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Structures of the *Leishmania infantum* polymerase beta

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Abstract

Protozoans of the genus *Leishmania*, the pathogenic agent causing leishmaniasis, encode the family X DNA polymerase Li Pol β . Here, we report the first crystal structures of Li Pol β . Our pre- and post-catalytic structures show that the polymerase adopts the common family X DNA polymerase fold. However, in contrast to other family X DNA polymerases, the dNTP-induced conformational changes in Li Pol β are much more subtle. Moreover, pre- and post-catalytic structures reveal that Li Pol β interacts with the template strand through a nonconserved, variable region known as loop3. Li Pol β loop3 mutants display a higher catalytic rate, catalytic efficiency and overall error rates with respect to WT Li Pol β . These results further demonstrate the subtle structural variability that exists within this family of enzymes and provides insight into how this variability underlies the substantial functional differences among their members.

Keywords

DNA repair; leishmaniasis; DNA polymerase; family X

Introduction

Leishmaniasis is a parasitic disease spread by the bite of certain species of sand fly and caused by the protozoan parasites of the genus *Leishmania*. It is an endemic disease in many

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tropical and subtropical regions of the world and affects about 12 million people worldwide (Desjeux, 2004a, b). Upon injection of the infective promastigote stage of the parasite in the hosts, the parasite is phagocytized by macrophages and delivered into the phagolysosome, where it transforms into the amastigote form. Amastigotes then survive and proliferate within the phagolysosome, and can then periodically escape the macrophage and infect additional host cells. The phagolysosome subjects the amastigotes to an acidic environment and frequent oxidative bursts. Despite the existence of antioxidant defense pathways in the parasite (Van Assche et al., 2011), this environment is likely to cause high levels of DNA damage. The necessity to thrive in this hostile environment is presumably the reason why the genome of the parasite encodes several enzymes related to DNA repair, in particular the enzymes necessary for Base Excision Repair (BER; (Gallego et al., 2005)). BER is a critical repair pathway that protects the cell against DNA base damage (David et al., 2007; Meira et al., 2005; Robertson et al., 2009). The classical BER pathway involves a DNA glycosylase, an AP endonuclease and a family X DNA polymerase.

Family X DNA polymerases play crucial and dual roles in BER; they are responsible for DNA synthesis and 5'-deoxyribose-phosphate (dRP) removal (dRP lyase activity). In addition to BER, these enzymes are involved in other DNA repair processes such as non-homologous end-joining (Yamtich and Sweasy, 2010) and lesion bypass (Hubscher and Maga, 2011). They are composed of a catalytic polymerase domain responsible for polymerization and a specialized 8 kDa domain that is critical for DNA binding, allowing them to simultaneously bind the 5' and 3' ends of a small gap. Moreover, in some of these enzymes, the 8 kDa harbors the dRP lyase activity (Garcia-Diaz et al., 2001; Saxowsky et al., 2002). The conservation of these enzymes in bacteria, archaea and eukaryotes and even in viruses (Aravind and Koonin, 1999; Burgers et al., 2001) suggests that the members of this family of enzymes play crucial roles for genome stability.

A wealth of structural studies has provided extensive insight into the structural characteristics that define each of the four mammalian family X members (Pol β , Pol λ , Pol μ and terminal deoxynucleotidyl transferase, TdT) and revealed how subtle structural alterations in these polymerases can have significant functional consequences (Beard and Wilson, 2006; Delarue et al., 2002; Garcia-Diaz et al., 2005; Moon et al., 2007; Nick McElhinny et al., 2005). The overall family X DNA polymerase fold is well conserved. However, a loop connecting two beta-strands in the palm subdomain differs widely in size. Variations in this loop have been correlated with drastic changes in substrate specificity. This is particularly evident in members of the family capable of template independent DNA synthesis such as TdT and Pol μ (Juarez et al., 2006; Romain et al., 2009).

The family X DNA polymerase from *Leishmania Infantum* (Li Pol β) is a nuclear enzyme (Taladriz et al., 2001). Its expression levels throughout the life cycle of the parasite are consistent with a role in the repair of oxidative damage, especially in the amastigote stage (Ramiro et al., 2002). The biochemical similarities with Pol β , together with the fact that it also contains a dRP lyase activity in its 8 kDa domain, suggest that it plays a role in BER (Alonso et al., 2006). Nevertheless, substantial sequence divergence suggested that structural and functional differences might exist between Li Pol β and the mammalian family X enzymes. In this study, we describe binary and ternary complexes of Li Pol β that

constitute the first structural characterization of this enzyme, revealing a conserved polymerase fold but an active site that nevertheless presents clear differences with that of human Pol β . Interestingly, Li Pol β contains an extension in its thumb subdomain that interacts with the template strand and likens Li Pol β to mammalian TdT and Pol μ .

Results and Discussion

Given that all existing evidence points to a role of Li Pol in BER, and the fact that single-nucleotide DNA gaps are the preferred substrates for family X polymerases, we decided to crystallize this enzyme in the presence of a single-nucleotide gap substrate (see Methods). Our crystallization attempts initially yielded crystals of a binary complex that diffracted to 2.45 Å (see Table I). The resulting electron density maps were of sufficient quality to build the backbone and most side chains of the polymerase domain, the template (TB) and primer (PBT) oligos, but no density was observed for the downstream oligo (DB) or the 8-kDa domain. Moreover, in this structure the template strand is located in a non-catalytic conformation, partly occupying the dNTP binding pocket, suggesting that the observed conformation of the template strand is not catalytically relevant and partially modulated by crystal packing (Sup. Fig. 1). Nevertheless, this initial structure allowed us to determine a more suitable substrate size for crystallization. Using shorter oligos (11-mers) we were able to obtain three additional structures of Li Pol β : a ternary gap complex (2.30 Å), ternary P/T complex (1.90 Å) and a nick complex (2.30 Å). In all the structures the polymerase catalytic domain is well resolved, although only fragments of the 8-kDa domain could be built in two of the three structures due to disorder.

Ternary Gap Complex

The structure (Fig. 1A) represents the pre-catalytic conformation of the enzyme when polymerizing across a gap. All active site residues are in a catalytically relevant conformation (Sup. Fig. 2) consistent with the conformations observed in a pre-catalytic complex of human Pol β (2FMS) (Batra et al., 2006). The 8-kDa domain is engaged in binding the 5'-phosphate. The conformation of the polymerase in this structure is similar to that observed for other family X polymerases in complex with a 1-nt gap (Garcia-Diaz et al., 2005; Moon et al., 2007; Sawaya et al., 1997).

Ternary P/T Complex

The ternary P/T complex was crystallized in an identical manner to the ternary gap complex, except that the crystals were grown at 4°C. As a result, incorporation of the first ddTTP in the 2-nt gap was not catalyzed. The resulting structure (Fig. 1B) shows a ddTTP residue being incorporated opposite the first gap base of a 2-nt gap. All active site residues are in conformations indistinguishable from those observed in a single-nucleotide gap structure, indicating that this structure represents a functional complex. However, the binding mode is distinct from that observed in the other example of a family X polymerase bound to a two-nucleotide gap. When polymerizing across a 2-nt gap, Pol λ binds both the 3' and 5'-ends of short gaps through the polymerase and 8-kDa domains respectively. This is achieved by “scrunching” mechanism that involves rotating the second single-stranded nucleotide of the template into a binding pocket. Binding of the base in Pol λ is stabilized by three conserved

amino acids (Garcia-Diaz et al., 2009). Interestingly, these residues are absent in Li Pol β . Together, this suggests that Li Pol β may not be capable of binding the 5'-end of a 2-nt gap, but instead exclusively relies on contacts made between the polymerization domain and 3'-end of the gap during polymerization. Consistently, the downstream duplex and the 8-kDa domain are disordered in the structure. Thus, the observed conformation might reflect what would happen when polymerizing on an open template/primer.

Nick Complex

The nick complex represents the post-catalytic conformation of the enzyme after polymerization across a gap (Fig. 1C). In contrast to what was first observed with mammalian Pol β (Pelletier et al., 1996), Li Pol β appears to be in a closed conformation with respect to the position of the thumb subdomain. However, this conformation appears to be consistent with recent observations of catalysis by human Pol β *in crystallo* (Freudenthal et al., 2013). Moreover, two of the three catalytic residues overlay well with the ternary gap and P/T complex. However, a slight rotation with respect to the ternary gap and P/T complex is observed in one of the catalytic aspartates (see below). As a result, the active site residues are not in a catalytically relevant conformation.

Organization of the active site and interaction with DNA—Our four crystal structures reveal a catalytically relevant high-resolution view of the active site of Li Pol β . Interestingly, no obvious structural differences are observed among the four complexes (Fig. 1D). Moreover, in both of our Li Pol β ternary complexes, the position of most residues and the nucleic acid substrates can be well overlaid. As in other family X enzymes, three universally conserved catalytic aspartates, Asp 194, Asp196 and Asp 271 (corresponding to Asp190, Asp192 and Asp256 in human Pol β), responsible for coordinating two divalent metal ions are located in conformations that are consistent with their proposed roles in catalysis (Brautigam and Steitz, 1998). The dNTP-binding metal (metal B) can be observed coordinating the phosphate groups of the incoming triphosphate as expected from other family X structures (Batra et al., 2006). In ternary gap complex, a water molecule is occupying the site of the catalytic metal (metal A), consistent with the absence of the 3'OH group from the 3' nucleotide of the primer (Fig. 2A). Interestingly, a 3'-OH group is present in ternary P/T complex and yet no density is observed in the site of metal A (Fig. 2B). This is consistent with the fact that the 3'-OH is located away from the α -phosphate, presumably stabilized through an interaction with Arg273. Arg273 could thus play an analogous role to Lys472 in Pol λ and prevent the 3'OH from adopting a catalytic conformation until the catalytic Mg^{2+} ion (metal A) is bound (Bebenek et al., 2010). Nevertheless, the conformation of the catalytic aspartates in both structures overlay well with those of Pol β (Fig. 2C) (Pelletier et al., 1996). As a result, the catalytic residues in both the ternary gap and ternary P/T complexes are in catalytically relevant conformations.

Arg298 in Li Pol β is a well-conserved residue that is key for fidelity in other family X enzymes (Bebenek et al., 2008). Similar to Pol λ , Arg298 appears to be within hydrogen-bonding distance (3.0 Å) of N3 of the templating adenine and also establishes a hydrogen bond with O2 of the thymine upstream of the adenine (the template base of the primer

terminus). These interactions are thought to be crucial to stabilize the primer terminus and the incoming nucleotide and facilitate catalysis.

Despite the extensive similarities, the active site of Li Pol β also presents significant differences with respect to that of Pol β . In Pol β , a handful of residues are critical for positioning the substrates and catalysis. Most of these residues are conserved in other family X enzymes, but several of these residues are not conserved in Li Pol β . Asp276 and Lys280 form one side of the nascent base pair binding pocket and are replaced in Li Pol β by Lys291 and Val295. These residues nevertheless appear to be playing similar roles in Li Pol β (Fig. 2C) (Vande Berg et al., 2001).

Another important residue in Pol β that is not conserved in Li Pol β is Tyr271 (Thr 286 in Li Pol β). Together with Phe270, this residue is key for activation of Pol β : upon dNTP binding, repositioning of Tyr271 and Phe270 interrupts a salt bridge between Arg258 and Asp192 (one of the three catalytic aspartates) that maintains the latter residue removed from the active site. This allows proper assembly of the active site, and is considered a crucial step for activation of the polymerase (Beard and Wilson, 2006). Whereas Phe270 is conserved in Li Pol β (Phe287), the absence of Tyr271 suggests that the residue network that operates in Pol β is not conserved in Li Pol β . Nevertheless, a mechanism appears to exist to prevent Asp196 from adopting a catalytic conformation that is independent of Phe287 (see below).

In addition to mediating the activation of the enzyme in Pol β , Tyr271 plays a more general role in family X polymerases. In the active conformations of both Pol β and Pol λ , Tyr271 (Tyr505 in Pol λ) establishes minor groove interactions to the nucleotide in the 3' end of the primer. This interaction appears to be important to correctly position the 3'-OH for catalysis in Pol β and Pol λ , and yet no equivalent interaction is seen in Li Pol β . Furthermore, this residue has also been demonstrated to play an important role as a steric gate for ribonucleotide discrimination (Gosavi et al., 2012).

dNTP-induced conformational changes—The catalytic cycle in Pol β is known to involve a large scale conformational change that results in substantial subdomain motions that mostly involve rotation of the thumb subdomain and the 8 kDa domain (Beard and Wilson, 2006), leading to two structurally distinct (“open” and “closed”) states. By contrast, the conformational change in Pol λ is much more subtle, only involving DNA motions and repositioning of a number of protein side chains (Garcia-Diaz et al., 2005). The other two mammalian family X DNA polymerases, Pol μ and TdT, are thought to share this characteristic with Pol λ (Delarue et al., 2002; Gouge et al., 2013; Li and Schlick, 2010). Interestingly, the polymerase domains in all four Li Pol β structures superimpose well (Fig. 1D), suggesting that no major subdomain conformational changes occur upon dNTP binding (binary and ternary complex gap or P/T) or catalysis (ternary gap and nick complex). Unfortunately, it is not possible to judge from these structures whether the 8 kDa domain undergoes similar motions as those observed in Pol β due to disorder. Similar to what is observed in Pol λ , comparing the ternary pre-catalytic structures to the nick structure reveals very few differences. One of these differences is that both the nick and binary complexes appear to adopt an inactive conformation like in Pol β : Asp196 is, like Asp192 in Pol β , removed away from its catalytic conformation and interacts with Arg273, the equivalent

residue to Arg258 in Pol β (Fig. 2D). By contrast, in both ternary complexes, it is located in its expected catalytic conformation. Thus, a similar activation mechanism appears to exist in Li Pol β . However, given that a tyrosine residue is absent at the equivalent 271 position and that while Phe272 (Phe287) is conserved, it does not appear to change its conformation in any of the Li Pol β structures, the mechanism by which this repositioning is controlled in Li Pol β is not apparent from the crystal structures.

Variable Regions in Family X Polymerases—A sequence alignment between Li Pol β and the mammalian family X DNA polymerases (Fig. 3A) suggests extensive structural conservation among all five enzymes (Li Pol β , human Pol β and Pol λ , murine TdT and Pol μ). Indeed, the relatively good structural overlay (rmsds of 1.389 Å for 245 C- α atoms with Pol β , 1BPY, 1.582 Å for 243 C- α atoms with Pol λ , 1XSN, 1.839 Å for 234 C- α atoms with TdT, 1KDH and 1.977 Å for 235 C- α atoms with Pol μ , 2IHM) demonstrates that the overall fold is well conserved (Fig. 3B). However, a comparison of the different family X members allows to define four variable regions in between conserved secondary structure elements (Fig. 3C). Two of these regions, loop1 (between β -strands 3 and 4) and loop2 (between p-strand 4 and 5) are located in the palm subdomain and were originally identified when the TdT structure was solved (Delarue et al., 2002). A third one, loop3 (between P-strand 7 and α -helix O in the thumb subdomain) was observed to be highly divergent in human Pol λ . An additional variable loop is present between β -strand 2 and α -helix L (loop0).

Loop1 has received the most attention, as its length appears to be correlated with substrate specificity in family X DNA polymerases (Nick McElhinny et al., 2005). It occludes the template-binding site in TdT, making it a strictly template-independent enzyme. It is however more flexible in Pol μ , presumably serving to stabilize the primer in a template-independent context, while allowing template-dependent reactions (Juarez et al., 2006). On the other hand, it is short in Pol β (arguably the most strict of the Family X enzymes with respect to substrate specificity) and it has an intermediate size in Pol λ . Loop1 swaps have drastic effects on polymerase activity. For instance, replacing the TdT loop1 with the equivalent residues in Pol μ or Pol λ results in an enzyme that shares some of the properties of the latter enzymes, including the ability to catalyze template-dependent reactions (Romain et al., 2009). Replacing the Pol λ loop1 with the equivalent residues in Pol β , on the other hand, results in an extremely unfaithful DNA polymerase, suggesting that loop1 directly or indirectly modulates the dNTP-induced conformational change (Bebenek et al., 2010). The role of loop3 has been less clearly established, but in Pol λ it appears to be able to contact the template strand and has been implicated in modulating the ability of the polymerase to promote deletion errors (Garcia-Diaz et al., 2006). Loop2 protrudes towards the exterior of the protein and it is therefore disordered in all structures. Similarly, loop0 is located in the protein surface. This suggests that these two variable regions could serve to mediate differential protein-protein interactions, although loop2 mutations in Pol β have been shown to drastically alter its catalytic properties, including its fidelity of synthesis (Lin et al., 2009; Lin et al., 2007). Interestingly, the size of loop2 in Li Pol β is similar to that in TdT and Pol μ .

Loop 3 is a template-binding loop and its deletion results in higher catalytic efficiency—

In comparison to other family X DNA polymerases, loop3 in Li Pol β is extraordinarily long and adopts a distinct conformation (Fig. 4A). In Pol λ , this loop extends towards the template strand downstream of the nascent base pair. However, loop3 in Li Pol β interacts with the template strand upstream of the nascent base pair (Fig. 4B). The side chain residues of Asn335 and Glu338 hydrogen bond with the phosphate groups of the -2 and -3 template nucleotides respectively, while the main chain amide nitrogen atom of Ala336 is involved in hydrogen bonding with the phosphate of the -2 template nucleotide. Moreover, Glu338 also appears to interact with Asn309 in order to stabilize the observed conformation of loop3. Interestingly, with respect to its interaction with the template strand, the conformation of loop3 in Li Pol β is thus reminiscent of that of loop1 in other family X enzymes.

In order to examine the importance of loop3 for DNA polymerization, we decided to generate a chimeric protein in which loop3 was replaced with the equivalent residues in human Pol β . The swapping points were based on a structural alignment between both enzymes to ensure that the core fold would not be perturbed (see Methods). Initial primer extension assays using the mutant protein (Li Pol β loop) suggested that the mutant enzyme displayed an increased catalytic rate (not shown). We thus decided to analyze the activity of the mutant using a steady-state approach. As shown in Table II, steady state analysis revealed that both enzymes present a relatively low $K_{m(app)}$ value, suggesting similarities with human Pol λ (Garcia-Diaz et al., 2002). However, Li Pol β loop consistently displayed a higher catalytic rate, resulting in a modest but reproducible 4-fold increase in catalytic efficiency (Table II). This is once again extremely similar to what is observed when loop1 is deleted in Pol λ , further suggesting that both loops might indeed have equivalent functions.

DNA synthesis fidelity of Li Pol β and effects of loop3 deletion—As stated before, a clear correlation exists between the length of loop1 and fidelity of synthesis. In order to test whether this is also the case for loop3, we decided to characterize the fidelity of synthesis of Li Pol β using a well-validated genetic assay and examine how it is affected by deletion of loop3. For our analysis, we used a M13mp2 forward mutation assay capable of detecting a broad range of substitution, insertion and deletion errors in different sequence contexts ((Bebenek and Kunkel, 1995); see Table III). This assay requires the enzyme to fill in a 407-nucleotide gap. Both wt Li Pol β and the Li Pol β loop mutant were able to complete gap filling (not shown). The resulting products were introduced into *E. coli* and plated. Plates were then scored for total and mutant plaques (see Methods). Wild-type Li Pol β generated lacZ mutants with a 4.7% mutant frequency, which is only slightly higher to that observed for human Pol β (Osheroff et al., 1999). The majority of the mutations generated by Li Pol β were base substitutions, but numerous -1 deletions could also be observed (Table III). Calculation of the error rates for Li Pol β (Table IV) reveals that, as expected, the highest error rate corresponds to base substitutions. Further analysis of the specific errors made by the polymerase (Sup. Table 1) indicates that by far the highest error rate (27×10^4) corresponds to T to C transitions.

The error rate for base substitutions is the highest observed for any family X DNA polymerase, with the exception of the highly error prone Pol μ (Zhang et al., 2001). Family

X DNA polymerases, on the other hand, have been shown to be particularly unfaithful for deletion errors. Li Pol β thus appears unique in its ability to generate base substitutions while maintaining a relatively low error rate for insertion/deletion mutations.

We then tested the effect of swapping loop3 on polymerization fidelity. The effects observed were modest but reproducible: Li Pol β loop displayed a mutation frequency of 8.9%, and corresponding base substitution error rates that were roughly 2-fold higher than for wild-type Li Pol β . Interestingly, however, loop3 deletion moderated the error rates for deletion errors, while slightly increasing the error rate for single base insertions (Table IV). Hence, deletion of loop3 in Li Pol β results in an increase in catalytic efficiency that is comparable to that observed upon deletion of loop1 in human Pol λ , and in an overall decrease in DNA synthesis fidelity also as observed in a loop1 deletion mutant (a roughly 2-fold increase in overall mutation frequency was observed for the Pol λ loop1 deletion as well). For Pol λ , these effects were rationalized as a consequence of the role of loop1 as a kinetic checkpoint for fidelity during the conformational change of the polymerase, and it is reasonable to speculate that a similar mechanism might take place during the Li Pol β catalytic cycle. However, it is important to note that the alterations in fidelity observed for Li Pol β are substantially less drastic than what was observed upon deletion of loop1 in Pol λ . This might reflect the different nature of the catalytic cycle for Li Pol β (see below).

Conclusions

In this paper, we report the first crystal structures of a family X DNA polymerase from *Leishmania infantum*. Our structural characterization reveals that Li Pol β conserves the overall fold of Family X enzymes, and interacts with DNA in an analogous manner to the template-dependent members of the family, supporting its proposed role in DNA repair. However, the structures also reveal interesting and unique details of the Li Pol β catalytic cycle that suggest mechanistic differences with other members of the family. This represents an additional example of how a family of enzymes that share a common fold nevertheless presents a surprising amount of structural diversity to ultimately perform the same nucleotidyl transfer reaction.

Despite sharing a common strongly conserved fold, family X DNA polymerases are endowed with very different biochemical properties. Structural characterizations of every mammalian member of the family have led to a significant understanding of the subtle details of their catalytic cycles and unraveled some of the structural characteristics that make each enzyme unique. However, much uncertainty remains with respect to the nature of the conformational changes during polymerization or the mechanisms by which these enzymes discriminate against incorrect insertions. The discovery that loop1 in TdT is likely responsible for its template-independent behavior sparked an interest in understanding the roles of this divergent loop. It was then demonstrated that loop1 could exert an important influence on the substrate specificity of these enzymes. In fact, as we show in this paper, four divergent regions exist in family X DNA polymerases. Two of these divergent regions are located in the protein surface and therefore appear likely to either play a structural role or to mediate protein-protein interactions. Two other regions (loop1 and loop3) are however positioned to directly influence the interaction with the template strand and presumably

contribute to modulate substrate specificity. Li Pol β contains a very long and structured loop3. While it in part shares the conformation of loop3 in Pol λ , its much larger size results in the loop establishing extensive interactions with the template strand, in the same region as loop1 for other family X enzymes. Although Li Pol β is unable to catalyze template-independent insertions, this observation nevertheless suggests that loop3 plays an important functional role in Li Pol β . Deletion of this loop results in a surprising increase in the catalytic efficiency of the enzyme, an effect that is almost identical to that observed when deleting loop1 from human Pol λ . Moreover, deletion of loop3 also resulted in a decrease in DNA synthesis fidelity, although much more modest as compared to the effects of removing loop1 in Pol λ . This suggests that like loop1 in Pol λ , loop3 might be important to regulate the conformational change of the enzyme (Bebenek et al., 2010). These results further support the hypothesis that these variable loops play a role in the regulation of catalysis in family X DNA polymerases. Loop1 in Pol λ and loop3 in Li Pol β appear to serve as counterweights, helping to fine tune the delicate balance between activity and fidelity.

It is interesting to note that, at least in mouse cells, a TdT splicing variant results in an insertion in loop3 that would make it almost the same size as in Li Pol β . This splicing variant, TdTL, has been shown to display widely altered biochemical properties and does not appear to participate in V(D)J recombination (Doyen et al., 2004; Thai et al., 2002), implying that loop3 can indeed have a dramatic influence on the biochemical properties of the DNA polymerase.

The DNA repair pathways of *Leishmania* constitute a promising novel drug target to fight leishmaniasis. The structures of Li Pol β further confirm that this enzyme is well suited to perform small gap repair reactions that might be crucial for the survival of the parasite in macrophages. Despite the extensive conservation of the family X DNA polymerase fold in mammals, the existence of highly divergent, variable regions that play an important functional role suggest that designing inhibitors against these regions might lead to the development of effective and selective drugs to help combat the infection.

Experimental Procedures

Materials

Oligonucleotides were synthesized using an Expedite 8909 DNA synthesizer. Monomers and reagents for synthesis were from Glen Research. Nucleotides were from GE Healthcare. Primary crystallization screens were from Hampton Research.

Protein purification

Li Pol β (42.7 kDa) was cloned into pTEV-HMBP3, allowing expression of a fusion with his-tagged maltose binding protein (MBP) cleavable by TEV protease (Yakubovskaya et al., 2010). The protein was overexpressed in Arctic Xpress *E.coli* (DE3) cells (Stratagene) at 16°C for 20 h. Following lysis by sonication, the protein was purified using ProBond Resin (Invitrogen), subsequent overnight TEV protease cleavage, Heparin and Mono S chromatography. The protein was then concentrated using a 10,000 MWCO Spin-X UF 20 device (Corning) and stored in 20 mM HEPES (pH 8.0), 100 mM KCl and 1mM DTT. The

Li Pol β loop mutant was introduced using a nested pcr approach. Residues 323-349 in Li Pol β were replaced with alanine-glycine, corresponding to human Pol β . The mutant was purified as described for the wild-type polymerase.

Crystallization and structural determination

In all cases, the appropriate oligonucleotides were hybridized in a buffer containing 120 mM MgCl_2 , 0.4 M Tris-HCl, pH 7.5. The DNA substrate (final concentration 0.6 mM) was then mixed with the protein (final concentration 0.3 mM).

Binary complex—Li Pol β was crystallized in complex with oligonucleotides TB (5'-CCGGCAACGCATCAGC), PBT (5'-GCTGATGCG) and DB (5'-GCCGG) and ddTTP (10 mM). Crystals were grown at 23°C in a solution containing 0.1 M ammonium acetate, 0.02 M MgCl_2 , 0.05M Hepes, pH 7.0, 5% PEG 8000 and transferred to a solution containing 0.1 M ammonium acetate, 0.02 M MgCl_2 , 0.05M Hepes, pH 7.0, 5% PEG 8000, 25% ethylene glycol, 100 mM KCl, 1 mM DTT and 10 mM MgCl_2 .

Ternary Gap Complex—Li Pol β was crystallized in complex with oligonucleotides P5 (5'-CAGTA), T11dd (5'-CGGCAATACTG) and DT (5'-GCCG) and ddTTP (5 mM) to generate a ternary pre-catalytic intermediate after incorporation of ddTMP. Crystals were grown at 23°C using the hanging drop method in a solution containing 0.1 M magnesium formate. After harvesting, they were transferred to a solution containing 0.1 M magnesium formate, 50% glycerol, 100 mM KCl, 1 mM DTT and 10 mM MgCl_2 and cryocooled in liquid nitrogen.

Ternary P/T Complex—Crystallization was as above (ternary complex A), using the same oligo substrate, but crystals were grown at 4°C in a solution containing 0.1 M NaCl, 0.1 M Sodium acetate, pH 4.6 and 12% 2-methyl-2,4-pentanediol (MPD). Crystals were harvested and transferred to a solution containing 0.1 M NaCl, 0.1 M sodium acetate pH 4.6, 35% MPD, 100 mM KCl, 1 mM DTT and 10 mM MgCl_2 .

Nick Complex—Li Pol β was crystallized in complex with oligonucleotides T8XA (5'-CGGCAGTACTG), PGO/CN (5'-CAGTACT) and DT. Crystals grew at 4°C from a solution containing 0.2 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6 and 12.5% PEG 4000. Crystals were harvested and transferred to a solution containing 0.2 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6, 15 % PEG 4000, 25% ethylene glycol, 100 mM KCl, 1 mM DTT and 10 mM MgCl_2 .

Data collection was performed at -178°C at beamlines X25 and X29 at the National Synchrotron Light Source (BNL). The datasets were processed using HKL2000. To determine the binary structure, a search model for molecular replacement was created from PDB entry 1BPY. A molecular replacement solution was found using Phaser (McCoy et al., 2007) and refined using Phenix (Adams et al, 2010) following extensive model building in Coot (Emsley and Cowtan, 2004). The ternary and nick structures were solved by molecular replacement using the final binary model and refined using Coot and Phenix.

Kinetic analysis: Synthetic oligonucleotides were obtained from Operon. Oligonucleotide P (5' TAGCTAGCGACAGTAC) was ^{32}P -5' end-labelled and annealed to oligonucleotide T (5'-ACGTTCCGGCTGTACTGTCGCTAGCTA). Reactions (20 μl) contained 50 mM Tris, pH 7.5, 1mM DTT, 4% (v/v) glycerol, 0.1 mg/ml BSA, 10 mM MgCl_2 , 200 nM DNA and 6 nM wild-type or 1 nM Li Pol β loop. Reactions were initiated by adding dATP (1 μM -1 mM) and incubated at 37 °C for 6 min. Products were resolved by PAGE and gel band intensities were quantified using ImageQuant TL (GE Healthcare). The data were fit to the Michaelis-Menten equation using nonlinear regression.

Forward Mutation Assay: The assay scores errors generated in the LacZ α -complementation gene in M13mp2 during synthesis to fill a 407-nt gap. Reaction mixtures (25 μl) contained 2 nM M13mp2 gapped DNA substrate, 50 mM Tris-HCl (pH 8.5), 2.5 mM MgCl_2 , 1mM dithiothreitol, 2 μg of BSA, 4% glycerol and 50 μM each of dATP, dGTP, dCTP and dTTP. Polymerization reactions were initiated by adding wt Li Pol β (400 nM) or Li Pol β Dloop3 (500 nM), and were incubated at 37°C for 1 h, and terminated by adding EDTA to 15 mM. Reaction products were analyzed by agarose gel electrophoresis as described (Bebenek and Kunkel, 1995). Correct synthesis produces M13mp2 DNA that yields dark blue phage plaques upon introduction into an E. coli α -complementation strain and plating on indicator plates. Errors are scored as light blue or colorless mutant phage plaques. DNA from independent mutant clones was sequenced to define the lacZ mutation. The error rates are calculated as described (Bebenek and Kunkel, 1995). Shortly, the mutant frequency is multiplied by the percentage of mutants in a particular class and divided by 0.6, the minus-strand expression value, and divided by the number of sites at which the error can be detected.

Supplementary Material

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References

- Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, Grosse-Kunstleve RW, et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta crystallographica Section D, Biological crystallography*. 2010; 66:213–221.
- Alonso A, Terrados G, Picher AJ, Giraldo R, Blanco L, Larraga V. An intrinsic 5'-deoxyribose-5-phosphate lyase activity in DNA polymerase beta from *Leishmania infantum* supports a role in DNA repair. *DNA repair*. 2006; 5:89–101. [PubMed: 16174567]
- Aravind L, Koonin EV. DNA polymerase beta-like nucleotidyltransferase superfamily: identification of three new families, classification and evolutionary history. *Nucleic Acids Res*. 1999; 27:1609–1618. [PubMed: 10075991]

- Batra VK, Beard WA, Shock DD, Krahn JM, Pedersen LC, Wilson SH. Magnesium-induced assembly of a complete DNA polymerase catalytic complex. *Structure*. 2006; 14:757–766. [PubMed: 16615916]
- Beard WA, Wilson SH. Structure and mechanism of DNA polymerase Beta. *Chem Rev*. 2006; 106:361–382. [PubMed: 16464010]
- Bebenek K, Garcia-Diaz M, Foley MC, Pedersen LC, Schlick T, Kunkel TA. Substrate-induced DNA strand misalignment during catalytic cycling by DNA polymerase lambda. *EMBO reports*. 2008; 9:459–464. [PubMed: 18369368]
- Bebenek K, Garcia-Diaz M, Zhou RZ, Povirk LF, Kunkel TA. Loop 1 modulates the fidelity of DNA polymerase lambda. *Nucleic acids research*. 2010; 38:5419–5431. [PubMed: 20435673]
- Bebenek K, Kunkel TA. Analyzing fidelity of DNA polymerases. *Methods in enzymology*. 1995; 262:217–232. [PubMed: 8594349]
- Brautigam CA, Steitz TA. Structural and functional insights provided by crystal structures of DNA polymerases and their substrate complexes. *Current opinion in structural biology*. 1998; 8:54–63. [PubMed: 9519297]
- Burgers PM, Koonin EV, Bruford E, Blanco L, Burtis KC, Christman MF, Copeland WC, Friedberg EC, Hanaoka F, Hinkle DC, et al. Eukaryotic DNA polymerases: proposal for a revised nomenclature. *J Biol Chem*. 2001; 276:43487–43490. [PubMed: 11579108]
- David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. *Nature*. 2007; 447:941–950. [PubMed: 17581577]
- Delarue M, Boule JB, Lescar J, Expert-Bezancon N, Jourdan N, Sukumar N, Rougeon F, Papanicolaou C. Crystal structures of a template-independent DNA polymerase: murine terminal deoxynucleotidyltransferase. *The EMBO journal*. 2002; 21:427–439. [PubMed: 11823435]
- Desjeux P. Leishmaniasis. *Nat Rev Microbiol*. 2004a; 2:692. [PubMed: 15378809]
- Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis*. 2004b; 27:305–318. [PubMed: 15225981]
- Doyen N, Boule JB, Rougeon F, Papanicolaou C. Evidence that the long murine terminal deoxynucleotidyltransferase isoform plays no role in the control of V(D)J junctional diversity. *J Immunol*. 2004; 172:6764–6767. [PubMed: 15153493]
- Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta crystallographica Section D, Biological crystallography*. 2004; 60:2126–2132.
- Freudenthal BD, Beard WA, Shock DD, Wilson SH. Observing a DNA polymerase choose right from wrong. *Cell*. 2013; 154:157–168. [PubMed: 23827680]
- Gallego C, Estevez AM, Farez E, Ruiz-Perez LM, Gonzalez-Pacanowska D. Overexpression of AP endonuclease protects Leishmania major cells against methotrexate induced DNA fragmentation and hydrogen peroxide. *Molecular and biochemical parasitology*. 2005; 141:191–197. [PubMed: 15850702]
- Garcia-Diaz M, Bebenek K, Krahn JM, Kunkel TA, Pedersen LC. A closed conformation for the Pol lambda catalytic cycle. *Nature structural & molecular biology*. 2005; 12:97–98.
- Garcia-Diaz M, Bebenek K, Krahn JM, Pedersen LC, Kunkel TA. Structural analysis of strand misalignment during DNA synthesis by a human DNA polymerase. *Cell*. 2006; 124:331–342. [PubMed: 16439207]
- Garcia-Diaz M, Bebenek K, Kunkel TA, Blanco L. Identification of an intrinsic 5'-deoxyribose-5-phosphate lyase activity in human DNA polymerase lambda: a possible role in base excision repair. *The Journal of biological chemistry*. 2001; 276:34659–34663. [PubMed: 11457865]
- Garcia-Diaz M, Bebenek K, Larrea AA, Havener JM, Perera L, Krahn JM, Pedersen LC, Ramsden DA, Kunkel TA. Template strand scrunching during DNA gap repair synthesis by human polymerase lambda. *Nature structural & molecular biology*. 2009; 16:967–972.
- Garcia-Diaz M, Bebenek K, Sabariego R, Dominguez O, Rodriguez J, Kirchhoff T, Garcia-Palomero E, Picher AJ, Juarez R, Ruiz JF, et al. DNA polymerase lambda, a novel DNA repair enzyme in human cells. *The Journal of biological chemistry*. 2002; 277:13184–13191. [PubMed: 11821417]
- Gosavi RA, Moon AF, Kunkel TA, Pedersen LC, Bebenek K. The catalytic cycle for ribonucleotide incorporation by human DNA Pol lambda. *Nucleic acids research*. 2012; 40:7518–7527. [PubMed: 22584622]

- Gouge J, Rosario S, Romain F, Beguin P, Delarue M. Structures of Intermediates along the Catalytic Cycle of Terminal Deoxynucleotidyltransferase: Dynamical Aspects of the Two-Metal Ion Mechanism. *J Mol Biol.* 2013; 425:4334–4352. [PubMed: 23856622]
- Hubscher U, Maga G. DNA replication and repair bypass machines. *Curr Opin Chem Biol.* 2011; 15:627–635. [PubMed: 21889903]
- Juarez R, Ruiz JF, Nick McElhinny SA, Ramsden D, Blanco L. A specific loop in human DNA polymerase mu allows switching between creative and DNA-instructed synthesis. *Nucleic acids research.* 2006; 34:4572–4582. [PubMed: 16963491]
- Li Y, Schlick T. Modeling DNA polymerase mu motions: subtle transitions before chemistry. *Biophysical journal.* 2010; 99:3463–3472. [PubMed: 21081096]
- Lin GC, Jaeger J, Eckert KA, Sweasy JB. Loop II of DNA polymerase beta is important for discrimination during substrate binding. *DNA repair.* 2009; 8:182–189. [PubMed: 19013261]
- Lin GC, Jaeger J, Sweasy JB. Loop II of DNA polymerase beta is important for polymerization activity and fidelity. *Nucleic acids research.* 2007; 35:2924–2935. [PubMed: 17439962]
- McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. Phaser crystallographic software. *Journal of applied crystallography.* 2007; 40:658–674. [PubMed: 19461840]
- Meira LB, Burgis NE, Samson LD. Base excision repair. *Advances in experimental medicine and biology.* 2005; 570:125–173. [PubMed: 18727500]
- Moon AF, Garcia-Diaz M, Bebenek K, Davis BJ, Zhong X, Ramsden DA, Kunkel TA, Pedersen LC. Structural insight into the substrate specificity of DNA Polymerase mu. *Nature structural & molecular biology.* 2007; 14:45–53.
- Nick McElhinny SA, Havener JM, Garcia-Diaz M, Juarez R, Bebenek K, Kee BL, Blanco L, Kunkel TA, Ramsden DA. A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. *Molecular cell.* 2005; 19:357–366. [PubMed: 16061182]
- Osheroff WP, Jung HK, Beard WA, Wilson SH, Kunkel TA. The fidelity of DNA polymerase beta during distributive and processive DNA synthesis. *The Journal of biological chemistry.* 1999; 274:3642–3650. [PubMed: 9920913]
- Pelletier H, Sawaya MR, Wolfle W, Wilson SH, Kraut J. Crystal structures of human DNA polymerase beta complexed with DNA: implications for catalytic mechanism, processivity, and fidelity. *Biochemistry.* 1996; 35:12742–12761. [PubMed: 8841118]
- Ramiro MJ, Hanke T, Taladriz S, Larraga V. DNA polymerase beta mRNA determination by relative quantitative RT-PCR from *Leishmania infantum* intracellular amastigotes. *Parasitol Res.* 2002; 88:760–767. [PubMed: 12122435]
- Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells: Base excision repair: the long and short of it. *Cellular and molecular life sciences : CMLS.* 2009; 66:981–993. [PubMed: 19153658]
- Romain F, Barbosa I, Gouge J, Rougeon F, Delarue M. Conferring a template-dependent polymerase activity to terminal deoxynucleotidyltransferase by mutations in the Loop1 region. *Nucleic acids research.* 2009; 37:4642–4656. [PubMed: 19502493]
- Sawaya MR, Prasad R, Wilson SH, Kraut J, Pelletier H. Crystal structures of human DNA polymerase beta complexed with gapped and nicked DNA: evidence for an induced fit mechanism. *Biochemistry.* 1997; 36:11205–11215. [PubMed: 9287163]
- Saxowsky TT, Matsumoto Y, Englund PT. The mitochondrial DNA polymerase beta from *Crithidia fasciculata* has 5'-deoxyribose phosphate (dRP) lyase activity but is deficient in the release of dRP. *Journal of Biological Chemistry.* 2002; 277:37201–37206. [PubMed: 12151410]
- Taladriz S, Hanke T, Ramiro MJ, Garcia-Diaz M, Garcia De Lacoba M, Blanco L, Larraga V. Nuclear DNA polymerase beta from *Leishmania infantum*. Cloning, molecular analysis and developmental regulation. *Nucleic acids research.* 2001; 29:3822–3834. [PubMed: 11557814]
- Thai TH, Purugganan MM, Roth DB, Kearney JF. Distinct and opposite diversifying activities of terminal transferase splice variants. *Nature immunology.* 2002; 3:457–462. [PubMed: 11938351]
- Van Assche T, Deschacht M, da Luz RA, Maes L, Cos P. *Leishmania*-macrophage interactions: Insights into the redox biology. *Free Radic Biol Med.* 2011; 51:337–351. [PubMed: 21620959]

- Vande Berg BJ, Beard WA, Wilson SH. DNA structure and aspartate 276 influence nucleotide binding to human DNA polymerase beta. Implication for the identity of the rate-limiting conformational change. *The Journal of biological chemistry*. 2001; 276:3408–3416. [PubMed: 11024043]
- Yakubovskaya E, Mejia E, Byrnes J, Hambardjiev E, Garcia-Diaz M. Helix unwinding and base flipping enable human MTERF1 to terminate mitochondrial transcription. *Cell*. 2010; 141:982–993. [PubMed: 20550934]
- Yamitch J, Sweasy JB. DNA polymerase family X: function, structure, and cellular roles. *Biochimica et biophysica acta*. 2010; 1804:1136–1150. [PubMed: 19631767]
- Zhang Y, Wu X, Yuan F, Xie Z, Wang Z. Highly frequent frameshift DNA synthesis by human DNA polymerase mu. *Molecular and cellular biology*. 2001; 21:7995–8006. [PubMed: 11689691]

Highlights

- The first crystal structure of Li Pol β
- Li Pol β adopts the common family X DNA polymerase fold
- A nonconserved, variable loop (loop3) in Li Pol β interacts with the template strand
- loop3 mutants display a higher catalytic rate and overall mutation frequency

Figure 1. Structure of Li Pol β during gap repair

(A) Crystal structure of the Ternary Gap complex of Li Pol β. The 8-kDa domain is shown in grey and the 39-kDa catalytic domain is colored by subdomain: fingers (blue), palm (yellow) and thumb (red). The polymerase is in complex with ddTTP (pink) and a 1-nt gap 16-mer oligo (orange). The primer strand is terminated with a dideoxynucleotide (blue). (B) Crystal structure of the Ternary P/T complex of Li Pol β, consisting of a finger (blue) palm (yellow) and thumb (red) subdomains, in complex with a ddTTP (pink) and a 2-nt gap 16-mer oligo (orange). (C) Crystal structure of nick complex of Li Pol β, consisting of a finger (blue) palm (yellow) and thumb (red) subdomains, in complex with a 16-mer oligo (orange) containing a nick. (D) Overlay of the C-α traces of the four Li Pol β structures. The Ternary Gap (yellow), P/T (blue), binary (gray) and nick (green) complex structures have an rmsd (with respect to the Ternary Gap structure) of 0.616 Å (Ternary P/T), 1.341 Å (binary) and 0.506 Å (nick) for 269, 224 and 282 C-α atoms, respectively.

Figure 2. Active site of Li Pol β and dNTP-induced conformational changes

(A) dNTP-binding pocket in the ternary gap complex. The incoming ddTTP is shown in green, together with the complementary templating base (magenta), the three catalytic aspartates (yellow), a water molecule in the metal A site (red), and a Mg²⁺ ion (metal B; olive). A simulated annealing fo-fc electron density map is shown, contoured at 3σ (blue). (B) dNTP-binding pocket in the ternary P/T complex. The color coding is as in A. (C) Overlay of the active sites between the ternary gap complex of Li Pol β and the corresponding Pol β complex (1BPY). The DNA and incoming dNTP are shown for the Li Pol β structure. Relevant active site residues are shown in yellow (Li Pol β) or blue (Pol β). (D) Conformational change affecting Asp196 in Li Pol β. In the binary and post-catalytic complex, Asp196 is seen in an inactive conformation (yellow), hydrogen-bonding to Arg273, while in both pre-catalytic ternary complexes Asp196 is in a catalytic conformation (grey) coordinating metal B, suggesting that a similar switch exists as in Pol β that determines the activation state of the polymerase. The incoming ddTTP in the pre-catalytic complex is shown in magenta for orientation purposes.

Figure 3. Variable regions in Family X DNA polymerases

Structure-based multiple amino acid alignment of the mammalian family X enzymes and Li Pol β . Secondary structure elements conserved in all the members of the family are indicated above the alignment. Secondary structure elements not observed in Li Pol β due to disorder are indicated in gray. The four variable regions (Loops 0-3) are also indicated above the alignment. Identical residues are shown in white bold type over a black background. Conserved residues are bold. (B) Overlay of the C- α traces for Li Pol β (red), human Pol β (green, 1BPY), human Pol λ (blue, 1XSN), mouse Pol μ (purple, 2IHM), and mouse TdT (orange, 1KDH). Human Pol β , human Pol λ , mouse Pol μ , and mouse TdT have a RMSD (with respect to Li Pol β) of 0.727 Å, 0.873 Å, 1.267 Å, and 1.362 Å for 166, 161, 174, and 173 C- α atoms respectively. (C) Locations of the four variable regions in the catalytic domain of family X DNA polymerases. Loop0, loop1 and loop2 are located in the palm subdomain (yellow). A fourth variable loop is present in the thumb subdomain (purple). (C)

Figure 4. Loop1 and loop3 are template binding loops in Li Pol β

(A) Partial overlay of Loop 3 for Li Pol β (green), human Pol β (magenta, 1BPY), human Pol λ (yellow, 1XSN), mouse Pol μ (pink, 2IHM), and mouse TdT (purple, 1KDH). (B) Ala336/Glu338 of Loop3 (yellow), Asp335 of Loop3 and Leu270 of Loop1 (pink) interact with the phosphates of the -1, -2, and -3 template nucleotides respectively.

Table I
Data collection and refinement statistics

	Ternary Gap	Ternary P/T	Nick	Binary
PDB Code				
Data collection				
Space group	P 65 2 2	P 65 2 2	P 65 2 2	P 21
Cell dimensions (Å)	108.7, 108.7, 151.6	108.7, 108.7, 151.6	106.5, 106.5, 159.6	59.5, 166.3, 72.0
α, β, γ (°)	90,90,120	90,90,120	90, 90, 120	90, 113.9, 90
Wavelength	1.075	1.000	1.000	1.075
Resolution (Å)	2.30	1.90	2.30	2.45
Unique reflections	24191	41119	24544	45815
Redundancy	21.3	18.7	7.8	4.1
Rsym	0.136 (0.753)	0.065 (0.613)	0.069 (0.457)	0.105 (0.532)
I/ σ I	31.4 (4.5)	38.4 (4.1)	20.9 (3.0)	17.8 (2.1)
Completeness (%)	100 (100)	100 (100)	100 (99.9)	98.2 (85.8)
Refinement				
Rwork/Rfree	19.4/22.8	16.9/18.5	19.7/24.4	23.1/27.3
No. atoms	3043	2757	2867	4948
Water	237	332	250	146
Mean b-factors	32.9	33.2	36.1	58.2
Rmsds				
Bond lengths (Å)	0.008	0.009	0.006	0.007
Bond angles (°)	1.125	1.059	1.044	1.179

Table II
Steady state kinetic parameters

Protein	k_{cat} (s^{-1})	K_m (nM)	k_{cat}/K_m ($s^{-1} nM^{-1}$)
WT Li Pol β	0.033 ± 0.013	111.8 ± 31.6	$0.28 \pm 0.09 (\times 10^{-3})$
Li Pol β loop	0.123 ± 0.07	138.9 ± 93.1	$1.2 \pm 0.7 (\times 10^{-3})$

Li Pol β mutants**Table III**

	Enzyme	
	Wt Li Pol β	Li Pol β loop
Mutants sequenced	90	95
Single mutants	71	54
Multiples	19	41
Total mutations	110	145
Base substitutions	52 (40)*	88 (31)
- 1 bs frameshifts	28 (25)	14 (12)
- 2 bs frameshifts	2	2
+ 1 frameshifts	1	6
2< Deletions < 10	4	8
Deletions > 10	20	24
Complex	3	3

*
in parenthesis number of detectable mutations.

Table IV**Error rates**

Change	Error rate $\times 10^{-4}$	
	Wt Li Pol β	Li Pol β loop
Base sub	14 (2.8)	23 (7.1)
-1 bs frameshifts	7 (1.1)	3.6 (0.9)
Non-run	5.9	2.0
Run	9.4	5.2
+1 bs frameshifts	0.27	1.55

In parenthesis are error rates for detectable changes only.