’ was used as the nondamaged template. The same 30mer oligomers containing the CPD (29) and the (6-4)PP (30) at the underlined sites were synthesized as described. The 30mer oligomers, in which the underlined TT sequence of the “non-damaged TT” oligomers was changed to TC, were also used as nondamaged templates (designated “non-damaged TC”) and the 30mer oligomers containing (6-4)PP at the TC site were synthesized as described (31). Various lengths (16-18 mer) of oligomers with sequences complementary to the 30mer template were synthesized and used as primers. Each primer was labeled at the 5’-end using T4 polynucleotide kinase and [γ-32P] ATP and was annealed with the template at a molar ratio of 1:1. Standard reactions (10 µL) contained 40 mM Tris-HCl (pH 8.0), 5 mM MgCl2, 100 µM each of the four dNTPs, 10 mM DTT, 250 µg/mL BSA, 60 mM KCl, 2.5% glycerol, 40 nM primer-template, and the indicated amount of enzyme. After incubation at 37°C for 15 min, the reactions were terminated by the addition of 10 µL formamide followed by boiling. The products were subjected to 20% polyacrylamide/7M urea gel electrophoresis followed by autoradiography.

Detection of correctly replicated products opposite lesions.
Two kinds of oligomers were used: 49mers bearing the centrally located (6-4)PP or CPD at the TT site (32) and 30mers bearing the centrally located (6-4)PP at the TC site (31). The substrate sequence is as follows (the introduced thymine dimer is underlined):
49mer: 5’-
AGCTACCATGCGCTGCAGAATTAAAGCAATTCGTAATCATGTCATAGCT-3’
and 30mer: CCTGGTTAGTAGTACTTCGATTTAGGTGACACT. The photoproduct in the 49 base pair duplex DNA is at an MseI site (MseI cleaves between the two thymines in the TTAA sequence) and that in the 30 bp duplex DNA is at a TaqI site (TaqI cleaves between the two pyrimidines in the TCGA sequence). The assay measures the susceptibility of the bypass product to MseI cleavage. A 20mer oligomer (5’-ACCATGATTACGAATTGCTT-3’) and a 15mer oligomer (5’-AGTGTACCTAAATC-3’), with sequences complementary to the 49mer and 30mer damage containing strands, respectively, were synthesized and used as primers. After labeling with $\gamma^{32}$P-ATP, the primer was annealed with the template and was used as the substrate for DNA synthesis. DNA synthesis was carried out as described in the DNA polymerase assay. After DNA synthesis, the replication products were denatured by incubation at 95°C for 5 min, and then were annealed with a ten-fold excess of the 49mer non-damaged oligonucleotide (400 nM). The annealed duplex DNA was digested with one unit of MseI or TaqI for 90 min at 37°C or 65°C, respectively. The digested DNA was denatured at 95°C for 5 min in the presence of 50% formamide and was then separated on a 10% polyacrylamide sequencing gel. The MseI or TaqI susceptible and resistant fractions were quantified by scanning the gels with a Fuji Bioimage Analyzer (BAS-2000, Fuji Film, Japan) and the ratio was calculated as follows. The amounts of the undigested bypass products (Counts-bp) were obtained by measuring the radioactivity of the areas corresponding to the 24 - 42 base fragments or the 19 - 30 base fragments in each lane for the template containing no damage, TT-CPD and TT-(6-4)PP or TC-(6-4)PP, respectively. The radioactivity of the same area in the control sample, which did not contain enzyme, was used as the background for bypass products (Counts-bp-bg). The amounts of the bypass products susceptible to each enzyme were determined by measuring the radioactivity of the 19 base or 14 base fragments (Counts-dig). The radioactivity of the same area of the undigested reaction was measured and used as the background of the digested products (Counts-dig-bg). The “% restriction enzyme
sensitive bypass products” in the Table was calculated as (Count-dig)-(Counts-dig-bg)/(Counts-bp)-(Counts-bp-bg)x100.

RESULTS

\textit{Isolation of Drosophila drad30A and drad30B cDNAs}

Two \textit{Drosophila} cDNA clones were identified in the GenBank dbEST database which had significant homology to human Rad30A and Rad30B protein, and were designated \textit{drad30A} and \textit{drad30B}. DNA sequence analysis of the full length cDNAs revealed that \textit{drad30A} encodes a protein of 885 aa with 31\% identity to the human XPV/RAD30A protein (hpolt) and \textit{drad30B} encodes a protein of 737 aa with 30\% identity to the human RAD30B protein (hpolt). Previous studies have shown that the proteins in the UmuC/DinB/Rev1/Rad30 superfamily are most highly conserved in their N-terminal portion which includes five discrete conserved regions (1, 33). As shown in Figures 1A and 1B, these regions (I - V) are conserved in the N-terminal portion of all the RAD30-like proteins including the \textit{Drosophila} proteins encoded by \textit{drad30A} and \textit{drad30B}.

The amino acid sequences were aligned for all the members of the UmuC/DinB/Rev1/Rad30 protein family, and the alignment was used to generate a phylogenetic tree (Figure 1C). The tree reveals several distinct subgroups of proteins; the validity of these subgroups are convincingly supported by the bootstrap test. Since the Rad30B subfamily is equally distant from the RAD30A and DinB subfamilies, the superfamily can be classified into five subgroups; UmuC, DinB, Rev1, Rad30A, and Rad30B. The \textit{Drosophila} genes \textit{drad30A} and \textit{drad30B} belong to the Rad30A and Rad30B subfamilies, respectively. A \textit{Drosophila} homologue of human REV1 was also identified, which belongs to the REV1 subfamily as shown in Figure 1C; however, no \textit{Drosophila} homologue of the human DINB1 protein (hpolt) was identified in the cDNA
libraries that were searched. In addition, the sequence of the *Drosophila* genome (34) does not include a homologue of polκ.

*Overproduction and purification of dRAD30A and dRAD30B proteins*

The proteins encoded by *drad30A* and *drad30B* (*dRAD30A* and *dRAD30B*, respectively) were purified as recombinant fusion proteins with a GST tag at the N-terminus. Initially, the entire coding sequences of the *drad30A* and *drad30B* genes were fused with the *GST* gene; however, these constructs produced a very low yield of the desired recombinant proteins in *E. coli*. However, when a mutation was introduced in each gene that creates a stop codon in the C-terminal region, the resulting C-terminally truncated proteins were expressed at a higher level in *E. coli*. These truncated forms of the *drad30A* and *drad30B* gene products are similar in size to the truncated forms of hpolη and hpolκ proteins and include all of the N-terminal conserved regions of the UmuC/DinB/Rev1/Rad30 superfamily; thus, they were expected to be active polymerases despite the missing C-terminal region (5, 9, 14, 21). The truncated forms of dRAD30A (dRAD30AΔC) and dRAD30B(dRAD30BΔC) include the N-terminal 545 residues and 445 residues of each polypeptide, respectively. They were purified by GST-Sepharose affinity chromatography (see Materials and Methods) and characterized enzymatically as described below (Figure 2).

*DNA polymerase activities of dRAD30AΔC and dRAD30BΔC*

The dRAD30AΔC and dRAD30BΔC proteins were assayed for DNA polymerase activity using a primer extension assay with a 5′-endlabeled 16mer primer annealed to a 30mer template. As shown in Figures 2B and 2C, both dRAD30AΔC (Figure 2B, lanes 5-9) and dRAD30BΔC (Figure 2C, lanes 5-9) synthesize DNA in a template-dependent fashion, and the size of the replication products gradually increases with increasing
enzyme concentration in the reaction. The polymerase activity of dRAD30AΔC is 10 times higher than that of dRAD30BΔC.

If the observed DNA polymerase activity is intrinsic to the purified protein, then site-directed mutagenesis of amino acids in the enzyme active site should create a mutant deficient in DNA polymerase activity. This prediction was tested by carrying out site-directed mutagenesis on adjacent Asp and Glu residues within Motif III that are believed to be critical for the catalytic activity of the UmuC/DinB/Rad30 family proteins (4, 9, 10, 22, 28, 35, 36). The highly conserved Asp and Glu residues are present at positions 126 and 127 and at positions 115 and 116 in dRAD30A and dRAD30B, respectively (Figure 1B). In the mutants generated for this study, these residues were changed to alanine. The mutant proteins displayed the same chromatographic properties as the wild type protein, but they lacked DNA polymerase activity (Figures 2B and 2C, lanes 2-4). Therefore, the observed DNA polymerase activity is intrinsic to the dRAD30A and dRAD30B proteins.

We therefore propose that dRAD30A be renamed dpolη and dRAD30B be renamed dpolι. In the following text, dpolηΔC will be used to refer to the truncated protein dRAD30AΔC, and dpolιΔC will be used to refer to dRAD30BΔC.

The nucleotide selectivity of dpolηΔC and dpolιΔC was analyzed in DNA synthesis reactions using a non-damaged DNA template in the presence of only one deoxynucleotidetriphosphate (i.e., dA, dG, dC or T). Incorporation of the correct complementary nucleotides and to less extents incorrect nucleotides opposite each template occurred (data not shown), suggesting that both dpolηΔC and dpolιΔC have relatively low fidelity.

Translesion synthesis by dpolηΔC and dpolιΔC
To determine whether dpolηΔC and dpolιΔC bypass DNA lesions, polymerase assays were carried out using templates with TT-CPD or TT-(6-4)PP lesions. These templates were described in previous studies of hpolη (5 7), hpolι (26) and hpolκ (9). In addition, a template with TC-(6-4)PP in the same sequence context as the above two templates was constructed and used in this study. A 16mer primer was annealed to the template-containing lesion which terminates just before the lesion site (Figure 3).

dpolηΔC and dpolιΔC efficiently bypass TT-CPD (Figure 3A) and the efficiencies of DNA synthesis on the lesion-containing template were almost the same as on the non-damaged template. dpolηΔC showed some ability to bypass TT-(6-4)PP (Figure 3B, lanes 2-4) and TC-(6-4)PP (Figure 3C, lanes 2-4), but in most cases added one or two bases opposite the lesions before arresting DNA synthesis. dpolιΔC added one base opposite the 3’-T of TT-(6-4)PP (Figure 3B, lanes 6-8) or the 3’-C of TC-(6-4)PP (Figure 3C, lanes 6-8); in addition, it bypassed TC-(6-4)PP at a very low level when the enzyme was present at a high concentration in the reaction (Figure 3C, lane 8). As reported previously (7), hpolη did not bypass TT-(6-4)PP, arresting DNA synthesis after addition of one base opposite the 3’-T of the lesion. Therefore, the ability to bypass TT- and TC-(6-4)PP is a remarkable feature of dpolηΔC, which distinguishes it from other RAD30-like polymerases.

Nucleotide selectivity of dpolηΔC and dpolιΔC during incorporation opposite lesions

Polymerization reactions by dpolηΔC and dpolιΔC were performed with the lesion-containing template-primer substrates described above in the presence of a single deoxynucleotide. The reaction products were analyzed and are shown in Figure 4. On a
template with TT-CPD, dpolηΔC and dpolηΔC preferentially incorporated dAMP and arrest synthesis after adding four dAMPs opposite TT-CPD and the following CT residues (Figure 4A, CPD). dpolηΔC and dpolηΔC preferentially incorporated dAMP opposite the 3’T of TT-(6-4)PP, incorporating dGMP at a significant level (Figure 4A, TT-(6-4)PP). Both enzymes incorporated dGMP, dAMP and dTMP opposite the 3’-C of TC-(6-4)PP (Figure 4B, TC-(6-4)PP). Thus, the selectivity of correct nucleotide on the template containing (6-4)PP was lower than on the template containing CPD.

It should be noted that on the template containing no damage, both dpolηΔC and dpolηΔC incorporated not only A but also G opposite T (Figure 4A, no damage). Misincorporation of G opposite T is a common feature of the polη and polt family, and especially hpolτ incorporates G opposite T more efficiently than A opposite T (10, 13, 27, 37). However, dpolηΔC misincorporates G opposite T less often than it correctly incorporates A opposite T.

**Influence of base pairing at the lesion on elongation past the lesion by dpolηΔC and dpolηΔC**

The results shown in Figure 4 indicate that dpolηΔC and dpolηΔC can insert a variety of bases opposite the 3’ base of TT-CPD, TT-(6-4)PP or TC-(6-4)PP. However, it is possible that the misincorporated base may not be extended as efficiency as the correctly incorporated base is, as shown in the case of hpolη for the bypass of various lesions (7). To explore this question, primers were synthesized in which each of the four base would be correctly or incorrectly paired with the 3’ or 5’ base of the above three lesions. First, the ability of dpolηΔC to elongate such primers annealed to TT-CPD was tested and the
results are shown in Figure 5. dpol\(\eta\Delta C\) elongated DNA chains more efficiently from the correctly base paired primers, the 17mer with a 3’-terminal A opposite 3’ T of TT-CPD (Figure 5B) and the 18mer with AA opposite the lesion (Figure 5D). Similar results were obtained with dpol\(\Delta C\) (data not shown).

Next, such primers were annealed to TT-(6-4)PP or TC-(6-4)PP to examine whether they could be extended by dpol\(\eta\Delta C\). On a template with TT-(6-4)PP and a 17mer primer whose 3’-terminal nucleotide pairs with the 3’-T of the lesion, dpol\(\eta\Delta C\) elongated the DNA chain more efficiently from a primer with a 3’-terminal G (G17) than from a primer with a 3’-terminal A (A17) (Figure 6A). This makes a sharp contrast with the above result that in the case of TT-CPD, dpol\(\eta\Delta C\) carried out the elongation more efficiently from the A17 primer than from the G17 primer (Figure 5B). To examine which base is incorporated after the G residue opposed the 3’-T of TT-(6-4)PP, 18mers with GN (N, any base) opposite the lesion were synthesized and examined for the extension by dpol\(\eta\Delta C\). The result showed that the enzyme elongated the DNA chain most efficiently from the primer with GA opposite the lesion (Figure 6B). However, on a template with TC-(6-4)PP, dpol\(\eta\) did not show selective elongation from a specific 3’ end; it elongated the DNA chain with equal efficiency from 17mers with 3’-terminal A or G opposite the 3’-C of TC-(6-4)PP and from 18mers terminating with AA, AG, GA, or GG opposite the lesion (data not shown). Thus, the ability of dpol\(\eta\) to recognize the correctly inserted base opposite a lesion site is lost more severely in TT-(6-4)PP and TC-(6-4)PP than in TT-CPD.

*Direct analysis of bypass products synthesized by dpol\(\eta\Delta C\) and dpol\(\Delta C\)*
The above results suggest that dpolιΔC incorporates a significant level of incorrect nucleotides during bypass of (6-4)PP; and in contrast, correct nucleotides are selectively incorporated opposite CPD by dpolιΔC and dpolιΔC. To know whether dpolιΔC and dpolηΔC incorporate correct nucleotide opposite each damage, the bypass products were analyzed directly (Figure 7). For this experiment, three kinds of oligonucleotides containing TT-CPD, TT-(6-4)PP, or TC-(6-4)PP were synthesized. TT-CPD and TT-(6-4)PP were positioned at the TT of the TTAA sequence, and TC-(6-4)PP was positioned at the TC of the TCGA sequence, which are the recognition sequence for the restriction enzymes MseI and TaqI, respectively. If the bypass products by dpolηΔC or dpolιΔC have the correct AA or GA sequence, then they should be cleaved by MseI or TaqI after rehybridization with a non-damaged oligonucleotide; the product of this cleavage reaction can be readily detected as a 19 or 14 base fragment, respectively (Figure 7A). Almost all of the bypass products of TT-CPD by dpolηΔC or dpolιΔC were cleaved by MseI (Figure 7B, lanes 9-12); more than 90% of the bypass products have the AA sequence (Table 1). On the other hand, only some of the bypass products of either TT-(6-4)PP or TC-(6-4)PP were susceptible to restriction enzyme digestion (Figure 7B, lanes 15, 16, 19 and 20). The ratio of the correct base incorporated opposite (6-4)PP is much lower than for the CPD. Of the bypass products of TT-(6-4)PP by dpolηΔC, 22.6% are susceptible to MseI digestion, and 36% of the TC-(6-4)PP bypass products are susceptible to TaqI digestion (Table 1), indicating that bypass of (6-4)PP by dpolη is highly error-prone.

**DISCUSSION**
Damage-induced mutagenesis is an important biological process whose molecular mechanism is not yet understood, and which is the subject of much current research. Recently, the proteins of the UmuC/DinB/Rev1/Rad30 superfamily have been isolated and characterized biochemically, and it was shown that these proteins have a nonprocessive DNA polymerase activity that can bypass DNA lesions. Thus, these enzymes are central to the process of damage-induced mutagenesis. In this study, *Drosophila* homologues of this polymerase superfamily, dpol\(\eta\) and dpol\(\iota\), were identified and characterized with respect to their ability to bypass UV-induced DNA lesions.

*S. cerevisiae* and human pol\(\eta\) are considered to bypass TT-CPD in a mostly error-free manner (7, 15, 21). Similarly, dpol\(\eta\)\(_{\Delta C}\) and dpol\(\iota\)\(_{\Delta C}\) incorporate AA opposite TT-CPD more efficiently than other nucleotides (Figure 4A) and the elongation starts selectively from a primer ending in A opposite the lesion (Figure 5B and D). These results suggest that dpol\(\eta\)\(_{\Delta C}\) and dpol\(\iota\)\(_{\Delta C}\) bypass TT-CPD in a error-free manner as in the case of hpol\(\eta\). In addition, direct analysis of the products of lesion bypass also indicates that dpol\(\eta\)\(_{\Delta C}\) and dpol\(\iota\)\(_{\Delta C}\) carry out error-free bypass of TT-CPD (Figure 7). Thus, for these enzymes, TT-CPD maintains a structure that directs correct Watson-Crick base pairing, in spite of the base modification. In fact, Lawrence and co-workers have argued that the CPD must be an instructive lesion by virtue of its high coding specificity in *E. coli* (38) and structural studies have shown that the Watson-Crick base pair is still intact at the CPD site (39 - 41). Therefore, the TLS polymerases bypass this lesion by incorporating the correct bases on this instructional lesion.

However, evidence also indicates that dpol\(\eta\)\(_{\Delta C}\) does not carry out error-free bypass of (6-4)PP lesions. On templates with TT-(6-4)PP, dpol\(\eta\)\(_{\Delta C}\) did not show
selective elongation from the correct base-paired primer/template (Figures 6A).

Furthermore, the bypass products of TT-(6-4)PP by dpolΔC showed high levels of
misincorporation (Figure 7B). dpolΔC elongated DNA efficiently from the 17mer end
with a mismatched G opposite the 3’-T of TT-(6-4)PP (Figure 6A) and preferentially
from the 18mer with GA opposite the lesion (Figure 6C). The favored elongation from
the mismatched GA/TT-(6-4)PP terminus is consistent with structural studies. An NMR
study of a duplex DNA with (6-4)PP revealed that TT-(6-4)PP greatly distorts the DNA
structure and prevents base pairing with A in the complementary strand (41). However, a
mismatched base pair between the 3’-T of TT-(6-4)PP and G forms hydrogen bonds and
stabilizes the helix (42, 43). Furthermore, the G/T mispair at the 3’-T of TT(6-4)PP
retains the normal Watson-Crick type base pairing between the 5’-T of the TT-(6-4)PP
and an opposed A, and the resultant GA/TT-(6-4)PP duplex can assume the typical B-
form-DNA conformation (44). Our results also fit well with the specificity of base
alteration of UV-induced mutations in which a G residue is preferentially inserted
opposite the 3’-T of the (6-4) photoproduct during TLS in E. coli (19). These results
indicate that the TT-(6-4) photoproduct can be classified as a mis-instructional lesion.

In spite of the biological relevance of this DNA lesion, the tertiary structures of
oligonucleotide duplexes which include TC-(6-4)PP have not been well characterized.
Fujiwara and Iwai (1997) showed that the duplex containing G opposite the 3’-C of
TC-(6-4)PP is thermodynamically more stable than another duplex (42). Horsfall and
Lawrence (1994) have studied the mutagenecity of TC-(6-4)PP in E. coli cells and found
that 66% of the bypass products have the correct TC sequence, 28% of the mutants
having the TT sequence (20). Our observation that both G and A are incorporated
opposite the 3’-C of TC-(6-4)PP (Figure 4B) fit well with these results. On the other
hand, the nucleotide incorporated opposite the 5’-T of TC-(6-4)PP is not consistent with
the mutation spectrum. While the in vivo mutation spectrum indicates that A is
incorporated preferentially opposite the 5’-T of TC-(6-4)PP, our in vitro results indicated
that dpolηΔC elongated the DNA chain equally well from the 18mer primer with A or G opposite the 5’-T (data not shown). It will be necessary to determine the sequence of the bypass products directly or perform steady-state kinetics to clarify this point.

The mutagenic properties of dpolηΔC and dpolτΔC fit well with the mutation spectrum obtained using a defined photoproduct introduced into SOS-induced *E. coli* (19, 38). The mutation spectrum reported in those studies reflects the mutagenic properties of pol V (UmuD’₂C complex). Thus, the mutagenic properties of dpolηΔC on TT-CPD and TT-(6-4)PP are very similar to those of pol V. Both enzymes bypass TT-CPD in an error-free manner and incorporate GA opposite TT-(6-4)PP cells ((8) and this study). The similarity in the nucleotides incorporated suggests that structural information in the altered bases contribute to nucleotide selection during incorporation opposite these lesions by these polymerases.

In the Rad30 protein family, dpolηΔC seems to be an unusual member of the Rad30 family in having the ability to bypass (6-4)PP, although we cannot rule out a possibility that having a N-terminal GST tag and a C-terminal truncation have affected the TLS specificity and activity and we also have to keep in mind that we cannot draw a quantitatively definitive conclusion since the observations present in this study are qualitative. It should also be noted that the DNA repair capacity of *Drosophila* cells is somewhat different from that of human cells. The most striking difference is that *Drosophila* cells have a (6-4)photolyase, which specifically repairs (6-4)PP in a light-dependent manner, but human cells lack this enzyme (45). The (6-4)PP lesions are removed rapidly by nucleotide excision repair. However, a small amount of the lesion might remain which would be toxic because (6-4)PP is highly mutagenic. The remaining (6-4)PP lesions can be removed by the (6-4)photolyase in *Drosophila* cells, and thus the error-prone bypass activity of dpolη might not be so deleterious. On the other hand,
human cells lack the (6-4)photolyase, and thus more lesions might remain, so that error-
prone bypass of this lesion would have a deleterious effect. Thus, hpol\(\eta\) might have lost
the ability to bypass (6-4)PP because that loss enhances genetic stability.
Paradoxically, we have recently shown that in human cells, mutations are produced at the
(6-4)PP site, although the frequency is low (46). Thus, in human cells some DNA
polymerases bypass the (6-4)PP in an error-prone manner. Another enzyme, for example
hpolt, might bypass (6-4)PP in vivo, in conjunction with pol\(\zeta\) (27).

In this study, TT(6-4)PP was bypassed by dpol\(\eta\)\(\Delta\)C but not by dpolt\(\Delta\)C. The
TLS activity of dpol\(\eta\)\(\Delta\)C and dpolt\(\Delta\)C was also examined on chemically modified
damage, and all of the other lesions tested were bypassed by both enzymes (data not
shown). Thus, at present we cannot assign any specific biological function to dpolt.
Further analyses of the substrate specificity of these polymerases, by screening various
types of DNA lesions, may reveal differences in their substrate specificity. Other
possibility is that each polymerase interacts with other protein differently in vivo. The
recombinant protein used in this study has a C-terminal deletion. The deleted C-terminal
portion might have an important biological interaction with other protein in vivo, while it
is not required for polymerase activity in vitro. It is also possible that dpol\(\eta\) and dpolt
have different in vivo functions in Drosophila that have not been detected by in vitro
studies carried out to date. One enzyme may be important for the defense against DNA
damage and another may be involved in some biological process that requires DNA
replication on a primer/template complex containing an unusual mismatch. Detailed
analyses of the expression pattern of each gene at the tissue and cellular levels will
provide clues to the function of each gene. Furthermore, the phenotypes of transgenic
flies that over-express these genes and mutant flies in which each gene is disrupted by an
insertion or repressed by RNAi, will clarify the biological roles of these proteins in

*Drosophila*.

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**FOOTNOTES**

The abbreviations used are: CPD, cis-syn cyclobutane pyrimidine dimer; (6-4)PP, pyrimidine-pyrimidone (6-4) photoproduct; bp, base pair; kb, kilobase pair; GST, glutathione S-transferase.
FIGURE LEGENDS

Figure 1. Deduced amino-acid sequences of the dRAD30A and dRAD30B proteins. (A) Schematic representation of the conserved regions in the dRAD30A and dRAD30B proteins. The conserved regions I-V are indicated. Schematic representations of the carboxyl terminal deletion mutants are also shown, and the sites of the conserved DE sequences where the mutations were introduced are indicated by arrows. (B) Alignment of the region III amino acid sequences of the Rad30A proteins from *H. sapiens* (XPV), *A. thaliana* (AtRad30), *C. elegans* (CeRad30), *S. cerevisiae* (ScRad30), *S. pombe* (SpRad30), and *D. melanogaster* (DRad30A), and the Rad30B proteins from *H. sapiens* (hRad30B) and *D. melanogaster* (DRad30B). The alignment was performed using the MegAlign program (DNA star Inc., Madison, WI). The exact locations of the amino acids in each protein are indicated at the left-hand side of the figure. Residues identical to dRAD30A or dRAD30B are indicated in black. The conserved DE sequence, within which mutations are produced, is marked with asterisks below the sequence. (C) Possible phylogenetic tree of the DinB/UmuC/Rev1/Rad30 family proteins. The accession numbers in the Swiss-Prot and GENBANK for the sequences and the species names are as follows: *E. coli* (P04152), *A. thaliana* (T19K24.14 in AC02342), *C. elegans* (ZK675.2 in Z46812), *S. cerevisiae* (P12689), *S. pombe* (SPBC1347.01c in AC035548), *H. sapiens* (HsREV1, AF151538), *M. musculus* (MmREV1, AF179302), *D. melanogaster* (DmREV1, AB049435), *E. coli* (Q47155), *H. sapiens* (HsDINB1, AA576919), *M. musculus* (MmDINB1, AB027563), *S. pombe* (SPCC553.07c), *C. elegans* (F22B7.6), *A. thaliana* (T19K24.15 in AC02342), *C. elegans* (F53A3.2 in AF025460), *H. sapiens* (HsRAD30A (XPV) (H. sapiens, AB024313 and AF158185), *M. musculus* (MmRAD30A, AB027128), *D. melanogaster* (AB049433), *S. cerevisiae* (ScRAD30 (S. cerevisiae, S69702), *S. pombe* (SpRAD30 (S. pombe,
SPBC16A3.11 in AL021748), HsRAD30B (*H. sapiens*, AF140501), MmRAD30B (*M. musculus*, AF151691), DmRAD30B (*D. melanogaster*, AB049434).

Figure 2. Detection of DNA polymerase activity. (A) An SDS-PAGE analysis of the purified dRAD30AΔC and dRAD30BΔC protein is shown. Lane 1, molecular weight marker. 0.5 µg each of GST-dRAD30AΔC (lane 2), GST-dRAD30A-DE126AA (lane 3), GST-dRAD30BΔC (lane 4), and GST-dRAD30B-DE115AA (lane 5) were applied. The dRAD30AΔC and dRAD30BΔC proteins are indicated by the arrow. (B and C) DNA polymerase activities of dRAD30AΔC (B) and dRAD30BΔC (C). Three or five different amounts of dRAD30AΔC, dRAD30A-DE126AA, dRAD30BΔC, and dRAD30B-DE115AA (10, 100, and 500 fmol in lanes 2, 3, and 4, respectively and 0.1, 1, 10, 100, and 500 fmol in lanes 5, 6, 7, 8, and 9, respectively) were added in the reaction mixture. Control reactions with no enzyme are shown in lane 1.

Figure 3. Translesion synthesis by dpolηΔC and dpolιΔC. Increasing amounts of dpolηΔC (10, 100, and 500 fmol in lanes 2, 3, and 4, respectively) and dpolιΔC (30, 300, and 1500 fmol in lanes 6, 7, and 8, respectively) were incubated with the 5'32P-labeled primer-template indicated above the panel for 15 min at 37 °C in the standard reaction mixture. The products were subjected to polyacrylamide gel electrophoresis and the autoradiograms of the gel are shown. The DNA lesions on the templates are TT-CPD (A), TT-(6-4)PP (B), and TC-(6-4)PP (C). Control reactions with no enzyme are shown in lanes 1 and 5.
Figure 4. Selectivity of dpolη∆C and dpolτ∆C nucleotide incorporation opposite the lesions. dpolη∆C (20 fmol; upper panels) and dpolτ∆C (100 fmole; lower panels) were incubated with a 30mer template containing either a TT-(6-4)PP (A) or a TC-(6-4)PP (B) annealed to 5'-32P-labeled 16mer primer with one of the indicated dNTPs. The sequence of the template and the primer are the same as that used in Figure 3 and the partial sequences of the 3' ends of each primer and template are shown above each panel. No damage (TT) or (TC) are undamaged controls of TT-CPD and TT-(6-4)PP or TC-(6-4)PP, respectively.

Figure 5. Ability of dpolη∆C to elongate DNA chains past TT-CPD. Sets of 5'-32P-labeled 17mers (N17mer, A and B) or 18mer (AN18mer, C and D) primers, which contain different sequences at their 3' ends (indicated by N, where N is A, C, G or T as seen beneath each panel), were annealed to the 30mer template. Increasing amounts of dpolη∆C were incubated with these primed templates. The results of a set of three reactions for each A, C, G and T primer are shown. Each set contained three reactions with different amounts of enzyme; the one shown on the left (-) contained no enzyme and the middle and right ones contained increasing amounts of enzyme; 1 and 5 fmole (A and C), and 2.5 and 12.5 fmol (B and D). The template containing no damage (A and C) or TT-CPD (B and D), respectively. The autoradiograms of the gels are shown.

Figure 6. Ability of dpolη∆C to elongate DNA chains past TT-(6-4)PP. Sets of 5'-32P-labeled 17mers (N17mer, A) or 18mers (GN18mer, B) were annealed to the 30mer template. In GN18mer, G is opposite the 3'-T of (6-4)PP and N is opposite the 5'-T of (6-4)PP. Conditions are the same as in Figure 5, except for the amount of enzyme; middle and right lanes contain 50 and 250 fmol, respectively.
Figure 7. MseI or TaqI cleavage assay of the TLS bypass products by dpolηΔC and dpolΔC. (A) A schematic drawing of the experimental protocol of the MseI cleavage assay is shown. In TaqI assay, 30 mer substrate was used and a cleavage of the substrate produced a 14 base fragment. (B) The 49mer oligomer with no lesion (lanes 1 - 6), a TT-CPD (lanes 7 - 12), or a TT-(6-4)PP (lanes 13 - 16), or the 30mer oligomer with TC-(6-4)PP (lanes 17 - 20) were annealed to a 5′-32P-labeled primer and then were mixed with Klenow fragment (2U in lanes 1, 2, 7, 8, 13, 14, 17, and 18), dpolηΔC (500 fmol in lanes 3, 4, 9, 10, 15, and 16 or 1 nmol in lanes 19 and 20) or dpolΔC (500 fmol in lanes 5, 6, 11, and 12). The reaction products were denatured by heating at 95°C for 5 min and then excess amounts of the 49mer oligonucleotide (lanes 1 - 16) or the 30mer oligonucleotide (lanes 17 - 20), which does not contain a DNA lesion, were added and annealed to the newly synthesized DNA. The resultant duplex DNA was cleaved with MseI or TaqI, respectively, denatured in the presence of formamide, and then electrophoresed on a sequencing gel. The autoradiograms of the gels are shown.
Table 1. Ratio of bypass products with normal sequences.

|                  | % Restriction enzyme sensitive bypass products a, b, c |
|------------------|-------------------------------------------------------|
|                  | Klenow       | dpolηΔC | dpolΔC |
| no damage        | 84.2%        | 82.5%   | 80.3%  |
|                  | (1)          | (1)     |        |
| TT-CPD           | n.d.         | 77.9%   | 77.5%  |
|                  | (0.944)      | (0.965) |        |
| TT-(6-4)PP       | n.d.         | 22.6%   | n.d.   |
|                  | (0.274)      |         |        |
| TC-(6-4)PP       | n.d.         | 36.0%   | n.d.   |
|                  | (0.436)      |         |        |

a. Values are the average of counts of 19 base or 14 base fragments after digestion with *Mse* I or *Taq* I respectively, and are normalized to those of the undigested bypass products (see Materials and Methods). Data are the average of two independent experiments.

b. Values in parentheses are normalized to data for the no damage control of each enzyme.

c. n.d.: not determined.
Motif III

hRAD30A (XPV) 86 KANLTKYREASVEV----------MEIMSRFAVIERASIDEAYVDLTD
AtRAD30 91 KADLNLYRSAGSPVDGSGYYTVCVWSILAKSGKCEIRASIDEAYVDLTD
CeRAD30 85 KADIKYRDAASAY----------FVPNYYDSQIIIEIRASIDEAYVDLTD
ScRAD30 125 KVSLEPYRESRKA----------LKIFKSACDLVERASIDEAYVDLTD
SpRAD30 117 KTCLDPYRESHVKI----------LINIIKHA--PVVKAIDEYCFIELT
dRAD30A 95 KADTSKYRDAKRSV----------ANVLOFTQ--DLERASIDEAYVDLTD

**

hRAD30B 96 GEDLTRYREMSYKV----------TELIEPS--PVVERLGFENDFVDLTD
dRAD30B 83 GEDLAPYROMSQRI----------FDIIITY--PLVEKLGFDENDMDVT

**

Ishikawa et al. Fig. 1B
A

Marker
dRAD30AΔC
dRAD30A-DE126AA
dRAD30BΔC
dRAD30B-DE115AA
(kDa)

97.4
66.2
45

1 2 3 4 5

dRAD30A

dRAD30B

B

dRAD30A

dRAD30A

DE126AA
ΔC

1 2 3 4 5 6 7 8 9

C

dRAD30B

dRAD30B

DE115AA
ΔC

1 2 3 4 5 6 7 8 9

no damage (TT)

Ishikawa et al. Figure 2
5’-CACTGACTGTATGATG 3’-GTGACTGACATACTACTATCTACGACTGCTC-5’

TT-CPD or TT-(6-4)PP

5’-CACTGACTGTATGATG 3’-GTGACTGACATACTACTTCCTACGACTGCTC-5’

TC-(6-4)PP

A TT-CPD

B TT-(6-4)PP

C TC-(6-4)PP

Ishikawa et al. Figure 3
A. \[ \text{dNTP} \quad \begin{array}{cccc} 0 & A & C & G & T \end{array} \quad \begin{array}{cccc} A & C & G & T \end{array} \quad \begin{array}{cccc} A & C & G & T \end{array} \]

\[
\begin{array}{cccc}
\text{template} & \text{no damage} & \text{CPD} & (6-4)PP \\
\end{array}
\]

\[
\begin{array}{cccc}
\text{dPol} & \Delta C \\
\text{TT} \quad 5' - G \\ 3' - \text{CTCTA-5'} \\
\end{array}
\]

B. \[ \text{TC} \quad 5' - G \\ 3' - \text{CTCTA-5'} \]

\[
\begin{array}{cccc}
\text{template} & \text{no damage} & \text{CPD} & (6-4)PP \\
\end{array}
\]

\[
\begin{array}{cccc}
\text{dNTP} & \quad A & C & G & T \quad A & C & G & T \\
\end{array}
\]

\[
\begin{array}{cccc}
\text{template} & \text{no damage} & \text{CPD} & (6-4)PP \\
\end{array}
\]

\[
\begin{array}{cccc}
\text{dPol} & \Delta C \\
\end{array}
\]
TT-CPD

A no damage

Primer: N17m

B

-30

N= A C G T

$\text{[32P]}$-CTAGCTG ATG TGN

3'---GAGCAGATCTTTACGACGCT

5'---N

3'---TT---5'
TT-(6-4)PP

A

(6-4)PP

Primer: N17mer

5'3'
AATT

42 base uncleaved fragment

19 base cleaved fragment

Ishikawa et al. Figure 7A
Mutagenic and non-mutagenic bypass of DNA lesions by Drosophila DNA polymerases dpol and dpol<IMG SRC="/math/eta.gif" ALIGN="BASELINE" ALT="eta ">
and dpol<IMG SRC="/math/iota.gif" ALIGN="BASELINE" ALT="iota ">
Tomoko Ishikawa, Norio Uematsu, Toshimi Mizukoshi, Shigenori Iwai, Hiroshi Iwasaki,
Chikahide Masutani, Fumio Hanaoka, Ryu Ueda, Haruo Ohmori and Takeshi Todo

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