Published in final edited form as:
DNA Repair (Amst). 2007 February 4; 6(2): 213–223.

A unique error signature for human DNA polymerase ν

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Abstract

Human DNA polymerase ν is one of three A family polymerases conserved in vertebrates. Although its biological functions are unknown, pol ν has been implicated in DNA repair and in translesion DNA synthesis (TLS). Pol ν lacks intrinsic exonucleolytic proofreading activity and discriminates poorly against misinsertion of dNTP opposite template thymine or guanine, implying that it should copy DNA with low base substitution fidelity. To test this prediction and to comprehensively examine pol ν DNA synthesis fidelity as a clue to its function, here we describe human pol ν error rates for all 12 single base-base mismatches and for insertion and deletion errors during synthesis to copy the *lacZ* α-complementation sequence in M13mp2 DNA. Pol ν copies this DNA with average single-base insertion and deletion error rates of 7×10^{-5} and 16×10^{-5}, respectively. This accuracy is comparable to that of replicative polymerases in the B family, lower than that of its A family homolog, human pol γ, and much higher than that of Y family TLS polymerases. In contrast, the average single base substitution error rate of human pol ν is 3.5×10^{-3}, which is inaccurate compared to the replicative polymerases and comparable to Y family polymerases. Interestingly, the vast majority of errors made by pol ν reflect stable misincorporation of dTMP opposite template G, at average rates that are much higher than for homologous A family members. This pol ν error is especially prevalent in sequence contexts wherein the template G is preceded by a C-G or G-C base pair, where error rates can exceed 10%. Amino acid sequence alignments based on the structures of more accurate A family polymerases suggest substantial differences in the O-helix of pol ν that could contribute to this unique error signature.

Keywords

error-prone polymerase; infidelity; base substitutions

1. INTRODUCTION

Among the 16 DNA polymerases encoded by the human genome [1–4], three are members of the A family, composed of enzymes with homology to *Escherichia coli* (*E. coli*) DNA pol I. The most extensively studied of these three enzymes is DNA polymerase γ (pol γ). Consistent with its roles in replicating and repairing the mitochondrial genome, pol γ is a highly accurate enzyme due to the high nucleotide selectivity of the polymerase and the presence of an intrinsic

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3′ exonuclease that can proofread mistakes [5]. In fact, high nucleotide selectivity is a property shared by most other A family DNA polymerases, of which the most extensively studied are the prototypical *E. coli* DNA polymerase I [6–9], bacteriophage T7 DNA polymerase [10–12], *Thermus aquaticus* (*Taq)* DNA polymerase [13–16] and *Bacillus* fragment (BF) DNA polymerase [17,18]. The structural and kinetic bases for the high nucleotide selectivity of these A family enzymes have been studied in detail [7–15,17–25] and reviewed in [8,26–30], providing a wealth of information for comparison to more recently discovered A family members whose biological functions are currently unknown.

Family A members include two other human enzymes of unknown function, pol θ [31–33], and the subject of the present study, pol ν. Human pol ν was first described three years ago [34], as an A family DNA polymerase that is conserved in vertebrates but not in invertebrates, yeast or fungi. On that basis, it was proposed that pol ν could have a role related to organ systems that are especially developed in vertebrates. As one approach to investigate the possible functions of pol ν *in vivo*, several biochemical properties of pol ν have been described [34, 35]. Pol ν has moderate processivity, it has efficient strand displacement activity, and it can perform translesion synthesis past a thymine glycol in an oligonucleotide template [35]. Pol ν lacks intrinsic 3′ exonuclease activity and is therefore unable to proofread its own mistakes. This inability to proofread is not unprecedented among A family polymerases; e.g., *Taq* DNA polymerase [13] and BF DNA polymerase [17] are also 3′ exonuclease-deficient polymerases. Nonetheless, *Taq* and BF polymerases have high nucleotide selectivity [13–18]. In contrast, a recent kinetic analysis of single nucleotide incorporation at two template locations has demonstrated that pol ν discriminates very poorly against misinsertion of dTTP and dGTP opposite template G or T in an undamaged DNA oligonucleotide [35]. If the resulting T-G and T-T mismatches, or any others not yet examined, were extended by naturally proofreading-deficient pol ν, then synthesis by pol ν would be error prone for base substitutions, thus representing an exception among usually highly accurate A family polymerases. Here we test this prediction using an assay that monitors a wide variety of DNA synthesis errors in numerous sequence contexts. The results reveal that pol ν is the least accurate A family enzyme studied to date. For base substitutions, the low fidelity primarily pertains to one particular mismatch that is preferentially generated in certain sequence contexts. We discuss the possibility that this unusual error specificity may result from differences in the geometry of the nascent base pair binding pocket of pol ν, perhaps as determined by its O-helix, whose putative amino acid composition varies from those of more accurate A family members.

2. MATERIALS AND METHODS

Enzymes, reagents, strains

Recombinant human DNA pol ν was purified to near homogeneity as described [35]. The exonuclease-deficient, large Klenow fragment of *E. coli* DNA pol I and T4 polynucleotide kinase were purchased from New England BioLabs. [γ-^{32}P]ATP (4500 Ci/mmol) and unlabeled deoxyribonucleotide-triphosphates were from GE Healthcare Biosciences. All strains and materials for processivity analysis [36] and the M13mp2 fidelity assay were from sources described previously [37].

Processivity Analysis

Processivity was monitored using single-stranded M13mp2 DNA primed with a [γ^{32}P]ATP 5′ end labeled DNA oligonucleotide complementary to positions +173 to +192 of the *lac*Zα coding sequence. Reaction mixtures (30μl) for pol ν contained 20 mM Tris-HCl (pH 8.8), 8 mM magnesium acetate, 2 mM dithiothreitol, 4% glycerol, 80μg/ml bovine serum albumin and 100μM each dATP, dCTP, dGTP and dTTP. Reaction mixtures for exonuclease-deficient Klenow fragment (Klenow fragment exo-) pol contained 10 mM Tris (pH 7.5), 7.5 mM

dithiothreitol, 10 mM $MgCl_2$ and 25μM each dATP, dCTP, dGTP and dTTP. Both reaction mixtures contained primed M13mp2 ssDNA substrate in excess (200 fmol) of pol ν (40 fmol) or Klenow fragment exo- pol (160 fmol). Reaction mixtures were incubated at 37°C and 10μl aliquots were removed at 3, 6 and 9 min for pol ν and at 3 and 5 min for Klenow fragment exo- pol. Samples were mixed in a 1:1 ratio with stop buffer (99% formamide, 5mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue) and DNA products were analyzed by electrophoresis on a 12% denaturing polyacrylamide gel. Products were quantified by phosphorimagery using a Molecular Dynamics Typhoon 9400 and the ImageQuant software. Conditions were such that termination probabilities reflected one cycle of processive synthesis. The termination probability for each template position is defined as the ratio of products of a given length to the sum of that product plus all longer DNA products [38].

M13mp2 Fidelity Assay

DNA synthesis reaction mixtures for pol ν (25μl) were performed as described above but contained 0.2 nM M13mp2 (407-nucleotide gap) DNA substrate (from nucleotide −216 through +191 of *lac*Z gene) and 1 mM each dATP, dCTP, dGTP and dTTP. Polymerization reactions were initiated by the addition of pol ν (46 nM), incubated at 37°C for 30 min and terminated by the addition of 20 mM EDTA. Half of the reaction mixture was mixed with 3μl of SDS buffer (20 mM Tris pH 8.0, 5 mM EDTA, 5% SDS, 0.5 % bromophenol blue and 25% glycerol) and analyzed by agarose gel electrophoresis [37]. Pol ν reactions completely filled the gap (e.g., Fig. 1A). DNA products were also examined for the frequency of *lac*Z mutants by electroporation and plated onto indicator plates as previously described [37]. Errors were scored as light blue or colorless mutant plaques, while correct synthesis yields plaques that are dark blue. DNA from independent mutants was sequenced in order to identify the errors made during gap-filling synthesis. For sequence changes that yield light blue and colorless plaques, error rates per detectable nucleotide incorporated were calculated as described [37]. Error rates were also calculated by simply dividing the number of observed mutations of a particular type by the total number of nucleotides sequenced in the *lacZ* clones, a calculation used previously for particularly inaccurate polymerases [39].

3. RESULTS AND DISCUSSION

Fidelity of human pol ν in the M13mp2 forward mutation assay

We measured the fidelity of human pol ν during synthesis to fill a 407-nucleotide single-stranded template encoding the *lacZ* α-complementation gene within gapped M13mp2 DNA. In this assay, polymerization errors are detected as light blue and colorless *lacZ* mutant plaques among dark blue plaques resulting from correct synthesis. Since the assay monitors loss of a gene function that is not essential for M13 plaque formation, it scores a broad range of errors, including all 12 possible single-base substitutions in a variety of sequence contexts, additions and deletions of many different template nucleotides, and more complex errors [37]. The fidelity of DNA synthesis by other DNA polymerases has been determined using this assay, allowing comparisons to pol ν.

The products of two independent gap-filling synthesis reactions by pol ν (e.g. see Fig. 1A) yielded an average *lacZ* mutant frequency of 18%, (see Table 1 for mutant frequencies for independent experiments). These values are higher than observed for synthesis by other exonuclease-deficient A family polymerases, including Klenow fragment, *Taq*, bacteriophage T7 and pol γ. To determine the types and locations of errors generated by pol ν, 162 independent *lac*Z mutants were sequenced. The results (Table 1 and Figure 1B,C) were used to calculate specific error rates (Tables 1–4) and these were compared to the error rates of other polymerases (Table 2). The types of errors observed are discussed here in inverse order of prevalence.

Deletions and insertions of multiple nucleotides

Among 162 *lacZ* mutants sequenced, several contained deletions and/or insertions of multiple bases. Four mutants harbored a 317 base deletion that included loss of one of two direct repeats of 5′-CCCGC plus the intervening 312 bases. This deletion has been observed in the error spectra of several other DNA polymerases [40–42]. It can be explained by a model in which the first repeat sequence encountered by pol ν is copied, then the primer frays and relocates to the second repeat sequence to create a duplex primer-template for continued polymerization. This results in a misaligned intermediate that contains a loop of unpaired template strand bases that are eventually deleted. The high frequency with which pol ν generates this particular deletion ($4/162 \times 18\% = 4.4 \times 10^{-3}$) may be facilitated by an inverted repeat of three G-C base pairs present at the deletion junction that could stabilize the misaligned intermediate through formation of a stem at the base of the single-stranded loop (see Figure 2 in [41,43]). The ability to delete large numbers of nucleotides is consistent with the possibility that pol ν may contribute to larger forms of genome instability than simple point mutations. Other multiple-base errors include a 6-base deletion (+137 through +143), one large, complex error wherein 349 bases (−183 through +166, where +1 is the first transcribed base of the *lacZ* gene) were replaced with ‘GAC’, and another clone with ‘AC’ inserted at +172.

Error rates for single base insertions and deletions

Among many single base errors made by pol ν, a minority were single base additions and deletions (Figure 1C). These were generated at rates of 7.0×10^{-5} and 16×10^{-5}, respectively (Table 1). The single base deletion error rate for pol ν is 3–5 fold higher than those for exonuclease-deficient B family polymerases (Table 2) and ~20-fold higher than for exonuclease-deficient pol γ. On the other hand, the error rate is ~10-fold lower for pol ν compared to Y family pol η and pol κ (Table 2). The number of occurrences of additions and deletions is too few to suggest which of several possible mechanisms (reviewed in [44]) might be responsible for generating these deletion and addition errors, other than to note that a substantial proportion of these errors occur at non-repetitive sequences, and therefore do not result from classical strand slippage [35].

Error rates for single base substitutions

A total of 673 single base substitutions were recovered among 162 *lacZ* mutants sequenced (Table 1, Figure 1B,C). Of these, 226 were substitutions known to individually result in reduced plaque color (those depicted by the red letters in Figure 1C). From this information, the average base substitution error rate per phenotypically detectable nucleotide incorporated by pol ν was calculated to be 3.5×10^{-3}. A slightly higher error rate (1×10^{-2}) was calculated (see Materials and Methods) for all substitutions regardless of phenotype (i.e., detectable changes plus “silent hitchhikers”). These average error rate values indicate that pol ν is less accurate than other exonuclease-deficient DNA polymerases that have been examined in this same fidelity assay (Table II). For example, pol ν is about as inaccurate as Y family pol η and pol κ (Table II) that are implicated in TLS, consistent with the suggestion that pol ν may participate in bypass of lesions that have abnormal geometry [14]. In fact, it was because those TLS reactions [35] were conducted at pH 8.8 that we performed the current fidelity measurements at that same pH. Note however, that the base substitution error rates of several polymerases have been shown to vary with pH. For example, it has been demonstrated that the fidelity of two A family enzymes, *Taq* DNA pol [45] and the large Klenow fragment of *E. coli* pol I [35] decreases by several fold with increasing pH. Thus, when we succeed in obtaining more substantial quantities of purified pol ν, we plan to investigate pol ν fidelity and error specificity at lower pH, which may be more physiologically relevant and may reveal higher fidelity and/or altered specificity.

Mismatch and sequence context dependence of error prone synthesis by pol ν

Despite the fact that 12 different single base-base mismatches are possible, the vast majority of single base substitutions generated by pol ν (196 of 226 occurrences, 87%, Table 3) result from stable misincorporation (i.e., misinsertion followed by mismatch extension) of dTMP opposite template guanine. Such highly biased error specificity has not been seen before with any other polymerase when examined using this broad-spectrum fidelity assay. This error specificity, measured here in a complete polymerization reaction containing all four dNTPs in direct competition, is consistent with a very high relative rate of misinsertion of dTMP opposite one particular template guanine as determined by kinetic analysis of reactions containing single nucleotides [35]. In the present study, the average error rate for the 22 template guanines at which this error can be scored in the M13mp2 forward mutation assay is 1.7×10^{-2} (Table 2). The error rate for the second most common pol ν error, the reciprocal T-dGMP mismatch, is 1.3×10^{-3} (Table 3). Both rates are higher than for other A family members.

Among many template guanines in the *lacZ* template where the G-dTMP error can be scored, the error rate varies by more than 30-fold, from 0.6% at template guanine number −66 to an amazingly high 20% at template guanine +165 (Table 4 and Figure 1C). Very high rates for G to A substitutions were also seen at more than 20 phenotypically silent guanines (black letters above target sequence see Figure 1C). Given these hotspots and the wide variation in rates, we tested whether the error rate for the responsible G-dTMP mismatch might be higher or lower at sites where processive synthesis by pol ν terminates. Pol ν has previously been demonstrated to have moderate processivity similar to that of its A family homolog, the large Klenow fragment of *E. coli* DNA polymerase I [34]. That observation was confirmed here during processive synthesis to copy the *lacZ* template (Figure 2A). Moreover, the relative intensities of termination bands generated at some template positions (e.g., those bracketed in Figure 2A) during processive synthesis by pol ν (lanes 2–4) and Klenow fragment Exo- pol (lanes 6–7) differ, indicating that these two A family DNA polymerases interact somewhat differently with undamaged primer-templates during DNA synthesis. When we calculated (see Materials and Methods) the probability of termination of processive synthesis at each template base (Figure 2B), no obvious correlation was observed between error rates and termination probabilities for pol ν (Table 4). Nonetheless, when we considered the identity of the bases flanking the template guanines at which errors were observed, the highest rates were observed when the base pair located 3′ to the template guanine was C-G or G-C (Table 4, top), whereas rates were lower when the base pair located 3′ to the template guanine was T-A or A-T. This implies that sequence context-dependent differences in the rate at which pol ν misincorporates dTMP opposite G partly depends on the identity, and perhaps the stability, of the preceding, i.e., primer terminal base pair. We also note that there are some exceptions to this trend (e.g., guanine at −64, Figure 1C) that could indicate more distant and/or complex influences. For example, in 9 of 10 sequences in which the G to A change is prevalent, there is a pyrimidine two nucleotides 5′ to the template G, whereas there is a purine in 7 of 11 sequences in which the G is A change is less prevalent. A similar trend is seen four nucleotides 5′ of the template G. At this position, 9 of 10 nucleotides are purines when the G to A change is prevalent, while 7 of 10 nucleotides are pyrimidines at this location when the G to A change is less prevalent.

Controlling error prone synthesis by pol ν

One important implication of low fidelity synthesis by human pol ν is that its activity in cells should be tightly regulated. This could occur at several levels, including regulating mRNA expression in a tissue specific manner [46–48]. Based on the suggestion that pol ν may participate in TLS and exciting precedents with inaccurate Y family pols involved in TLS [49], it will be interesting to search for protein partners (e.g., polymerase clamps) and for post-translational modification of pol ν. These could perhaps involve residues in the amino terminal half of the pol ν open reading frame, whose functions are yet to be explored. It is also possible

that pol ν fidelity could be modulated in cells, either by accessory proteins that might enhance fidelity or via error correction by extrinsic proofreading [50,51] or by DNA mismatch repair [35]. The ability of pol ν to generate G-dTMP mismatches at a high rate also leads one to wonder if specialized forms of mismatch repair, e.g., initiated by thymine DNA glycosylase, might process pol ν errors in cells.

Implications for possible functions of pol ν

Its moderate processivity and strand displacement activity [35,52] suggest that pol ν operates in transactions requiring more extensive DNA synthesis than those repair pathways (e.g., short patch BER, NHEJ) performed by X family polymerases. In considering what these transactions might be, it is remarkable that pol ν shares several properties in common with Y family pol κ. Both enzymes are moderately processive (Figure 2, [35,53]), both can bypass thymine glycol lesions [52], and both are error prone, especially for G-dTMP and T-dGMP errors (Table 2 and [54]). It is worth noting that, in addition to the idea that both pols may participate in TLS, pol κ has recently been implicated [55] in gap-filling DNA synthesis during nucleotide excision repair (NER). This may include pol κ involvement in gap-filling during specialized NER to repair inter-strand crosslinks in DNA [56,57], a process thought to require lesion bypass [58, 59]. Notably, pol ν shares some sequence homology to *Drosophila melanogaster Mus308*, defects in which lead to sensitivity to killing by DNA crosslinking agents.

The unique error signature of pol ν could simply reflect use of a substrate *in vivo* that in some way mimics the structure of a G-dTMP mismatch. It is also possible that pol ν contributes to error prone DNA synthesis that is selectively advantageous to vertebrates. One currently unexplored possibility is that pol ν might participate in somatic hypermutation of immunoglobulin genes. Interestingly, the pol ν homolog DNA polymerase theta (pol θ) has already been proposed to introduce substitutions at G-C base pairs in immunoglobulin genes [60–62], and the TLS enzyme pol η has been implicated in generating substitutions at A-T base pairs during SHM [22,23,63].

The putative nascent base pair binding pocket of pol ν

Possible explanations for the unusual error specificity of pol ν can be considered in light of substantial structural and functional information on other, more accurate A family polymerases. The crystal structures of T7 DNA pol [17,64], BF DNA pol [15,65] and *Taq* DNA pol [18, 66] all reveal correct dNTPs that are tightly accommodated in a binding pocket at the active site, such that incorrect base pairs are expected to sterically clash. These and other structures involving mismatched bases [26–28], along with substantial biochemical evidence (reviewed in [27]) indicate that these A family members selectively incorporate correct dNTPs based partly on geometric complementarity in the nascent base pair binding pocket. We therefore asked which of the amino acid residues that contribute to the binding pocket in high fidelity A family members are conserved in low fidelity pol ν. The binding pocket of *Taq* pol I that is assembled by correct dNTP-induced conformational changes (Figure 3A) is comprised of a number of amino acids that are highly conserved in bacteriophage T7 DNA pol, Klenow fragment pol I, BF DNA pol I and human pol γ (Figure 3B). Numerous studies (reviewed in [67]), have shown that fidelity is reduced by non-conservative amino acid replacements for certain residues of this binding pocket (shown in blue in Figure 3B). As one example, a single Glu710Ala substitution in KF pol strongly reduces nucleotide selectivity [68,69]. Perhaps cogent to the unusual error specificity of pol ν, replacing KF Glu710 with alanine had an unanticipated and highly unusual error specificity, in that fidelity was reduced only for a subset of the 12 mismatches, especially those involving misinsertion of dTTP and dCTP. Kinetic analysis suggested that the Glu710 side chain in KF pol has an important role in excluding wobble base pairs in the binding pocket. Glu710 in KF pol is conserved as Glu628 in pol ν, and therefore does not itself account for the unique error signature of pol ν, but the Glu710Ala

results nevertheless serve as proof-of-principle that even a single amino acid difference could potentially account for low fidelity involving a subset of mismatches, as reported here for G-dTTP and T-dGTP mispairs generated at high rates by pol ν. Studies with *Taq* also highlight the importance of the O-helix and how perturbations of this helix affect the fidelity of the enzyme. On these bases, we note that among many amino acids that contribute to the binding pocket of the accurate A family polymerases, several that are located in the O-helix that contributes to binding pocket geometry of accurate A family polymerases are not conserved in pol ν (Figure 3B). Probing these differences may be useful for future attempts to understand the remarkable error specificity, and perhaps the *in vivo* functions, of pol ν.

Acknowledgements

We thank Drs. Katarzyna Bebenek, William C. Copeland and Stephanie Nick McElhinny for reading and critically commenting on manuscript. This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, by grant CA101980 from the NIH to RDW, and by the University of Pittsburgh Cancer Institute.

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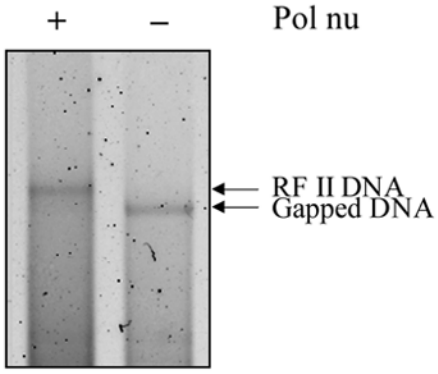
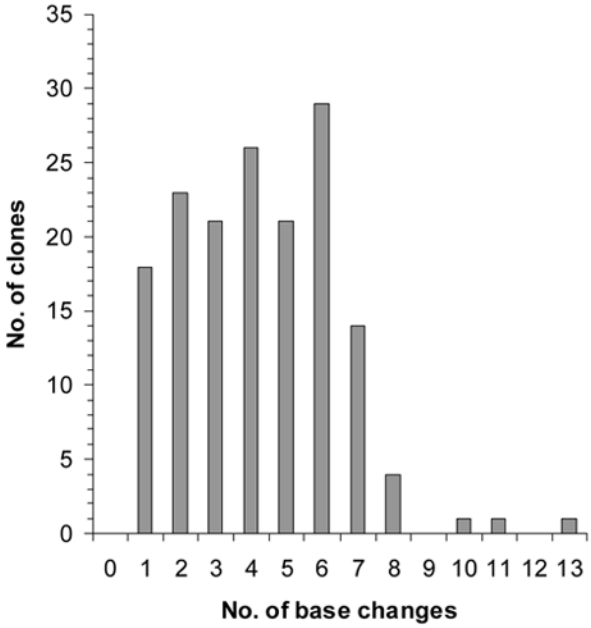


A.



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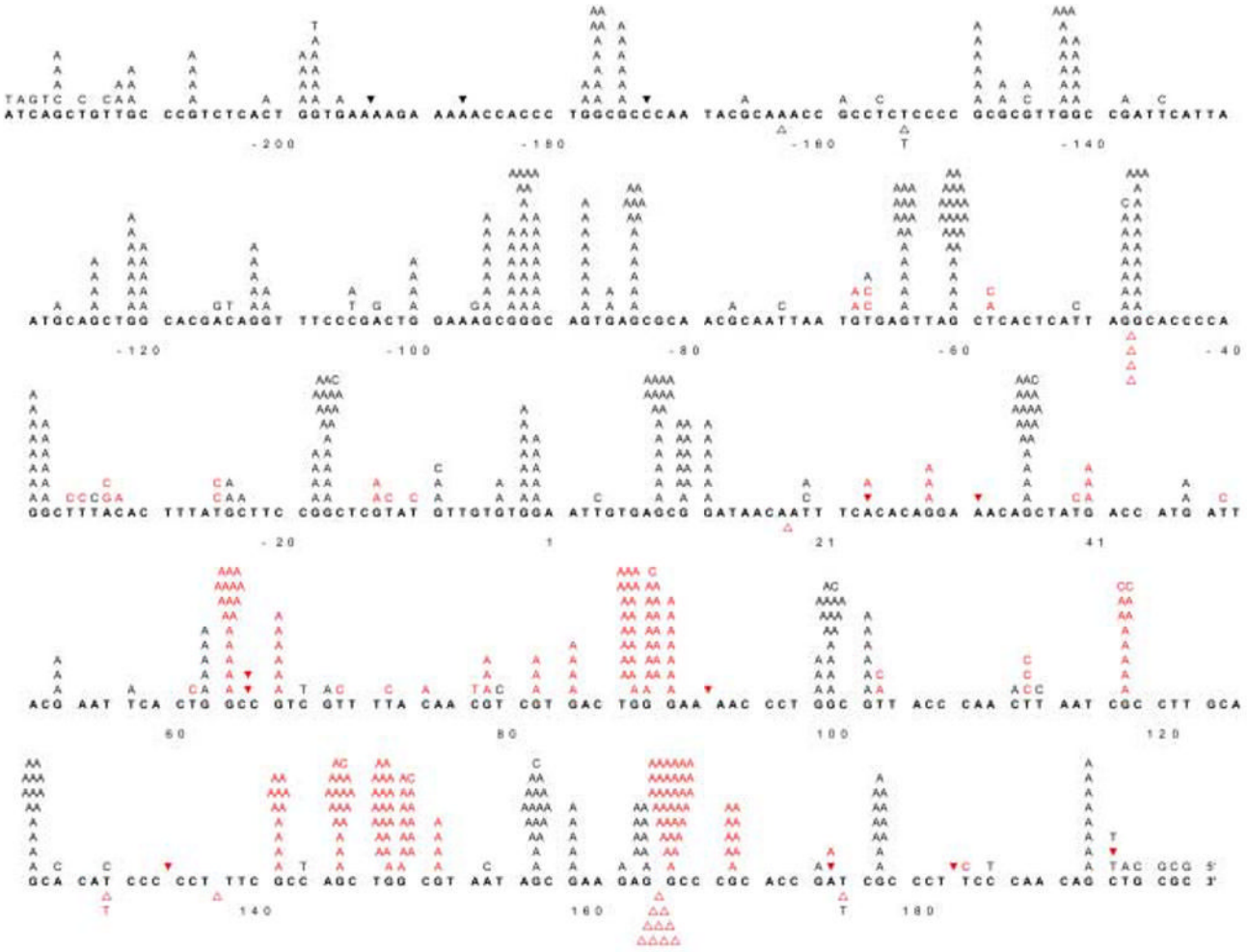
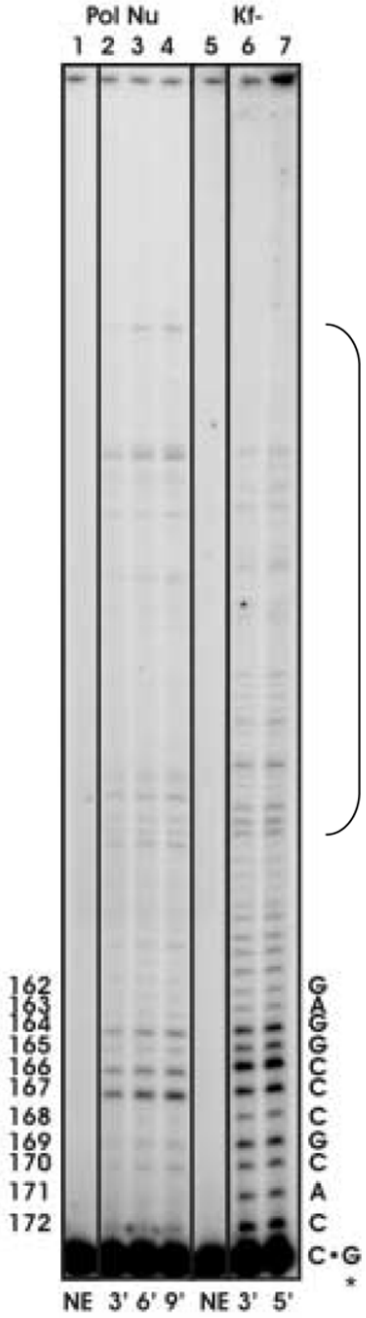


Figure 1.

(A) Analysis of gap-filling by agarose gel electrophoresis. Half of the reaction mixture was mixed with 3μl of SDS dye mix and analyzed by electrophoresis on an 0.8% agarose gel in TAE buffer. Lane A, gap-filling reaction with pol ν and lane B, includes the same reaction in the absence of pol ν. (**B**) Distribution of base substitutions per clone. 162 independent M13mp2 clones were sequenced and a total of 673 base substitutions were observed. (**C**) Spectrum of base substitution errors by human pol ν. The 407 template nucleotides within the single-strand gap of the M13mp2 substrate are shown as 5 lines of the template sequence. Base substitutions are indicated by letters above the target sequence. Deletion of a base is depicted by an open triangle whereas addition of a base is represented by a closed inverted triangle above the target sequence. *Red* characters represent phenotypically detectable changes in the gap region while *black* characters represent phenotypically undetectable changes found in association with detectable changes. +1 represents the first transcribed nucleotide of the *lacZα*-complementation region.



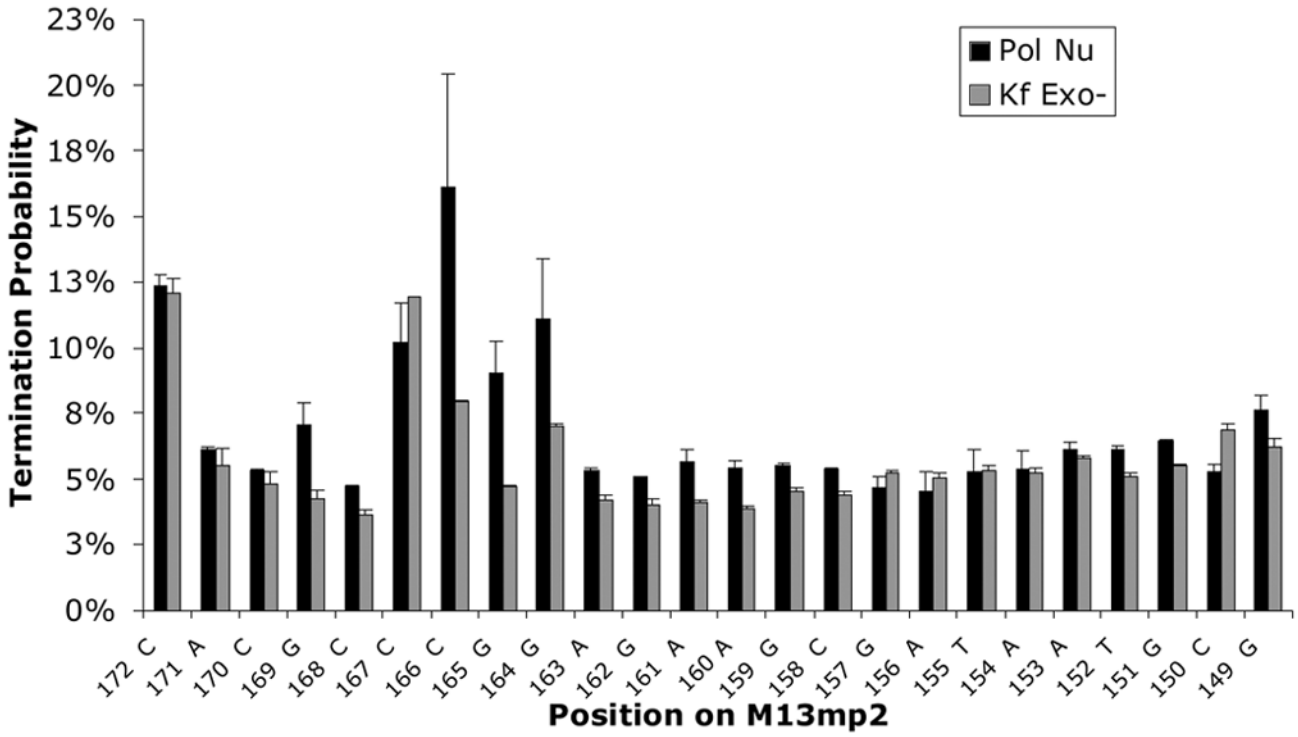


Figure 2.

Processive synthesis by pol ν and Klenow fragment exo$^-$. Reactions were performed as described in Materials and Methods. (**A**) Representative phosphor image of the reaction products of processive DNA synthesis resolved on a 12% denaturing polyacrylamide gel. Lanes 2–4, primer extension products by pol ν at 3, 6, and 9-min. Lanes 6–7, primer extension products by Klenow fragment exo$^-$ at 3 and 5-min. Lanes 1 and 5 represent negative control reactions. The sequence of the template strand is shown on the right. The numbers on the left indicate the positions along the *lac*Z gene. (**B**) Termination probability per nucleotide at each template position, 172 to 149. Numbers along the X-axis indicate nucleotide positions along the *lac*Z gene. The termination probability is defined as the amount of product of a given length divided by the sum of products of that length plus all greater length products. The values plotted are the average of six values for pol ν (for time points 3, 6, and 9 minutes for pol ν DNA at two different ratios) and an average of four values for Klenow fragment exo$^-$ (time points 3 and 5 minutes at one ratio) and the error bars represent the standard deviation.

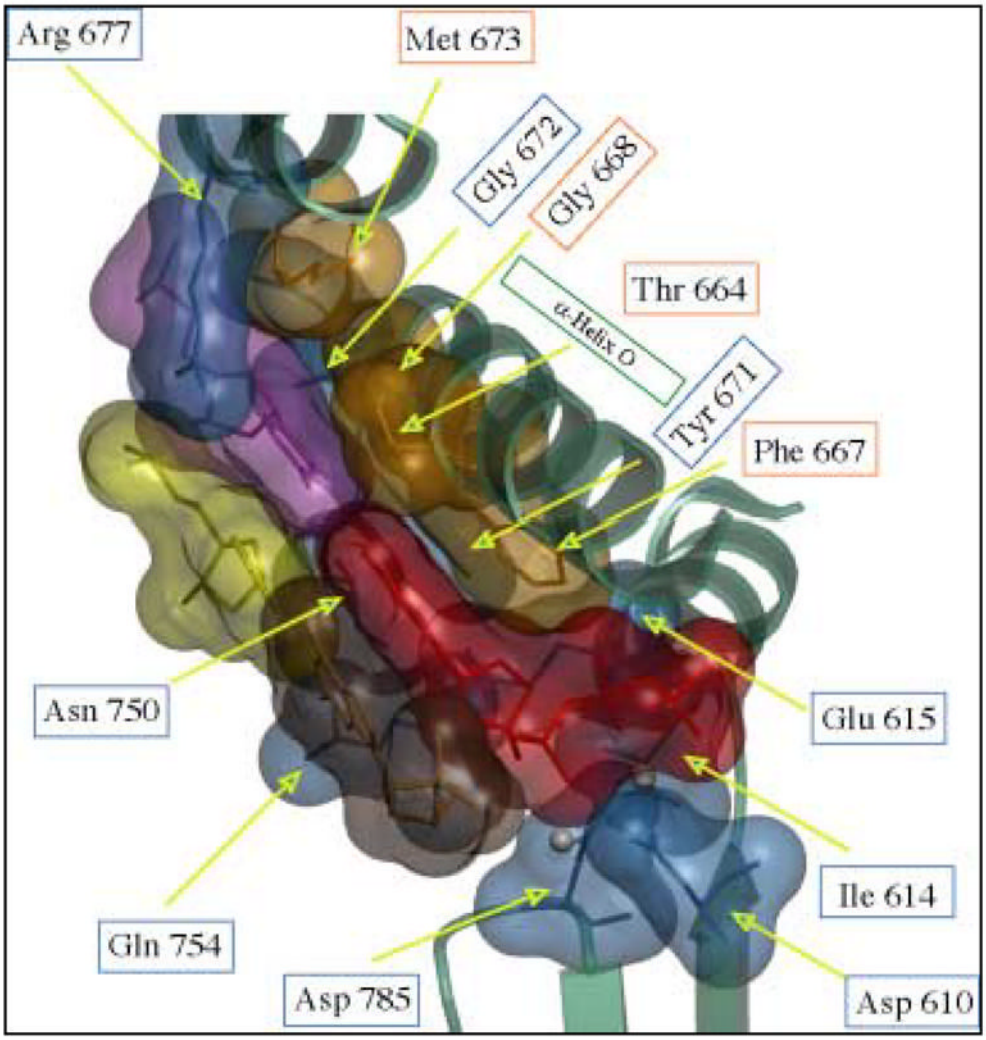


Figure 3.

(**A**) The nascent base pair binding pocket at the active site of *Taq* DNA pol I. Amino acids of *Taq* DNA pol I that interact with the incoming nucleotide (*red*) and the templating base (*magenta*) that are conserved in pol ν are colored in *blue*. Amino acids that are not conserved are represented in *orange*. The figure was created based on the structure of *Taq* DNA pol I in a ternary complex with DNA and incoming ddGTP (Protein Data Bank accession number 1QSS). (**B**) Conserved and non-conserved amino acids in Family A DNA polymerases including: Human pol ν, *Taq* DNA pol I, *E. coli* DNA pol I (Kf-), Bacteriophage T7 DNA pol, *Bacillus stearothermophilus* DNA pol I (BF) and human pol γ. Amino acids of *Taq* DNA pol I that are conserved in pol ν are white in a *blue background*. Amino acids that are not conserved

are white in an *orange background*. Conserved amino acids with respect to pol ν are indicated in bold.

Table I

Summary of sequence changes generated by Pol ν

LacZ Mutant Frequency	0.18[a]
Total mutants sequenced	162
Total bases sequenced	65, 934
Total sequence changes	712

	Total changes	Detectable changes	Error Rate ($\times 10^{-4}$)[b]
Base substitutions	673	226	35
Frameshifts (−1)	20	16	1.6
Frameshifts (+1)	12	7	0.7
Other[c]	7		

[a] Error rates and mutant frequencies were calculated using data from two independent experiments. Experiment 1: Mutant frequency was 17.6% (317 mutant plaques from a total of 1797). Experiment 2: Mutant frequency of 18.0% (1278 mutant plaques from a total of 7081).

[b] Error rate calculated from only detectable changes (see Materials and Methods).

[c] These errors are further discussed in text.

Table II

Single base error rates for Pol ν and others

	Polymerase Error rates (× 10^{-6})			
DNA Polymerase	**Single base deletions**	**Base Substitutions**	**G:dTMP**	**T:dGMP**
hPol ν[a]	160	3500	17000	1300
hPol γ (Exo-) (+p140 and p55)[b]	8	41	16	21
Kf (Exo-)[c]	6	25	5	45
hPol α[d]	28	75	13	62
yPol δ (Exo-)[e]	57	130	56	140
yPol ε (Exo-)[f]	56	240	51	75
hPol β[*]	140	230	220	100
hPol λ[#]	4500	900	310	1400
hPol η[§]	2400	35000	1200	5900
hPol κ[‡]	1800	5800	4400	2100

[a] hPol ν: Error rates reported are from this study.

[b] hPol γ (Exo-) (+p140 and p55): single base deletion error rate from Longley *et al*., 2001. Base substitution error rates (average and G:dTMP and T:dGMP) are unpublished results from Pursell, ZF and Kunkel TA.

[c] Kf (Exo-): single base deletion error rate from Table III, Minnick *et al*., 1996. Base substitution error rates (average and G:dTMP and T:dGMP) are from Bebenek *et al*., 1990.

[d] hPol α: Error rates are unpublished results from Kokoska RJ and Kunkel TA.

[e] yPol δ (Exo-): Error rates are taken from Fortune *et al*., 2005.

[f] yPol ε (Exo-): Error rates are taken from Shcherbakova *et al*., 2003.

[*] hPol β: Error rates are taken from Bebenek and Kunkel, 2003.

[#] hPol λ: Error rates are taken from Bebenek *et al*., 2003.

[§] hPol η: Error rates are taken from Matsuda *et al*., 2001 and 2002.

[‡] hPol κ: Error rates are taken from Ohashi *et al*., 2001.

Table III

Base substitution errors generated by Pol ν

Base	Mutation	Mispair	Number	Pol Nu Error rate ($\times 10^{-5}$)[a]
	From → To	Template ·dNMP		
A	A → G	A · dCMP	1	10
	A → T	A · dAMP	0	≤8
	A → C	A · dGMP	1	11
T	T → C	T · dGMP	18	130
	T → A	T · dTMP	2	24
	T → G	T · dCMP	0	≤8
G	G → A	G · dTMP	196	1700
	G → C	G · dGMP	5	51
	G → T	G · dAMP	0	≤8
C	C → T	C · dAMP	1	8
	C → G	C · dCMP.	0	≤22
	C → A	C · dTMP.	2	24

[a]Base substitution error rates were calculated using results from two independent experiments. All data are from the forward mutation assay. Error rates for individual base substitutions were calculated as previously described (36).

Table IV

Sequence Context Dependence for G→A base substitutions by Pol ν

Plaque Phenotype	Templated G		No. of G→A changes at site	ER[a] (%)	TP[b] (%)
2+	AGCGAAGAG G_{165}	CCCGCACCG	33	20	9.0
CL	TCGCCAGCT G_{148}	GCGTAATAG	19	12	
CL	GTCGTGACT G_{88}	GGAAAACCC	19	12	
2+	AATTCACTG G_{63}	CCGTCGTTT	17	11	
1+	CTTTCGCCA G_{145}	CTGGCGTAA	16	10	
CL	TCGTGACTG G_{89}	GAAAACCCT	15	9.0	
CL	CGCCAGCTG G_{149}	CGTAATAGC	12	7.4	7.7
3+	CCCCCTTTC G_{141}	CCAGCTGGC	11	6.7	
2+	AAGAGGCCC G_{169}	CACCGATCG	9	6.0	7.1
3+	AACTTAATC G_{118}	CCTTGCAGC	9	6.0	
3+	CGTGACTGG G_{90}	AAAACCCTG	7	4.3	
CL	TCACTGGCC G_{66}	TCGTTTTAC	6	4.0	
3+	CCAGCTGGC G_{151}	TAATAGCGA	4	2.5	6.5
2+	CAACGTCGT G_{84}	ACTGGGAAA	4	2.5	
3+	AACAGCTAT G_{41}	ACCATGATT	3	1.9	
3+	TTTTACAAC G_{79}	TCGTGACTG	3	1.9	
2+	TTTCACACA G_{29}	GAAACAGCT	3	1.9	
2+	TACAACGTC G_{82}	TGACTGGGA	3	1.9	
2+	GCAATTAAT G_{-68}	TGAGTTAGC	2	1.2	
2+	TTCCGGCTC G_{-13}	TATGTTGTG	2	1.2	
2+	AATTAATGT G_{-66}	AGTTAGCTC	0	≤0.6	

[a] Error rate calculated from number of G→A changes at a site, divided by total number of mutants analyzed (see Table 1).

[b] Termination probability value taken from Figure 2B. Values shown only for detectable templated G for which termination probability was calculated from processivity assay (Figure 2B).