Minor Groove Interactions at the DNA Polymerase β Active Site Modulate Single-base Deletion Error Rates*

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The structures of open and closed conformations of DNA polymerase β (pol β) suggests that the rate of single-nucleotide deletions during synthesis may be modulated by interactions in the DNA minor groove that align the templating base with the incoming dNTP. To test this hypothesis, we measured the single-base deletion error rates of wild-type pol β and lysine and alanine mutants of Arg283, whose side chain interacts with the minor groove edge of the templating nucleotide at the active site. The error rates of both mutant enzymes are increased >100-fold relative to wild-type pol β. Template engineering experiments performed to distinguish among three possible models for deletion formation suggest that most deletions in repetitive sequences by pol β initiate by strand slippage. However, pol β also generates deletions by a different mechanism that is strongly enhanced by the substitutions at Arg283. Analysis of error specificity suggests that this mechanism involves nucleotide misinsertion followed by primer relocation, creating a misaligned intermediate. The structure of pol β bound to non-gapped DNA also indicates that the templating nucleotide and its downstream neighbor are out of register in the open conformation and this could facilitate misalignment (dNTP or primer terminus) with the next template base.

One source of the nucleotide deletion and addition mutations often associated with human diseases is inaccurate DNA replication. Single-base deletions are among the most common replication errors (1). The intermediates for deletions and additions were initially suggested to result from slippage of the two DNA strands (2). When this occurs in a homopolymeric sequence, misaligned template-primers may be stabilized by correct base pairs (Fig. 1A). As the run length increases, there is a comitant increase in the number of correct base pairs that can stabilize the misalignment. This is illustrated in Fig. 1A for runs of 4, 6, and 8 template thymidine residues. There is also an increase in the distance between the 3′ terminus and the extra base and an increase in the number of possible intermediates that can form; only one intermediate is shown for each run length. These features predict that the single-base deletion error rate during DNA synthesis will increase as the length of homopolymeric runs increases. Previous studies have shown that the error rate of several DNA polymerases does indeed increase with increasing run length (reviewed in Ref. 3), such that single-base deletion rates can substantially exceed single-base substitution error rates. The relationship between deletion rate and run length implies that an extra template nucleotide in a misaligned duplex template-primer resides some distance from the polymerase active site, and that deletion error rates may be affected by changes distal to the polymerase active site. This is supported by studies of HIV-1 reverse transcriptase (RT)1 (4) and Klenow fragment polymerase (5). These studies have shown that the rates of single-base deletions and additions in homopolymeric sequences are modulated by polymerase interactions with the DNA minor groove three to five base pairs upstream of the active site.

Several distinct events may initiate formation of single-base deletion intermediates. Slippage of the template and primer strands has been suggested to occur during dissociation and/or reassociation of the DNA polymerase with the DNA (6, 7). This idea is supported by a correlation between the processivity of polymerization and single-base deletion and addition error rates (reviewed in Ref. 3). For those polymerases with intrinsic exonuclease activity, misalignments may form (8) or perhaps be realigned (9) during movement of the primer strand between the polymerase and the exonuclease active sites. Frameshift intermediates can also be corrected by exonucleolytic proofreading (9), albeit sometimes with lower efficiency that for base substitutions (10–12).

DNA polymerases also delete non-iterated template nucleotides during DNA synthesis. As an alternative to strand slippage, deletions of either iterated or non-iterated nucleotides may be initiated by nucleotide misinsertion (13). Misinsertion is generally rare because the active site of a DNA polymerase is designed to accept geometrically equivalent Watson-Crick base pairs and to reject base pairs differing from this geometry (see Ref. 3 for a recent review). Occasional failure of geometric selection can result in a base substitution if a mismatched terminus is extended to leave a mismatched base pair in duplex DNA. However, misinsertion immediately followed by relocation of the primer strand can result in a misaligned template-primer containing an unpaired template-strand nucleotide adjacent to a correct terminal base pair that facilitates further polymerization (Fig. 1B). The idea that misinsertion happens first, with the mismatched product then initiating a deletion mutation, is supported by several studies of DNA polymerases copying undamaged or damaged DNA. Polymerase structure-function studies indicate that nucleotide insertion fidelity de-
hinges on DNA polymerase interactions in the DNA minor groove in the active site (reviewed in Ref. 3). Here we provide a further analysis of the misinsertion-primer relocation model by determining if altered DNA polymerase interactions with the DNA minor groove in the active site also influence single-base deletion error rates.

We also consider a third model (Fig. 1C) for the origin of single-base deletion intermediates. This model is suggested by comparison of the position of template strand nucleotides in the crystal structure of DNA polymerase β (pol β) in different conformations. The crystal structures of several DNA polymerases with and without substrates indicate that they are either in an “open” or a “closed” conformation (reviewed in Ref. 14). In the closed conformation, DNA polymerases are poised for catalysis with the base of the incoming nucleoside triphosphate hydrogen bonded to the templating base. For example, the crystal structures of the closed ternary pol β-DNA-ddCTP complexes (15, 16) indicate that the templating guanine is correctly aligned and paired with a complementary incoming ddCTP. An important feature of the closed structure of pol β bound to a one-nucleotide DNA gap is that the next template base to be copied (i.e. one base 5′ to the templating base, designated n + 1 in Fig. 2, panels A and B) is moved out of the DNA helix axis (Fig. 2A). The nucleotide is stabilized by a histidine side chain in the amino-terminal lyase domain (not shown) of pol β (see Ref. 17, for a review). This displacement gives polymerase side chains access to the minor groove at the growing end of the duplex, allowing correct alignment that depends on Arg283, located on α-helix N (Fig. 2A) in the carboxyl-terminal subdomain of pol β. In a closed conformation, the side chain of Arg283 (purple ball-and-stick in Fig. 2A) makes van der Waals contacts with the minor groove edge of the templating base and can form a hydrogen bond with the sugar of the n − 1 template nucleotide. In contrast, in the open conformation (green in Fig. 2), the side chain of Arg283 does not interact with DNA (16, 18, 19). Thus, when pol β is bound to template-primer DNA and is in an open conformation, Arg283 cannot interact with the minor groove edge of the template nucleotide and the n + 1 nucleotide is not stabilized by an aromatic side chain. In this case, the N3 atom of the template guanine (green “n” in Fig. 2B) is moved 3.0 Å relative to its location in the closed complex (purple “n,” Fig. 2B). This difference in location is similar to the 3.4 Å difference between the position of the N3 of the templating guanine in the closed complex compared with the position of the adjacent template base (n + 1) of the open complex (Fig. 2B). This comparison suggests that as α-helix N rotates to a closed position, the incoming nucleotide (purple ddCTP in Fig. 2B) could on occasion pair with the n + 1 base, generating a single-base deletion intermediate (Fig. 1C).

To test these ideas, here we compare the single-base deletion error rate of wild-type human pol β to those of mutants in which the Arg283 side chain has been changed to either lysine or alanine. These amino acid replacements alter interactions between pol β and the DNA minor groove such that the R283K and R283A mutants have strongly increased misinsertion rates (20, 21) and strongly reduced base substitution fidelity (18), but distinctly different base substitution specificities (22). This provides an opportunity to examine the importance to frameshift fidelity of DNA minor groove interactions at the active site. Moreover, the error specificity data can be interpreted within the context of the three models shown in Fig. 1. As discussed

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**A. By Strand Slippage**

| A-A-A-T-T-5' | C-T-5' |
| G-T-T-T-A-3' | misinsertion |

**B. By Misinsertion**

| A-A-A-T-T-5' | C-T-5' |
| G-T-T-T-A-3' | relocation |

**C. By dNTP Misalignment**

| A-C-T-5' | C-T-5' |
| C-A-G-A-3' | C-A-G-A-3' |

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**FIG. 1.** Three models for initiation of misaligned intermediates for single-nucleotide deletions. For description, see “Introduction.”

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**FIG. 2.** Structural comparison of pol β and HIV-1 RT complexes in the open and closed conformations. A, structures of open (green) (18) and closed (purple) (16) complexes of pol β bound to template-primer (green) or a one-nucleotide DNA gap (purple). The polymerase structures were superimposed by the Cαes of their catalytic subdomains. Arg283 interacts with the minor groove edge of the template strand in the closed conformation and is illustrated in a ball-and-stick representation. The n + 1 template nucleotide is flipped out of the DNA helix axis in the closed conformation of pol β bound to a one-nucleotide gap, but not in the open conformation of pol β bound to template-primer (i.e. no DNA gap). B, positions of templating (n) and adjacent (n + 1) nucleotides in the open and closed conformations of pol β. The N3 atoms (gold) of the templating purines (n and n + 1) in the open conformation are about equidistant from the N3 of the templating guanine (n) in the closed conformation. C, structures of open (green) (38) and closed (purple) (39) conformations of HIV-1 RT and the positions of the templating (n) nucleotide. This figure was made with Molscript (42) and rendered with Raster3D (43).
The experiment was performed as described under “Experimental Procedures.” The revertant frequencies of single-stranded DNAs used to construct the gapped substrates, but not copied in vitro, are all much lower than the values listed (10). Deletion rates are errors per nucleotide incorporated, calculated as described (10, 23). A, rate for deletion of a T from the TTTT run in this experiment is similar to that observed in the run within the 6-nucleotide gap (Table II) and in a forward mutation assay (25). B, rates of dCTP misinsertion opposite the template T (underlined) by rat pol β (21, 44). C, rate of stable (i.e. misinsertion and extension) dCTP misincorporations by human pol β opposite template T (underlined) (25). When the 3'-flanking template strand base is also a T, some portion of the T9 near the amino terminus of the frame are seen as blue revertants. The target sequences are located indicator plates, polymerase errors that restore the correct reading 9-neighboring bases, hence the X.

3. Stranded gaps of base excision repair.
4. Man pol gapped DNA substrates similar to those used by pol detection of single-nucleotide deletions during synthesis to fill the 6-nucleotide single-stranded template gaps in the coding sequence of substrates and product analysis to determine error rates were per-
5. been described (10, 22, 23).

6. Deletion of Reiterated Nucleotides by Wild-type Pol β—In an attempt to distinguish between the three models for deletion errors illustrated in Fig. 1, we first determined the single-base deletion error rates for wild-type pol β during copying of runs of three to eight template thymines located within single-stranded gaps of >300 nucleotides (10). The results (Table I, A) indicate that the deletion error rate increases with increasing run length. This relationship has also been observed with other DNA polymerases (3) and is predicted by the model wherein misaligned intermediates in repetitive sequences are stabilized by correct base pairing, as in Fig. 1A. In considering how misalignments might be initiated, note that all the substrates used in this analysis (Table I, A) contained a guanine as the 5'-template base flanking the run. In this case, single-base deletions initiated by misinsertion as is illustrated in Fig. 1B, requires misinsertion of dCMP opposite the 5'-most T in the run followed by pairing of this dCMP with the flanking guanine. However, as the length of the run increases beyond four thymines, the rate of single-base deletions (Table I, A) exceeds the previously measured misinsertion and stable misincorporation rates for T-dCMP mispairs (Table I, B and C). This implies that the majority of single-base deletions generated by wild-type pol β in homopolymeric sequences need not be initiated by misinsertion.

7. Modulation of Deletion Error Rates by Minor Groove Interactions in the Active Site—Next we tested the hypothesis that frameshift errors are modulated by polymerase interactions in the DNA minor groove that align the template base with the incoming dNTP. We compared the single-base deletion error rate of wild-type pol β to those of R283K and R283A mutants that have altered interactions with the minor groove in the active site. These experiments were conducted with substrates containing gaps of only 6 nucleotides, since the catalytic efficiency of the R283K and R283A mutant polymerases is reduced (18). Thus, they will not fill gaps of >300 nucleotides but readily filled one to five nucleotide gaps such as those typically encountered during base excision repair (18, 22). DNA synthesis by wild-type pol β to copy a 5'-GTTTTA-3' template sequence within a 6-nucleotide gap generated products with a revertant frequency of 37 \times 10^{-4} (Table II). This value is consistent with previous studies (24, 25) indicating that pol β has lower frameshift fidelity than any of the eukaryotic DNA polymerases except pol η (26). Synthesis by the R283K and R283A mutants generated revertant frequencies that were 16- and 38-fold higher, respectively, than for wild-type pol β (Table II). This suggests that the ability of pol β to prevent single-base deletions is modulated by the Arg283 side chain. Sequence analysis of 32 LacZ revertants generated by wild-type pol β showed that 31 had lost one of the T residues in the TTTT run (Fig. 3), yielding an error rate of 150 \times 10^{-5} for wild type pol β (i.e. one deletion for every 670 template thymines copied). All revertants generated by the mutant polymerases were missing one thymidine (Fig. 3). One revertant from the R283K reaction with the 5'-GTTTTA-3' substrate contained a T deletion in combination with a T → G transversion (Fig. 3). Six of 30 revertants from the R283A reaction contained a T deletion in combination with a single-base substitution, and one contained a T deletion in combination with a tandem double-base substitution (Fig. 3). The appearance of these base substitution errors in combination with the deletions is not unexpected, since the R283K and R283A mutant DNA polymerases have reduced base substitution fidelity (18, 22).

8. Deletion of Non-iterated Nucleotides by Wild-type and Mutant Pol β—The above studies involve a repetitive template sequence. Previous studies have shown that pol β also generates deletions of non-iterated nucleotides; these comprise 4–7% of all errors generated by wild-type pol β when copying undamaged DNA templates (25, 27, 28). In contrast to the situation at repetitive sequences, these non-iterated single-base deletions
TABLE II

| Revertant frequency | Ratio of mutant to wild-type | Non-iterated sequence, 5'-cagTGA-3' | Revertant frequency | Ratio of mutant to wild-type |
|---------------------|-----------------------------|-----------------------------------|---------------------|-----------------------------|
| Control DNA         | 0.03                        | 5'-GTTTTA-3                       | 0.02                | 5'-CAGTGA-3                 |
| Wild-type pol β     | 37 ± 10                     | 2.2 × 10⁻⁴                        | 230 ± 10            | 100                         |
| R283K pol β         | 610                         | 100                               | 410 ± 10            | 190                         |
| R283A pol β         | 1400                        | 100                               | 100                 | 100                         |

would seem less likely to be initiated by strand slippage. This is because a one-nucleotide slip at a non-iterated sequence would yield a template-primer containing both a terminal mismatch and an unpaired template-strand nucleotide (e.g. Fig. 1A, last intermediate shown). To examine possible alternatives to the slippage model, we next measured the single-base deletion error rates of wild-type pol β and the R283K and R283A mutants when copying a non-iterated template sequence in a 6-nucleotide gap (Table II). The base substitution specificity of the mutant polymerases was already known for a TGA codon in a 5-nucleotide gap (22). We therefore attempted to distinguish between the misinsertion-primer relocation model (Fig. 1B) and the dNTP misalignment model (Fig. 1C) by measuring deletion rates using a template that also contained a TGA sequence, 5'-cagTGA-3'. Deletion of a single T, G, or A will yield blue plagues, whereas deletion of any of the first three nucleotides (i.e. cag) generates nonsense codons in the correct reading frame and therefore does not yield a blue plaque.

DNA synthesis by the wild-type enzyme generated products with a revertant frequency of 2.2 × 10⁻⁴ (Table II), and 11 of 12 revertants subjected to sequence analysis had lost one nucleotide (Fig. 3). This yields an error rate of 11 × 10⁻⁸, a value 5-fold lower than for deletions by wild-type pol β in the TTTT run (Table II). This difference between a TTTT run and a non-iterated sequence is consistent with the strand slippage model (Fig. 1A) and the relationship between run length and error rate shown above (Table II).

We then determined the single-base deletion error rates for synthesis by the R283K and R283A mutants. These mutant polymerases generated DNA products with revertant frequencies that were elevated by 100- and 190-fold, respectively, compared with the revertant frequency observed with wild-type pol β (Table II). When 46 LacZ revertants were analyzed by DNA sequence analysis, all were found to contain single-base deletions (Fig. 3), including seven that also contained a single-base substitution. Thus, the R283K and R283A mutants are strong mutator polymerases for loss of single, non-iterated nucleotides. This further supports the general hypothesis that interactions of the Arg283 side chain with the minor groove edge of the template strand modulate the rate of single-base deletions generated by pol β.

**DISCUSSION**

This study of the single base deletion fidelity of human pol β suggests three major conclusions. First, replacement of the Arg283 side chain with either lysine or alanine strongly increases error rates (Table II), suggesting that the DNA minor groove interactions in the polymerase active site provided by Arg283 are important for proper substrate alignment that prevents formation of deletion intermediates. Second, the correlation between increasing deletion error rate and increasing length of a homonucleotide run (Tables I and II), and the fact that the single-base deletion error rates greatly exceed misincorporation rates (Table II), strongly support the strand slippage hypothesis for deletions in homonucleotide runs generated by wild-type pol β (Fig. 1A). Third, wild-type pol β also deletes non-iterated nucleotides, and the error rate is increased by 100-fold or more upon replacement of the Arg283 side chain (Table II). This implies that some deletions likely result from a non-slippage mechanism that is mediated by DNA minor groove interactions at the active site. We consider two possible models, misinsertion-primer relocation (Fig. 1B) and dNTP misalignment (Fig. 1C).

In an attempt to distinguish between these two models, we used the following logic. If single-base deletions are initiated by misinsertion, then the deletion rate should not exceed the known rate for misincorporations that could realign to form a deletion intermediate (Fig. 1B). For example, the rate of loss of the T in the 5'-cagTGA-3' sequence should not exceed the rate at which dCMP is misinserted opposite the T. This is because this misinsertion is needed to pair with the adjacent template G in order to generate the deletion intermediate (Fig. 1B). On the other hand, if deletions are initiated by dCTP pairing with the adjacent template guanine (Fig. 1C), then misincorporation of dCMP opposite T is not necessary. In this case, the deletion error rate could, but need not necessarily, exceed the base substitution error rate. With this logic in mind, we used the data in Table II and Fig. 3 to calculate the rates at which wild-type pol β and the R283K pol and R283A mutants deleted a T, G, or A nucleotide from the non-iterated sequence. We then compared these rates to the known base substitution error rates of these same polymerases at the TGA codon in a five-nucleotide gap (22). The results (Table III) reveal that base substitution error rates exceed deletion rates for all three nucleotides with wild-type pol β, for one of three nucleotides (loss of A) with R283K pol β and for two of three nucleotides (loss of G or A) with R283A pol β. In other words, in these situations misinsertion rates are more than sufficient to explain the deletions by the model in Fig. 1B. However, the rate at which the R283K mutant deletes T or G and the rate at which the R283A mutant deletes T are similar to the base substitution error rates. Some of these deletions may be initiated via pairing of the incoming dNTP with the next base, as illustrated in Fig. 1C.
and suggested by structural considerations (see below).

Superimposing the structures of the open conformation of pol β bound to non-gapped template-primer (18) with the closed ternary complex of pol β bound to one-nucleotide gapped DNA (16) indicates that the template nucleotides (n and n+1) of the open conformation are not in register relative to those in the closed form (Fig. 2, panels A and B). Similarly, superimposing this open conformation with the closed ternary complex of pol β bound to non-gapped DNA (15) results in the same observation (not shown). The “vicinity” of the n+1 templating nucleotide in the open conformation with the templating nucleotide (i.e., n) in the closed form suggests that there are opportunities for the incoming nucleoside triphosphate to base pair with the n+1 template base (dNTP misalignment, Fig. 1C). Additionally, if an incorrect nucleotide is inserted opposite the templating nucleotide (i.e., n) and the misinserted nucleotide is complementary to the n+1 templating nucleotide, then the vicinity of the n+1 template base to the dNTP-binding site could facilitate primer relocation following misinsertion (Fig. 1B). As illustrated by comparing the last intermediates shown in Panels B and C of Fig. 1, an important distinction between the misinsertion-primer relocation model and the dNTP misalignment model for single-base deletions is whether the misalignment occurs before or after phosphodiester bond formation. Making this distinction will require approaches beyond measuring deletion errors among the completed products of DNA synthesis, including examination of intermediate steps in the process. Relevant to this point are studies of the rate at which pol β inserts nucleotides when copying DNA templates containing abasic sites (29) or propanodeoxyguanosine adducts (30). In these circumstances, catalytic efficiencies were greatest for insertion of nucleotides that were complementary to the n+1 template base. On that basis, it was suggested that pol β generates base substitutions at sites of DNA damage by dNTP misalignment. In like manner, dNTP misalignment could theoretically explain some of the base substitutions generated by pol β (25, 27, 28) or by E. coli DNA Pol III (31, 32) when copying undamaged DNA.

The balance among the deletion models shown in Fig. 1 likely depends on both the template sequence and the DNA polymerase. Pol I family (9, 10, 33, 34), pol α family (10, 35, 36) and RT family polymerases (37) and human DNA polymerase η (28) all generate single-nucleotide deletions, but at quite different rates. Some of these are deletions of non-iterated nucleotides that occur at rates consistent with the misinsertion-primer relocation model (Fig. 1B). However, structural considerations of frameshift fidelity differ among the polymerases. For example, in contrast to the situation with pol β (Fig. 2B), the templating nucleotide in the open complex of HIV-1 RT bound to DNA (38) is located in a position similar to that seen in the closed ternary complex poised for catalysis (39). In other words, the templating base in the open complex of HIV-1 RT is in register. This is illustrated by comparing the position of the templating base n in Fig. 2, panels B and C. This difference between pol β and HIV-1 RT may relate to differences in the nature of the subdomain movements that occur following dNTP binding. For example, the template strand in the pol β complex may be minimally constrained until the dNTP binds and helix N rotates to a closed conformation. This motion allows the Arg283 side chain to contact the minor groove edge of the template strand (Fig. 2A), thus aligning it for correct pairing. However, the opposite symmetry holds for HIV-1 RT, i.e., the axis for rotation to a closed conformation is on the other side of the binding pocket for the nascent base pair (Fig. 2C). This may allow stabilizing contacts between the enzyme and the templating strand even in the open conformation, with closure of the β3-β4 loop of RT eventually correctly positioning the incoming dNTP. The axis of rotation to a closed conformation is similarly located on the templating-nucleotide side of the binding pocket in pol I family DNA polymerases (reviewed in Ref. 3). Also, in the ternary closed complex of T7 DNA polymerase (40), HIV-1 RT (39), and pol β bound to a one-nucleotide DNA gap (16), the n+1 nucleotide is flipped out of the DNA helix and stacks with an aromatic amino acid side chain. Thus, depending on yet to be determined conformational dynamics as these polymerases move from open to closed conformations following dNTP binding, the incoming dNTP may not have the opportunity to pair with the n+1 nucleotide. Differences in the symmetry of subdomain closure, in template nucleotide register and in template nucleotide flipping, may explain why the insertion specificity of Klenow fragment polymerase (a homolog of T7 pol) and HIV-1 RT do not suggest that the incoming dNTP has paired with the n+1 nucleotide when these enzymes copy damaged templates (30, 41). Flipping of the n+1 nucleotide may or may not modulate the single-nucleotide deletion error rate of pol β. The n+1 nucleotide is displaced and stacked with His34 in a closed ternary complex of pol β bound to DNA containing a single-nucleotide gap (16). However, no such nucleotide displacement was observed when the downstream DNA was single-stranded (15, 18), as is the case for the gapped substrates used in this study. In this case, the amino-terminal 8-kDa domain (i.e., His34) is not observed to be interacting with the DNA.

The strong frameshift mutator phenotypes of the R283K and R283A mutants (Table II) imply that prevention of single-base deletions by wild-type pol β is modulated by side chain interactions with the DNA minor groove at the active site. This may explain the elevated rates of single-nucleotide deletions observed with two other pol β mutants, Y265C (27) and P272L (28). Both Tyr265 and Phe272 occupy positions in the subdomain that rotates as helix N opens and closes. Since Tyr265 and Phe272 could indirectly alter the ability of the Arg283 side chain to correctly align the template strand.

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REPRESENTATIVE

1. Kunkel, T. A. (1990) Biochemistry 29, 8003–8011
2. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., and Inouye, M. (1966) Cold Spring Harbor Symp. Quant. Biol. 31, 77–84
3. Kunkel, T. A., and Bebenek, K. (2000) Annu. Rev. Biochem. 69, in press
4. Bebenek, K., Beard, W. A., Darden, T. A., Li, L., Prasad, R., Luxon, B. A., Gorenstein, D. G., Wilson, S. H., and Kunkel, T. A. (1997) Nat. Struct. Biol. 4, 194–197
5. Minnáč, T., Astatke, M., Joyce, C. M., and Kunkel, T. A. (1996) J. Biol. Chem. 271, 24954–24961
6. Kunkel, T. A. (1985) J. Biol. Chem. 260, 12866–12874
7. Bebenek, K., Abbotts, J., Wilson, S. H., and Kunkel, T. A. (1993) J. Biol. Chem. 268, 10324–10334
8. Fuji, S., Akizawa, M., Aoki, K., Sugaya, Y., Higuchi, K., Hiraoka, M., Miki, Y., Saitoh, N., Yoshiyama, K., Aihara, K., Seki, M., Ohtsubo, E., and Mak, H. (1999) J. Mol. Biol. 289, 835–850

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**Table III**

Base substitution and deletion error rates

| Enzyme   | Error | dCTP-T | dATP-G | dCTP-P |
|----------|-------|--------|--------|--------|
| Wild type| Substitution | 0.2 | 0.2 | 0.3 |
|          | Deletion | 0.12 | 0.06 | 0.03 |
| R283K    | Substitution | 24 | 1.3 | 38 |
|          | Deletion | 35 | ±1.6 | 3.2 |
| R283A    | Substitution | 30 | 15 | 60 |
|          | Deletion | 37 | ±3.1 | 31 |

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**REFERENCES**

1. Kunkel, T. A. (1990) Biochemistry 29, 8003–8011
2. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., and Inouye, M. (1966) Cold Spring Harbor Symp. Quant. Biol. 31, 77–84
3. Kunkel, T. A., and Bebenek, K. (2000) Annu. Rev. Biochem. 69, in press
4. Bebenek, K., Beard, W. A., Darden, T. A., Li, L., Prasad, R., Luxon, B. A., Gorenstein, D. G., Wilson, S. H., and Kunkel, T. A. (1997) Nat. Struct. Biol. 4, 194–197
5. Minnáč, T., Astatke, M., Joyce, C. M., and Kunkel, T. A. (1996) J. Biol. Chem. 271, 24954–24961
6. Kunkel, T. A. (1985) J. Biol. Chem. 260, 12866–12874
7. Bebenek, K., Abbotts, J., Wilson, S. H., and Kunkel, T. A. (1993) J. Biol. Chem. 268, 10324–10334
8. Fuji, S., Akizawa, M., Aoki, K., Sugaya, Y., Higuchi, K., Hiraoka, M., Miki, Y., Saitoh, N., Yoshiyama, K., Aihara, K., Seki, M., Ohtsubo, E., and Mak, H. (1999) J. Mol. Biol. 289, 835–850
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