Low-fidelity DNA synthesis by human DNA polymerase theta

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ABSTRACT

Human DNA polymerase theta (pol θ or POLQ) is a proofreading-deficient family A enzyme implicated in translesion synthesis (TLS) and perhaps in somatic hypermutation (SHM) of immunoglobulin genes. These proposed functions and kinetic studies imply that pol θ may synthesize DNA with low fidelity. Here, we show that when copying undamaged DNA, pol θ generates single base errors at rates 10- to more than 100-fold higher than for other family A members. Pol θ adds single nucleotides to homopolymeric runs at particularly high rates, exceeding 1% in certain sequence contexts, and generates single base substitutions at an average rate of $2.4 \times 10^{-3}$, comparable to inaccurate family Y human pol κ ($5.8 \times 10^{-9}$) also implicated in TLS. Like pol κ, pol θ is processive, implying that it may be tightly regulated to avoid deleterious mutagenesis. Pol θ also generates certain base substitutions at high rates within sequence contexts similar to those inferred to be copied by pol θ during SHM of immunoglobulin genes in mice. Thus, pol θ is an exception among family A polymerases, and its low fidelity is consistent with its proposed roles in TLS and SHM.

INTRODUCTION

The human genome encodes numerous DNA polymerases that have key roles in DNA replication and the repair and recombination reactions needed to maintain the integrity of genetic information (1–4). These polymerases are classified by sequence homology into families A, B, X and Y. Family A members include human DNA polymerase γ (pol γ), pol ν and pol θ, the subject of this study. Pol θ is encoded by the POLQ gene that was initially described (5) as an open reading frame in humans with homology to the founding member of family A polymerases, Escherichia coli DNA polymerase I. Subsequent studies (6,7) demonstrated that POLQ encodes a 290-kDa protein with template-dependent DNA polymerase activity. The amino terminus contains seven motifs characteristic of DNA and RNA helicases, although no helicase activity has been reported. The carboxy-terminal half contains the residues required for polymerase activity (6). Recombinant pol θ lacks detectable 3' exonuclease activity, rendering it incapable of exonucleolytically proofreading any errors that it may make during DNA synthesis. Also, steady-state kinetic studies demonstrate that recombinant human pol θ misinserts incorrect dNTPs at rates that are higher than those of several other family A polymerases (7). Pol θ can also efficiently insert a nucleotide opposite an abasic site or a thymine glycol lesion (7–9), and it can extend the resulting primer terminus. In addition, pol θ can extend a primer terminus generated after another polymerase has inserted a nucleotide opposite a 6–4 photoproduct, a lesion that pol θ itself cannot bypass (7,8). Mice with a T to C base substitution (the chaos1 mouse) or a deletion in the Polq gene (10) have elevated levels of spontaneous and radiation-induced micronuclei in erythrocytes (10). When the chaos1 mutant mouse was crossed with an Atm-deficient mouse, the number of the double homozygous mice was lower than expected (11), indicating that pol θ is important for survival in the absence of ATM-dependent checkpoint signaling. Additional studies suggest that pol θ may participate in cellular responses to DNA damage, possibly including TLS (10,12).

Pol θ has also been suggested to be involved in somatic hypermutation of immunoglobulin genes, although a consistent model for a possible role has not yet emerged. It is highly expressed in lymphoid tissues and regulated in the germinal centers of B cells where SHM takes place (12).
In one study, mice expressing catalytically inactive pol B had reduced SHM at C:G base pairs (13); in another study, pol B null mice showed a reduction in SHM at both C:G and A:T base pairs accompanied by an increase of G to C transversions (14) and in a third study, pol B was proposed to be responsible for the residual A:T mutations observed in the absence of pol α (15).

Previous work has shown that mammalian polymerases implicated in TLS and/or SHM, such as the family Y members pol η, pol κ and pol λ, and the family B member pol μ, all have relatively high error rates when copying undamaged DNA templates (16). Analysis of the error specificity of DNA polymerases can provide insights into the mechanisms by which they avoid or generate errors, and clues about their biological functions. For these reasons, we analyzed the single base deletion, insertion and substitution error rates and specificity of recombinant human pol B during copying of undamaged DNA.

MATERIALS AND METHODS

Enzymes, reagents and strains

Recombinant full-length human DNA pol B was purified to near homogeneity as described (6,8). Materials for the M13mp2 fidelity assay were from sources described previously (17). The exonuclease-deficient, large Klenow fragment of E. coli DNA pol I and T4 polynucleotide kinase were purchased from New England BioLabs, Ipswich, MA, USA. [γ-32P]ATP (4500 Ci/mmol) and unlabeled deoxyribonucleotide-triphosphates were from GE Healthcare Biosciences, Piscataway, NJ, USA. Materials for processivity analysis were described previously (18).

M13mp2 fidelity assay

Gap-filling reactions for pol B (25 µl) contained 20 mM Tris–HCl (pH 7.5), 8 mM MgCl₂, 0.1 mM EDTA, 4% glycerol, 80 µg/ml bovine serum albumin and 1 mM each dATP, dCTP, dGTP and dTTP. Reaction mixtures for Klenow fragment exo-pol were as described above for pol B except 8 mM magnesium acetate was used. Both reaction mixtures contained primed M13mp2 ssDNA substrate in excess (6.6 nM) of pol B (2.8 nM) or Klenow fragment exo-pol (5.3 × 10⁻⁹ nM). Reaction mixtures were incubated at 37°C and 10 µl aliquots were removed at 2, 5, 10, 15 and 30 min for pol B and at 1, 3, 4 and 5 min for Klenow fragment exo-pol. Samples were mixed in a 1:1 ratio with stop buffer (99% formamide, 5 mM 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. 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.

Table 1. Error Rate for pol B

| Total changes | Error rate (×10⁻³)³⁴⁵ |
|---------------|----------------------|
| Frameshifts (−1) | 112                  |
| Frameshifts (+1) | 270                  |
| Base substitutions | 194                  |
| Other          | 29                   |
| ≥ 1 bases      | 16                   |

³Error rates are calculated from all observed changes (see Materials and Methods section). As mentioned in the text, mutant frequencies were calculated for two experiments. Experiment 1: mutant frequency was 27.0% (267 mutant plaques from a total of 997). Experiment 2: mutant frequency was 31.0% (873 mutant plaques from a total of 2835). Error rates are calculated from Experiment 1.
⁴Error rates calculated for detectable changes are: frameshifts (−1) 0.9 × 10⁻³, frameshifts (+1) 1.9 × 10⁻³, base substitutions 1.4 × 10⁻³.
⁵These errors are further discussed in the text and Table 2.
⁶Among the 200 clones sequenced, several clones (13) contained deletions of two or more bases, while three clones contained insertions of two or more bases.

RESULTS

Fidelity of human pol B in the M13mp2 forward mutation assay

The fidelity of human pol B was determined during synthesis to fill a 407-nt single-stranded gap within a circular duplex M13mp2 DNA substrate. The gap contains the lacZ α-complementation sequence that serves as the target for detecting polymerization errors. From the independent lacZ mutants sequenced, error rates have been calculated for the 12 single base-base mismatches and for addition and deletion errors, all in a variety of sequence contexts (17). This comprehensive
description of pol θ error rates and error specificity can then be compared to results obtained with other DNA polymerases using the same approach.

The DNA products of complete gap filling by human pol θ [data not shown, but for example see Figure 3 in ref. (17)] yielded a lacZ mutant frequency of 27% in the first experiment and 31% in a second experiment. These frequencies are much higher than those generated by most other exonuclease-deficient family A polymerases, e.g. 0.57% for exonuclease-deficient Klenow fragment polymerase (20), 0.75% for Thermus aquaticus (Taq) polymerase (21), 1.6% for exonuclease-deficient T7 polymerase (20,21) and 0.62% for exonuclease-deficient pol γ (22). The least accurate family A member studied previously was human pol ν (23), which generated a lacZ mutant frequency of 18%. That value was obtained from reactions conducted at pH 8.8, the pH initially used to study its TLS capacity (24). Here, we decided to perform a pol ν fidelity measurement at pH 7.5, to permit direct comparison to pol θ and the other family A polymerases, all of which were analyzed at neutral pH. Consistent with previous studies showing that Klenow fragment polymerase (25) and Taq pol (21) have higher fidelity at neutral compared to alkaline pH, the products of a pol ν reaction conducted at pH 7.5 yielded a lacZ mutant frequency of 2.3%. Thus, the lacZ mutant frequencies generated by pol θ are more than 10-fold higher than for pol ν or any other exonuclease-deficient family A polymerase examined in this assay, i.e. pol θ is error-prone.

Error rates and error specificity

To determine the types and positions of errors made by pol θ, the sequence of the 407 template bases within the gap was determined for 200 independent lacZ mutants. A total of 621 sequence changes were detected, yielding an average of 3.1 sequence changes per lacZ mutant. Among these were 112 single base deletions, 270 single base additions and 194 single base substitutions (Table 1), 29 other, more complex changes (Table 2) plus 16 deletions or additions of two or more bases (Table 1). The single base changes were distributed throughout the 407 base target sequence and included changes known to result in a colorless or light blue phenotype (Figure 1, mutations in red) and changes that are known to be, or may be, phenotypically silent (Figure 1, mutations in black). When error rates were calculated from these data (as described in Materials and Methods section), overall average rates were $1.4 \times 10^{-3}$ for single base deletions and $3.3 \times 10^{-3}$ for single base additions (Table 1). Error rates for single base deletions and additions were sequence context-dependent, increasing as the number of bases within homopolymeric runs increased from one [i.e. a noniterated sequence to two and then three bases (Figure 2)]. There was little or no further increase in error rates in runs of four or five of the iterated bases. The overall average single base substitution error rate was $2.4 \times 10^{-3}$ and error rates for the 12 individual mismatches varied over at least a 100-fold range, from $\leq 0.4 \times 10^{-4}$ for C-dCTP to $42 \times 10^{-4}$ for the T-dGTP (Table 3).

| # | From | nt | To |
|---|------|----|----|
| 10 | 127 | CACAT | CCCCCCTTTT | CGCCA | 144 |
| 2 | 127 | CACAT | CCCCCCTTTT | CGCCA | 144 |
| 2 | -202 | ACTGG | TG | AAAAG | -190 |
| 1 | -217 | GCTGT | TGGCCCCGT | CTCAC | -201 |
| 1 | -212 | TGCCC | GTCTC | ACTGG | -198 |
| 1 | -189 | AAAAA | CCA | CCCCCG | -178 |
| 1 | -118 | ACAGG | T | TTCCC | -105 |
| 1 | -38 | GCTTT | ACAC | TTTAT | -25 |
| 1 | -34 | TACAC | TTTATGCTCC | GGCCT | -14 |
| 1 | 94 | CTGCC | GTC | ACCCA | 113 |
| 1 | 116 | TGCC | TT | GACGC | 127 |
| 1 | 121 | TTGCA | GCCACATCCCCCCTTTT | GCCGA | 140 |
| 1 | 125 | AGCAC | AT | CCCCCC | 136 |
| 1 | 148 | CCGTA | ATAGC | GAAGA | 163 |
| 1 | 162 | CCCGC | ACCGATC | GCCCT | 186 |
| 1 | 173 | CGATC | GCCCTTCCCC | AACGAG | 191 |
| 1 | 176 | TCGCC | CTT | CCCAC | 188 |

For each single event listed, the top sequence shows the original template sequence (Figure 1) including the five nucleotides on the 5' and 3' sides of the sequence that was changed. The bottom sequence shows the observed changes highlighted in red. For example, among 200 independent clones, 10 contained an insertion of a C at position 132–136 and a T at position 137–139.

Base substitution errors were also generated at higher than average rates in certain sequence contexts (arrows in Figure 1, and see Discussion section).

Processivity

We next determined the processivity of synthesis by pol θ when copying the LacZ template sequence starting at nucleotide number 145 (where +1 is the first transcribed base of LacZ), which is adjacent to hot spots for two different single base additions and two different single base substitutions (arrows in Figure 1). Reactions were
performed under conditions of primer-template excess, such that only a small proportion of primers are extended and DNA products therefore primarily reflect one cycle of synthesis (see Materials and methods section). Under these conditions, pol \(\text{poly} \) generates chains that vary in length from one to more than \(\sim 75 \text{ nt} \) (Figure 3A). When individual band intensities were quantified to calculate the probability of termination of processive synthesis at multiple positions, termination probabilities for pol \(\theta \) ranged from 2% to 7%. At each of these positions, pol \(\theta \) is less likely to terminate processive synthesis than is exonuclease-deficient Klenow fragment polymerase (Figure 3B), i.e. pol \(\theta \) is slightly more processive. Termination probabilities at the hot spots for additions (CCCCC at positions 132–136 and TTT at positions 137–139) and substitutions (T to C at position 131 and C to T at position 136) were similar to those observed at other positions. The implications of these results are discussed subsequently.

**Pol \(\theta \) base substitution specificity and a possible role in SHM**

During somatic hypermutation of immunoglobulin genes, mammalian pol \(\eta \) is responsible for a large proportion of mutations occurring at A-T base pairs (26–28). Even so, SHM spectra in XPV patients or mice lacking functional pol \(\eta \) still retain a small fraction (5–15%) of mutations at A-T bases (26,28–30) implicating another polymerase
in SHM at A-T bases. This is interesting in light of the fact that 132 of 194 single base substitutions (68%) made by pol θ in vitro were generated when copying a template A or T (Table 4), including substitutions at several hot spots (arrows in Figure 1). This specificity prompted the search for a signature mutable motif for pol θ-independent substitutions at A-T pairs. Using the largest available spectrum of unselected somatic mutations located in the Jh intronic region from XP-V patients (30), the sequences ADK/MHT and AA/TT (D = A/G/T; K = G/T; M = A/C; H = A/C/T; mutable positions are underlined) were found to be the most likely mutable motifs (Table 4) (31).

When these motifs were compared with the distribution of base substitution errors made by pol θ, three hot spots in the pol θ error spectrum significantly correlated with the AA and ADK motifs (Table 4), consistent with a possible contribution of pol θ to somatic mutations in immunoglobulin genes in vivo (13,14). This result is further supported by the absence of correlation between

Table 3. Base substitution error rates for pol θ

| Base | Mutation | Mispair | Template·dNMP | All observed | Error rate ($\times 10^{-5}$) |
|------|----------|---------|---------------|--------------|-------------------------------|
| A    | A → G    | A·dCMP  | 11            | 5.6          |
| A    | A → T    | A·dAMP  | 9             | 4.5          |
| A    | A → C    | A·dGMP  | 7             | 3.5          |
| T    | T → C    | T·dGMP  | 77            | 42.0         |
| T    | T → A    | T·dTMP  | 24            | 13.0         |
| T    | T → G    | T·dCMP  | 4             | 2.2          |
| G    | G → A    | G·dTMP  | 16            | 8.4          |
| G    | G → C    | G·dGMP  | 4             | 2.1          |
| G    | G → T    | G·dAMP  | 5             | 2.6          |
| C    | C → T    | C·dAMP  | 35            | 14.0         |
| C    | C → G    | C·dCMP  | 0             | $\leq 0.4$   |
| C    | C → A    | C·dTMP  | 2             | 0.8          |

*Base substitution error rates were calculated using results from Experiment 1 (Table 1). All data are from the forward mutation assay. Error rates for all observed individual base substitutions were calculated as previously described (20).
the mutable motifs and spectra of somatic mutations in \( \eta^-/0^-/^- \) mice (Table 4).

**DISCUSSION**

The results presented here demonstrate that human pol \( \theta \) synthesizes DNA with moderate processivity and very low fidelity, and that it has a unique error specificity. This combination of properties is unusual, and can be considered in light of mechanisms by which polymerases generate errors and in light of pol \( \theta \)'s proposed biological functions.

**Insertion/deletion errors**

The most unusual and unanticipated aspect of pol \( \theta \)'s error specificity is the high rate at which single base insertions and deletions (indels) are generated. In the M13 forward mutation assay, most DNA polymerases have higher error rates for base substitutions than for single base insertions and deletions, whereas pol \( \theta \) generates all three types of errors at similar rates (Table 1). In fact, pol \( \theta \) is among the least accurate polymerases for indels, with an average deletion rate rivaling that of the notoriously inaccurate Y family polymerases (34) containing an extra base in the template strand (for deletions) or in the primer strand (for additions) that is not at the active site but is rather several correct base pairs upstream of the active site (33). It is, therefore, interesting that error rates for additions exceed those for deletions (Figure 2, black versus open bars), and that the error rate for additions is highest for runs of three identical bases and does not increase in longer runs (Figure 2). This pattern suggests that pol \( \theta \) may preferentially stabilize an extra base in the primer strand located two correct base pairs upstream of the active site.

It is entirely possible that particular residues of pol \( \theta \) may contribute to stabilizing misaligned intermediates, allowing pol \( \theta \) to generate single base indels at unusually high rates. Sequence alignments reveal that pol \( \theta \) contains extra amino acids, designated Inserts 1, 2 and 3 [see Figures 5 and 6 in ref. (7)], that are not found in other family A polymerases. While Insert 3 is unlikely to be near enough to interact with the DNA [see Figure 6 in ref. (7)], Inserts 1 and 2 may be at locations relevant to indel errors. Insert 1 is predicted to be near the tip of the polymerase thumb subdomain that in other family A members interacts with the primer template. This is interesting because deleting the tip of the thumb of Klenow fragment pol increases the error rate for indels, especially single base additions (35). Also, when T7 DNA polymerase lacks thioredoxin, its accessory subunit that interacts with the thumb, it is error-prone for single base indels, especially additions (36). An insert at this position is also found in a plant plastid A-family DNA polymerase (37). Insert 2 is located between conserved amino acids that are known to form secondary structural elements in T7 pol and *Taq* pol. It is interesting to note that some of these residues
interact with an extrahelical template strand base just upstream of the active site, in a manner suggested to preferentially contribute to single base deletions in two-base runs.

The rate and specificity of indel errors are also relevant to how misalignments may form during synthesis by pol θ. As mentioned above, one perhaps unlikely possibility is that the misalignment arises within the polymerase active site, with the unpaired intermediate possibly stabilized by pairing of a correct incoming dNTP with the next template base. (39,40). Another possibility is that misalignment initiates with dNTP misinsertion, followed by primer relocation to generate a misaligned intermediate with a correctly paired primer terminus (33). Although these possibilities cannot be excluded, they seem less likely because pol θ indel error rates in certain sequence contexts (Figure 1) are extraordinarily high (e.g. 2–7%, Figure 3B) and often exceed base substitution error rates (Table 3).

A third possibility, suggested by earlier studies of other polymerases (33), is that DNA misalignments arise as the polymerase dissociates and/or re-associates with the primer template. In order to test this possibility we examined the processivity of DNA synthesis by pol θ as it copied the lacZ template nucleotides that were hot spots for single base additions at a CCCCC run (position 132–136) and a TTT run (position 137–139). Site-specific termination probabilities at these two hot spots are between 1% and 4% (Figure 3B), similar to the respective 2% and 7% error rates for additions within these runs (Figure 3B). This implies that essentially all cycles of dissociation–reassociation would need to result in misalignments that were ultimately extended, in order to account for the extraordinary pol θ addition rates. This could be so, but would be unprecedented, because previous studies of other polymerases showed that at least 100 dissociation–reassociation cycles were observed for each addition error generated (41). Thus there may be additional opportunities for misalignments to form even during processive synthesis by pol θ. One possibility is suggested by the fact that forming an addition intermediate within a homonucleotide run requires (on average) disruption of one more correct base pair than does formation of a deletion intermediate (42). This energetic difference is consistent with the observation that addition rates of numerous DNA polymerases are consistently lower than are deletion rates [Figure 4 and (33)]. Here, we observe that pol θ is an exception; it is the only polymerase that generates additions at a higher average rate than deletions. One possible explanation is that during synthesis by pol θ, fraying at the primer terminus may involve multiple bases, such that subsequent re-annealing could provide a similar opportunity for forming either an addition or a deletion intermediate. Fraying of multiple base pairs might occur during movement of the primer strand between two separate DNA binding sites. This was previously proposed to account for the difference in indel error rates between the large Klenow fragment of *E. coli* DNA polymerase I, which can partition the primer strand between the polymerase active site and the exonuclease active site, and a derivative containing the polymerase domain alone,
i.e. one completely lacking the 3’ exonuclease domain (20). The idea is nicely articulated in the melting-misalignment model of Maki and colleagues (43,44), who proposed that the tendency of E. coli DNA polymerase III to generate single base additions (albeit at much lower rates than seen here), results from misalignments that arise during partitioning of the primer strand between the polymerase and proofreading exonuclease active sites. Unlike E. coli pol III, recombinant pol θ lacks a detectable proofreading exonuclease activity (6), but the enzyme purified from human HeLa cells has been reported to have an associated 3’ exonuclease (9). It is also possible that the pol θ catalytic subunit itself could have a second DNA-binding site encoded by residues in its large open reading frame, which contains helicase motifs that are unique to pol θ in comparison to other polymerases, including its closest but more accurate homolog, pol ν.

**Base substitution errors**

The observation that the average base substitution error rate of human pol θ is high (Figure 4C) is consistent with low nucleotide selectivity (7) and promiscuity in extending mismatched primer termini (8). Pol θ’s closest homolog, pol ν, also generates base substitutions at unusually high rates in polymerization reactions conducted at pH 8.8 (23), a condition where the enzyme has maximum activity (24). Here we compared the fidelity of the two enzymes in reaction mixtures at pH 7.5, and pol θ was about 10-fold less accurate than pol ν (240 × 10⁻⁵ versus 24 × 10⁻⁵, Figure 4). This difference is seen despite the strong conservation of key residues in and around the putative nascent base pair-binding pockets of pol θ and pol ν (see Figure 7 in Supplementary Data in ref. 45). Pol θ’s remarkable infidelity among family A members is further highlighted by comparison to the exonuclease-deficient derivative of Klenow fragment polymerase, which is fully 10-fold more accurate (Figure 4, substitution error rate of 2.5 × 10⁻⁵). It is particularly interesting that pol θ is almost as inaccurate as pol κ (Figure 4), a family Y polymerase whose members are characterized by open, solvent accessible active sites. Pol θ generates many of the 12 possible base-base mismatches at high rates (Table 3), including three pyrimidine–pyrimidine mismatches that may be solvated (46). These facts imply that the active site of pol θ may be more solvent accessible than is typical of accurate family A polymerases known to have closed, relatively solvent inaccessible active sites.

**Biological implications**

The low fidelity of pol θ when copying undamaged DNA is generally consistent with its proposed role in lesion bypass [e.g. of AP sites, (7)], similar to several other DNA polymerases (e.g. pol η) that are implicated in bypass of lesions that perturb DNA helix geometry, which also synthesize undamaged DNA with low fidelity. The high-base substitution error rates of human pol θ and its error specificity in comparison to the SHM specificity at A-T base pairs in humans and mice lacking functional pol η (Table 4) are also consistent with previous reports implicating pol θ in SHM of immunoglobulin genes (13,14,47).

As is typical of family A members, human pol θ is more processive than are certain other polymerases (pol η, pol α, some bacterial pols) implicated in TLS. In fact, at all template positions examined here (Figure 3B), pol θ is slightly more processive than Klenow fragment polymerase, and can even generate chains exceeding 71 nt in length during a single cycle of processive synthesis. The combination of low fidelity and moderate processivity by human pol θ is shared by both pol κ (48) and by pol ζ (49,50). The latter two enzymes have been implicated in TLS, reputedly as promiscuous mismatch extenders (51). Perhaps relevant to their moderate processivity, pol κ and pol ζ are implicated in DNA transactions that may require filling of gaps of about 30 nt. For example, pol κ has recently been implicated in nucleotide excision repair of UV photoproducts (52) and pol ζ is reported to have a role in gap filling associated with repair of interstrand cross-links (53,54). In like manner, the processivity of pol θ would make it well suited for specialized transactions requiring more extensive DNA synthesis, e.g. SHM downstream of the initiating deamination event, during a sub-pathway of base excision repair (55) or nucleotide excision repair, or perhaps during reactions involving the helicase motifs of pol θ. The very low fidelity but moderate processivity of pol θ may also be relevant to the recovery of lacZ mutants containing multiple single base changes within a few nucleotides of each other (Table 2). Such ‘complex’ events are also generated by pol ζ (49) which, as mentioned earlier, shares with pol θ the properties of processive but inaccurate synthesis.

Although the low fidelity and moderate processivity of human pol θ is potentially beneficial for certain specialized DNA transactions, this combination could be adversely mutagenic if pol θ synthesized DNA at the wrong time or place. For example, one study reports that expression of pol θ mRNA is upregulated in human cancers, and that patients expressing high levels of pol θ have poorer clinical prognoses than do patients expressing lower levels of pol θ (12). Given its low fidelity and moderate processivity, pol θ activity may be tightly regulated, which could occur at any of several levels. For example, pol θ is most highly expressed in the germinal centers of splenic B cells where antibody maturation takes place (6,11,12). By analogy to an elegant recent study demonstrating that UmuD and RecA proteins modulate the mutagenic potential and the fidelity of the bacterial family Y DNA polymerase DinB (56), accessory proteins may exist that interact with and regulate pol θ, perhaps decreasing its potential for indel mutagenesis.

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