DNA Polymerase Family X: Function, Structure, and Cellular Roles

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Abstract

The X Family of DNA polymerases in eukaryotic cells consists of Terminal Transferase, and DNA polymerases β, λ, and μ. These enzymes have similar structural portraits, yet different biochemical properties, especially in their interactions with DNA. None of these enzymes possesses a proofreading subdomain, and their intrinsic fidelity of DNA synthesis is much lower than that of a polymerase that functions in cellular DNA replication. In this review, we discuss the similarities and differences of three members of Family X: polymerases β, λ, and μ. We focus on biochemical mechanisms, structural variation, fidelity and lesion bypass mechanisms, and cellular roles. Remarkably, although these enzymes have similar three-dimensional structures, their biochemical properties and cellular functions differ in important ways that impact cellular function.

Pol X Family Members

Eukaryotic cells contain at least 15 DNA polymerases, which have been grouped into families based on sequence similarity. The X family of DNA polymerases contains DNA polymerases β, λ and μ and terminal transferase (Tdt). DNA polymerase β (Pol β) was the first X Family polymerase discovered [1] and has structural similarities with the CCA-adding enzymes [2]. X family polymerases are widely distributed among species, with all family members being found in vertebrates and one or two being found in plants in fungi (reviewed in [3]. They function in DNA repair rather than replication and have a variety of accessory functions, such as dRP lyase activity, to add in these functions [3]. In the following sections we will discuss the mechanism of X family polymerases, focusing on Pols β, λ, and μ and their roles in DNA repair.

The structures of the Pol X family of DNA polymerases are remarkably similar considering that primary amino acid sequence homology is somewhat divergent. Pols λ, μ, and Tdt each have amino-terminal BRCA1 c-terminal protein-protein interaction (BRCT) domains that are important for interaction with DNA and protein partners during Non-homologous end-joining (NHEJ) and VDJ recombination (for an excellent review see [4]). A symbolic alignment of these proteins is presented in Figure 1. The polymerase domains of these proteins range in primary sequence similarity from 23-44% with Tdt and Pol μ being most

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similar to each other at this level. Members of the Pol X family have a structurally similar 8 kDa domain, although it encodes an active dRP lyase activity for only Pols β and λ. The 31-kD polymerase fragment is comprised of the thumb, palm, and fingers subdomains. The polymerases contain two Helix-Hairpin-Helix motifs that function in the interaction of the polymerase with DNA. One of these motifs is in the 8 kDa subdomain and interacts with the DNA downstream of the gap. The other is located in the thumb subdomain, which is important for interaction with the primer strand of the DNA. The palm subdomain contains three conserved catalytic aspartic acid motifs as part of the active site of these enzymes. The fingers subdomain includes Helices M and N which contain amino acid residues that interact with nucleotide.

DNA Repair and the Pol X Family

Base Excision Repair

Base excision repair (BER) is the DNA repair pathway primarily responsible for repairing lesions caused by reactive oxygen species and alkylating agents. This pathway functions to repair at least 20,000 endogenous DNA lesions per cell per day [5]. It has three subpathways, short-patch (SP-BER), long-patch base excision repair (LP-BER), and an APE1 independent subpathway, as shown in Figure 2 [6]. In SP-BER, a damaged base is excised by a DNA glycosylase. The DNA backbone is then incised 5′ to the abasic site by an AP endonuclease (APE1), leaving a nicked intermediate, which is a substrate for Pol β's dRP lyase and polymerase activities. Pol β fills the single nucleotide gap, generating a nicked duplex that either DNA Ligase I or Ligase III/XRCC1 complex seals. When the 5′ flap is modified, making it refractory to the dRP lyase function of Pol β, the LP-BER subpathway repairs the damaged site. In LP-BER, several nucleotides are resynthesized and additional proteins, such as FEN1 and PCNA, are required [7]. Pol β is a key enzyme in BER. It acts as the main gap-filling enzyme in both SP-BER and in some cases LP-BER (reviewed in [8]) via its strand-displacement synthesis [7, 9-11], and the dRP lyase activity of Pol β is necessary for the APE1 independent pathway [6].

While SP-BER can be reconstituted *in vitro* with only four proteins and LP-BER with six, over 30 proteins can be involved *in vivo*. The interactions between and regulation of these proteins is an on-going area of research (reviewed in [12]). Pol β interacts with other proteins during BER including APE1, PCNA, FEN1, PARP1, XRCC1, WRN, HMGB1 and APC to regulate the overall activity, strand-displacement synthesis and dRP lyase activity of the polymerase [10, 11, 13-17]. One model of BER regulation has been proposed in which the proteins pass the damaged DNA from one protein to the next, using the unnatural bending of the DNA as a signal to recruit specific proteins [18]. In this way, protein interaction would alleviate the mutagenic and cytotoxic effects of free BER intermediates.

Non-Homologous End Joining

The non-homologous end-joining (NHEJ) pathway functions to repair double-strand breaks (DSBs) (for an excellent review see [19]). NHEJ is active throughout the cell cycle. The simplest form of NHEJ is the ligation of DNA ends. If genetic information has been lost from the ends via end-processing, mutations and/or genomic instability, including the formation of chromosomal translocations, can occur. NHEJ requires the Ku 70/80 heterodimer, DNA-dependent protein kinase (DNA-Pkcs), Xray-cross-complementing gene 4 (XRCC4), DNA ligase IV (Lig IV), Artemis, and XRCC4-like factor (XLF), which is also called Cernunnos. The DSB is detected by Ku, which when bound to DNA, translocates inward to leave a free DNA end so that other proteins can bind, as shown in Figure 2. Ku then recruits DNA-PKcs to the DNA and two DNA-PKcs molecules interact to bridge the DNA ends. DNA-PKcs is then phosphorylated and it functions to protect the ends from

degradation. In the presence of DNA-PKcs and Ku, Artemis acquires endonuclease activity which functions in end remodeling on a variety of substrates including 3′-phosphoglycolate groups. End-processing of DSBs can lead to the formation of gaps, which are filled in by terminal transferase (Tdt), Pol λ, or Pol μ. The size of the gap and presence of microhomology likely influence the choice of polymerase that is used to fill the gap. This will be discussed later in the review. Once the DNA ends are processed and the gap is filled in, XRCC4/LigIV catalyzes ligation of the nicks.

DNA Polymerase β

DNA polymerase β (Pol β) is a 39 kD polymerase with 335 amino acids encoded by the *POLB* locus, which maps to the p11 region of chromosome 8 [20](Entrez Gene, GeneID: 5423). It has one subunit, which through partial proteolytic cleavage can be separated into two domains. The N-terminal 8 kD domain contains the residues important for the dRP lyase activity of the polymerase, and the C-terminal 31kD domain contains the residues for the nucleotidyl transferase activity. Pol β has been used extensively as a model to study polymerase activity and fidelity due to its small size, the relatively easy ability to overexpress the protein and purify it from *E. coli*, an abundance of crystal structures available of the polymerase alone and bound to a variety of substrates, and its lack of intrinsic exonuclease activity [8].

Pol β DNA Interactions

Pol β synthesizes DNA in a template-directed manner and can bind to a variety of DNA structures. While it is capable of synthesizing DNA on recessed, nicked, or gapped DNA, it prefers DNA with small gaps with a 3′ hydroxyl group on the primer and a 5′ phosphate on the downstream oligo, which is bound by the 8kD domain of the protein [21-23]. Binding to the 5′ phosphate occurs via a lysine-rich 5′-phosphate binding pocket located in the 8kD domain and is crucial for polymerase activity, as it both increases DNA binding and polymerase processivity [24]. Pol β is processive on short gaps (2-6 nt), although its processivity is not as great as that of some other polymerases and varies based on the DNA template [22, 25-27]. Pol β is capable of conducting strand-displacement synthesis, in which it displaces the downstream oligo to synthesize beyond the length of the initial gap [25]. This strand-displacement synthesis is modulated in a cell through interaction with a variety of other proteins, including X-ray repair cross complementing protein 1 (XRCC1), flap endonuclease 1 (FEN-1), Poly (ADP-ribose) polymerase family, member 1 (PARP1), AP endonuclease 1 (APE1), adenomatous polyposis coli (APC), Werner helicase (WRN), telomeric repeat binding factor 2 (TRF2) and ligase III (LIGIII) [10, 28, 29] [30-34].

Pol β Activity

The first presteady-state kinetic analysis of Pol β showed using a recessed primer/DNA template that Pol β follows a kinetic scheme similar to other polymerases and that under pre-steady state conditions, product formation fits to a biphasic burst [35]. The burst rate of Pol β ranges from 10 to 14 s^{-1} [35, 36] followed by a slow steady-state rate that is governed by product release, which is likely the rate limiting step of the overall reaction. In short, the overall catalytic mechanism of Pol β follows an ordered addition of substrates (Figure 3). First, Pol β binds the DNA substrate, preferring DNA with short gaps with a 3′-OH and a 5′ phosphate. The enzyme then binds incoming dNTP, preferentially binding to the correct deoxynucleoside triphosphate that maintains hydrogen bonding with the templating base. Upon dNTP binding, the enzyme undergoes a conformational change including large subdomain motions as well as smaller side chain rearrangements [37]. This conformational change positions the substrates optimally for an in-line nucleophilic attack of the O3′ on the α P of the incoming nucleotide, following a standard two-metal-ion catalytic mechanism

using three catalytic aspartates (190, 192, and 256) [38]. After chemistry, in which nucelotidyl transfer occurs, there is possibly a second conformational change involving subdomain opening allowing for pyrophosphate release and product dissociation.

Using stop flow fluorescence with 2-AP located at various positions in the template strand of the DNA, two major fluorescence transitions were identified. It was thought that the fast rate corresonded to a conformational change that occurred before chemistry due to the finding that the rate was unchanged with the use of a dideoxy-terminated primer [39, 40]. The slow step following this conformational change was not present when a dideoxy terminated primer was employed, suggesting that it corresponded to or was dependent upon nucleotidyl transfer. In addition, the rate of the slow step was similar to what was measured in the rapid quench flow apparatus. Numerous additional publications have addressed this issue of Pol β's kinetic mechanism, and for an excellent review of Pol β's structure and catalytic mechanism, please see [8].

While it was initially thought that a conformational change other than the large subdomain movement was the rate-limiting step of the reaction, recent studies are pointing towards chemistry as being at least partially rate limiting in Pol β catalysis. Using stopped-flow fluorescence assays, it was suggested that the stopped flow slow rate constant, which is similar to what is measured using rapid kinetics methodology, measures some enzyme movement occurring after chemistry, and that chemistry is actually the rate-limiting step [41]. Work using dGTP analogues with modified leaving groups in single-turnover kinetics experiments supports the hypothesis that chemistry rather than a conformational change is rate-limiting [42, 43].

In addition to its nucleotidyl transferase activity, Pol β has also been shown to have both dRP and AP lyase activities, although the dRP lyase activity is more efficient [44, 45]. Residue Lys72, located in the 8kD domain, is critical for the dRP lyase activity [46]. This activity has been shown to play a key role in the cell, as the dRP lyase activity and not the polymerase function of the enzyme are essential in order to rescue the MMS sensitivity of Pol β$^{-/-}$ mouse embryonic fibroblasts (MEFs) [47]. Although critical for repair, it has been suggested that the dRP lyase activity may lag behind the polymerase activity of Pol β [13].

Pol β Fidelity

It has been estimated that cells generate at least 20,000 endogenous lesions per cell per day that are substrates for the BER pathway [5, 48]. Given this high level of endogenous lesions, it is important to understand the inherent fidelity of the main repair polymerase in the pathway, Pol β. In the first major study of Pol β fidelity, an M13 gap-filling assay was used to characterize the frequency and types of errors made by Pol β during DNA synthesis. While copying the *lacZ* target, Pol β made a variety of errors, including both frameshifts and base substitutions, with an overall mutation frequency of 5×10^{-4}. Of the frameshifts produced, most were single base pair deletions, and most of the base substitutions were transitions [49]. Since this initial study, many labs have characterized the mutation frequency and spectra generated during DNA synthesis by Pol β. One assay of particular note was the *in vitro* HSV-*tk* forward mutation assay, which is different from the M13 assay in that it allows for the selection of, rather than screening for, mutants. Work using this assay showed that Pol β had an error frequency of 24×10^{-4}, which is consistent with the previous M13 assay result, and made predominantly single base deletions in runs of like nucleotides [50]. More recently, it has been shown that overexpression of Pol β in cells results in an increase in the frequency of frameshift mutations [51]. In the same study, the authors demonstrated that addition of excess Pol ß to whole cell extracts resulted in strand slippage that results from stimulation of strand displacement synthesis by Pol ß.

In addition to the types of general mutations created by Pol β, DNA structure and sequence have been shown to have an effect on the enzyme's fidelity. First of all, it was demonstrated that Pol β exhibits the highest fidelity on a single nucleotide gap with a 5′-phosphate on the downstream oligo [27]. Additionally, variation in the DNA duplex upstream of an abasic site and around a DNA lesion can affect the efficiency and fidelity of Pol β [52]. Pol β amino acid composition can also affect sequence specificity of the polymerase, as two cancer-associated single amino acid variants, Ile260Met and Lys289Met, have been shown to have sequence-specific mutator activity due to misalignment of the DNA within the active site [53, 54].

Given the many factors such as the DNA template that can affect polymerase fidelity, and the fact that the free energy difference between a correct Watson-Crick base pair and an incorrect pair is not sufficient to account for the relatively low error rate obtained for DNA polymerases [55-57], it becomes critical to understand what parts of the underlying catalytic mechanism of a polymerase contribute to its fidelity. In the case of Pol β, fidelity can be measured using single turnover kinetic assays, allowing for the calculation of both the maximum rate of polymerase catalysis (k_{pol}) and the apparent equilibrium dNTP dissociation constant (K_d). Selectivity at the level of ground state dNTP binding is the ratio of the equilibrium binding affinity of the incorrect base pair to the correct base pair and varies according to sequence context [57]. Pol β selectivity during dNTP binding is 5-30 fold, which is low compared to T7 and RB69, which have ratios of 100 to 400 [58, 59]. Selectivity at the level of k_{pol} is the ratio of the polymerization rate of the correct versus the incorrect nucleotide, and ranges from 680 to 1,030 for Pol β misincorporation opposite a template A [60]. Again, the exact selectivity values vary depending on the sequence context and are different from the enzyme's efficiency of misincorporation, which is the value obtained by dividing k_{pol} by K_d. Overall, the fidelity of Pol β (the ratio of the efficiency of insertion of a correct versus an incorrect base) ranges from 1,600 to 51,000, depending on the base pair studied [61].

Nucleotide Selectivity during dNTP Binding—Selectivity during dNTP binding is dependent upon the proper formation of the dNTP binding pocket. In Pol β, the dNTP binding pocket is formed of the amino acid residues Tyr271, Phe272, Asp276, and Asn279, as shown in Figure 4B, as well the templating base of the DNA and the incoming dNTP. Residue Tyr271 is important for both chemistry and dNTP binding, as alteration of the tyrosine to phenylalanine or histidine results in polymerases with altered steady-state catalytic efficiencies. Mutation to alanine results in a polymerase with tighter binding of both the correct and incorrect incoming dNTP as well as decreased k_{pol} for most base pairs tested [62, 63]. The phenolic ring of Tyr271 is involved in hydrogen bonding with the base of the primer in the DNA minor groove [64]. This interaction with the primer could be important for fidelity as well as activity, as the Tyr271Phe and —Ser mutants have slightly decreased accuracy due to loss of discrimination during both ground state binding and chemistry, depending on the sequence context studied [62].

Three of the residues, Phe272, Asp276 and Asn279, contact the incoming nucleotide [64]. Mutation of these residues can affect Pol β fidelity, as shown using both single-turnover and steady-state assays [62, 65, 66]. In a genetic screen to identify mutator mutants of Helix N (residues 274-278), many active variants of Asp276 were identified, suggesting that this residue is not critical for activity [66]. Additionally, mutation of this residue to valine resulted in a variant with an increased catalytic efficiency due to tighter ground state dNTP binding [67]. The relationship between Asn279 and the incoming nucleotide is important for Pol β activity, as molecular dynamics simulations suggest that the strength of this hydrogen bond correlates with the catalytic efficiency of Pol β [68]. Together, the three residues that

contact the incoming dNTP, Phe272, Asp276, and Asn279, are important for both Pol β's activity and fidelity due to their influence on nucleotide binding.

Although not technically in the dNTP binding pocket, Arg283 and the residues thought to bind the dNTP phosphate moiety are also important for nucleotide binding, as shown in Figure 4C. Arg283 hydrogen bonds the template base opposite the primer terminus and stabilizes the position of the template DNA, which is part of the dNTP binding pocket [35, 64]. Mutations of Arg283 to lysine and leucine result in polymerases with significantly decreased catalytic efficiency, primarily due to decreases in k_{pol} [69]. However, the Arg283Ala mutant exhibits reduced fidelity due to loss of discrimination during ground state binding in addition to decreased selectivity at the level of k_{pol} [61]. Four additional residues that may be involved in binding the triphosphate moiety, Arg149, Ser180, Arg183 and Ser188, as shown in Figure 4C, were studied by mutating them to alanine, one at a time. Two of the residues, Ser180 and Arg183, seem to be involved in the step of the reaction reflected by k_{pol}, as mutation to alanine resulted in a decrease in k_{pol}. However, it is recognized that decreased binding of the dNTP could also lead to a change in the overall reaction rate. This suggests that these residues are important for stabilizing the dNTP during the transition state. Two of the mutants, Arg183Ala and Arg149Ala, had decreased fidelity for the dGTP:T mispair due to loss of discrimination during ground state binding. This suggests that these residues may be important for conferring specificity during nucleotide binding, at least in some cases [70].

Another region important for Pol β fidelity is the hydrophobic hinge region located between the palm and fingers subdomains. This region is important for the large subdomain movement that occurs upon binding nucleotide, when the enzyme moves from the open (no dNTP bound) to closed (dNTP bound) state [64] [71]. This enzyme closure is important for the proper geometric alignment of the catalytic aspartates, incoming nucleotide, magnesium ions, DNA and other residues of Pol β that are critical for enzyme catalysis. The hinge contains both an outside lining, comprised of residues Ile174, Thr196 and Tyr265, and an inner lining, comprised of residues Leu194, Ile260 and Phe272, as shown in Figure 4D. Together, these residues contribute to Pol β fidelity by helping form the dNTP binding pocket [40, 60, 65, 72-74].

The role of the hinge in Pol β fidelity was primarily determined by analyzing the effect of single amino acid substitution variants on enzyme activity and fidelity. Genetic screens were used to identify active mutator mutants of hinge residues, often followed by single turnover kinetic studies to determine which step of Pol β's catalytic mechanism was affected by the mutation [75]. One of the earliest Pol β hinge mutator mutants identified was Tyr265Cys, which makes both misalignment-mediated and misincorporation-mediated errors [72] and can cause a mutator phenotype when expressed in cells [73], demonstrating that residue Tyr265 is critical for Pol β fidelity. Studies of an additional Tyr265 mutant, Tyr265His, using transient kinetic analysis at both 37°C and 20°C found that this residue is critical for the proper geometric alignment of the polymerase active site [40]. Structure/function studies of residue Ile260 identified the Ile260Asp, -Glu, -Lys, -Asn, and —Arg mutants as having decreased activity compared to WT, suggesting that the hydrophobic nature of this hinge residue is necessary for polymerase catalysis. This screen also identified Ile260His and —Gln variants as active mutator polymerases, and in the case of Ile260Gln this mutator phenotype is due to loss of discrimination during ground state binding [60, 74]. Finally, the leucine mutant of Phe272, a residue which is also a part of the dNTP binding pocket, has impaired fidelity due to loss of discrimination during dNTP binding [65]. Taken together, these findings strongly support the role of the hinge in maintaining the proper geometry of the dNTP binding pocket.

Nucleotide Selectivity during the Steps Reflected in k$_{pol}$—Polymerases can also discriminate the correct from incorrect nucleotide during the chemistry step of the catalytic mechanism. In this case, the enzyme will bind the incorrect nucleotide, but the molecules will not be properly positioned to allow for the nucleotidyl transfer reaction to occur. For Pol β, this is reflected in altered rate constants obtained from presteady-state and single-turnover kinetic assays (as described in "Pol β Activity"). However, it is not certain exactly which step of the catalytic mechanism corresponds to these rate constants, and it is likely that they reflect a combination of partially rate-limiting steps including both nucleotidyl transfer and small side-chain readjustments necessary for proper active site geometry.

A recent study designed to understand how the leaving group effects selectivity characterized analogues of the β-γ leaving group bridging oxygen. The results of this study suggest that the incorporation of incorrect or correct nucleotide is governed by a chemical step, rather than by a conformational change [42]. This shows that Pol β is capable of discriminating correct from incorrect nucleotides during the catalytic steps reflected by k$_{pol}$, but it does not exclude the possibility that dNTP binding plays a role in Pol β fidelity. Other work using stopped-flow fluorescence experiments found that for WT Pol β, incorporation of an incorrect dNTP follows the same general pathway as incorporation of the correct nucleotide. Binding of an incorrect nucleotide by a mutator variant of Pol β, Ile260Gln, results in a decrease in K$_d$, which was interpreted as reflecting an increase in the stability of the mismatched ternary complex [60, 76-78]. These results suggest that there are no gross changes in Pol β's catalytic scheme or subdomain movement upon binding a mismatch and that alterations in the active site geometry limit the maximum rate of incorporation in the presence of an incorrect nucleotide.

The Role of Subtle Conformational Changes in Pol β Fidelity

Much research has been conducted to determine if Pol β conformational changes are critical for the discrimination of correct from incorrect dNTP by this enzyme. Although it was first thought that the large subdomain motions could be contributing to fidelity through slowing the binding of incorrect dNTP, it is now thought that these movements are probably too fast to contribute to nucleotide discrimination [67, 79-82]. However, in addition to the large subdomain motions, there are more subtle conformational changes involving rearrangements of the polymerase side-chains upon dNTP binding [37, 64]. Current data suggests that these subtle side-chain rearrangments could be key determinants in Pol β fidelity.

Dr. Samuel Wilson's group suggested that side-chain rearrangements are necessary for the formation of a transition state and could contribute to polymerase fidelity through an induced-fit mechanism [8]. Experiments using dGTP with modified β,γ leaving groups and theoretical investigations based on the crystal structure of a G:A mismatch found that discrimination during substrate binding rather than during chemistry and the free energy required for the formation of the pre-chemistry state, which involves the subtle side-chain rearrangements, are key determinants of polymerase fidelity [42, 83]. Work conducted by our lab studying the effects of single amino acid substitution variants on Pol β fidelity supports the hypothesis that subtle side chain rearrangements can affect fidelity. In many of these cases, substitution of residues distant from the active site cause aberrant Pol β fidelity. Some of these variants work by altering the positioning of the DNA template within the active site, such as the Lys289Met, Asp246Val and Thr79Ser variants [53, 84, 85]. Others have decreased ability to discriminate the correct from incorrect incoming dNTP during ground state binding, as shown through the use of single turnover kinetic analysis. These variants include the previously mentioned hinge residues as well as the Met282Leu mutator mutant [40, 54, 60, 65, 72, 73, 86]. The importance of side-chain geometry on polymerase fidelity can also be observed for other polymerases [87].

One of the reasons Pol β is an ideal model polymerase is because it has no intrinsic exonuclease activity [88, 89]. However, despite this lack of intrinsic proofreading, it is thought that there may be extrinsic factors in the cell that can act as proofreading enzymes for Pol β DNA synthesis. Since Pol β is slow to extend mispaired primer termini, it is thought that this slowness of mispair extension may allow time for the polymerase to dissociate from the substrate DNA, allowing access for an extrinsic exonuclease to access and remove the mispair [8]. Also, in the case that Pol β misinserts a nucleotide, it has been shown that DNA Ligase I and Ligase III are slow to repair the nick in the DNA backbone, possibly allowing time for an extrinsic exonuclease to remove the mismatch [90]. It has been proposed that this extrinsic exonuclease may be APE1, which has exonuclease activity at a mismatch located at the 3′ ends of nicked or gapped DNA and has been shown to be capable of acting as a proofreading enzyme in an *in vitro* reconstituted BER assay [91].

Extension of Mispaired Primer Termini

In the case that a misincorporated nucleotide is not removed by an extrinsic exonuclease, polymerases can also demonstrate reduced fidelity through an increased ability to extend mispaired primer termini. In the case of WT Pol β, molecular dynamics simulations used to investigate the effect of a mismatch on DNA extension revealed that altered geometry of the active site in the presence of a mispair, as well as altered dynamics of Arg258 in its rotation towards Asp192, probably contribute to the low ability of WT Pol β to extend a mispair [79]. Several single amino acid substitution variants of Pol β have been identified with this ability. In one case, the Glu249Lys mutant demonstrated a decreased ability to discriminate nucleotides during ground state binding without affecting incorporation fidelity. In this case the altered nucleotide binding led to an increased ability to extend mispairs compared to WT [92]. The Tyr265Phe, —Trp and Ile260Gln hinge mutants also extend certain mispairs more proficiently than WT due to their ability to bind the incoming nucleotide more tightly, supporting the role of the hydrophobic hinge in shaping the dNTP binding pocket and contributing to fidelity both during nucleotide incorporation and mispair extension [77, 93]. Finally, a mutant selected as being AZT-resistant in a genetic screen, His285Asp, extends mispaired primer termini more efficiently than WT due to decreased discrimination during nucleotide binding. Molecular dynamics and modeling simulations suggest that the His285Asp substitution is working through several other amino acids to alter the flexibility of the c-terminal tail resulting in a decrease in fidelity in the presence of a mispair [36].

Lesion Bypass by Pol β

Pol β is capable of bypassing several types of DNA lesions, including 8-oxo-7,8-dihydrodeoxyguanine (8oxoG), Cisplatin-induced lesions and UV-induced lesions, as summarized in Figure 7. Regarding the oxidative lesion 8oxoG, it was first shown using steady-state kinetic analysis that Pol β can both misincorporate 8oxodGTP *in vitro* and can insert dCTP and dATP opposite an 8oxoG lesion in the template DNA. Incorporation opposite an 8oxoG lesion occurred with a 2:1 preference for dCTP under these conditions, while 8oxodGTP was preferentially misincorporated opposite template A [94]. Interestingly, mutation of Asn279 to Ala resulted in the preferential incorporation of C opposite 8oxoG [94]. In later experiments using single-turnover kinetic analysis, insertion of 8oxodGTP by Pol β occurred with relatively low fidelity, as "correct" insertion opposite a templating base C was less efficient than insertion opposite a template A. However, translesion synthesis by Pol β occurred with relatively high fidelity, as Pol β had the highest efficiency inserting the correct nucleotide dCTP opposite the lesion [95]. Use of dynamics simulations suggests that the motion of the fingers subdomain and destabilization of the closed form of the polymerase in the presence of the mismatched lesion are key determinants for the fidelity of translesion synthesis opposite 8oxoG [96, 97].

Pol β has been shown to bypass a cisplatin adduct *in vitro* [98], and this translesion synthesis is greater when the lesions are located in gapped DNA [99]. This bypass results in the creation of frameshift mutations [100]. Specifically, Pol β makes one base deletions in runs of nucleotides that surround cisplatin and oxaliplatin adducts *in vitro* [101]. In addition to bypassing the above-mentioned platinum adducts, Pol β is also capable of conducting bypass synthesis across both cyclobutanol pyrimidine dimers (CPD) and (6-4) TT lesions, especially if these lesions occur in gapped DNA. Cells overexpressing Pol β 2-3-fold have a modest increase in UV resistance and mutagenesis following UV exposure compared to the isogenic cell line not exogenously expressing Pol β [102]. Finally, Pol β can conduct strand displacement synthesis past a tandem lesion composed of a thymine glycol followed by a 5′-adjacent 2-deoxyribonolactone or tetrahydrofuran abasic site, a type of clustered lesion that could be produced by ionizing radiation or other oxidizing agents, *in vitro* [103].

Crystal Structures of Pol β

The protein structure of Pol β has been well characterized, with crystal structures of the enzyme alone and in complex with various substrates. The first ternary complex crystal structure of a DNA polymerase was that of Pol β and an overview of this is presented in Figure 4A [71, 104]. Alignment of the Pol β structures with structures of other polymerases showed that Pol β followed a standard two-metal ion catalytic mechanism, as previously mentioned. Subsequent structures of Pol β in complex with gapped and nicked DNA, both in the binary complex without nucleotide and in the ternary complex bound to ddCTP, revealed the configuration of Pol β in complex with its prefered *in vivo* substrate [64]. In these structures the DNA is bent 90°. In the binary complex, the enzyme is in an "open" conformation. Upon binding the correct dNTP, the fingers subdomain rotates approximately 30° around α-helix M, assuming a "closed" position that poises the active site for catalysis. In the ternary complex, the 8kD domain is found close to the fingers subdomain, causing the enzyme to form a donut shape [64]. The fingers subdomain, which moves approximately 12 angstroms between the open (binary) and closed (ternary) complexes, is involved in dNTP binding [64].

Extensive characterization of the Pol β crystal structures has been conducted, identifying residues key to various polymerase functions [8, 64]. Residues Arg283 and Tyr271 are important for the protein-template interactions, with Arg283 hydrogen bonding in the DNA minor groove with the template base opposite the primer terminus in the closed conformation and Tyr271 hydrogen bonding with the templating base in the open conformation. Tyr271 also hydrogen bonds with the primer terminus in the minor groove, while a helix-hairpin-helix (HhH) motif in the thumb subdomain (residues 92-118) binds the primer backbone. Another HhH motif in the 8 kD domain (residues 55-79) interacts with the downstream strand of a gapped or nicked DNA substrate. The previously mentioned dNTP binding pocket, as shown in Figure 4B, which contains residues Tyr271, Phe272, Asp276 and Asn279, is responsible for the protein-dNTP interaction, with Asn279 hydrogen bonding in the DNA minor groove with the incoming nucleotide.

In addition to these key structural residues, Pol β has three loops. Loop II, which contains residues 240-253, has been shown to be important for polymerase fidelity. This was first shown by the selection of AZT-resistant Pol ß variants, which turned out to be mutator polymerases. Selection for Pol ß variants that are AZT resistant likely identified mutants with altered substrate specificity. Several AZT-resistant mutator mutants have been identified in this region. Alteration of the length of the loop has also been shown to affect polymerase activity and fidelity [84, 105-108]. Loops can also be important domains for protein-protein interaction, and residues in Loop III of Pol β (residues 301-316) mediate the interaction with XRCC1 [109].

Crystal structures have been solved of Pol β in complex with a variety of lesion-containing substrates. One of the most notable was a structure of Pol β in complex with DNA containing an 8oxoG lesion, the first crystal structure of a polymerase with a premutagenic lesion in the active site, as shown in Figure 5. This structure showed that when the 8oxoG was paired with dCTP, residue Lys280 helped keep the 8oxoG in the *anti* conformation, while modeling of 8oxoG paired with dATP showed that Lys280 and Tyr271 both stabilized the *syn* conformation of the lesion [110]. A second notable structure consists of Pol β complexed with a tumorigenic bulky DNA adduct, benzo[c]phenanthrene diol epoxide, which would be a substrate for bypass synthesis. When present on a templating guanine, the bulky adduct causes a distortion of the dNTP binding pocket and impairs the polymerase from being able to adopt a fully closed conformation [111].

More recent structures of Pol β complexed with a terminal mispair suggest that this partially open structure is typical when Pol β binds to an incorrect substrate, as shown in Figure 6. In a binary nicked DNA structure with a terminal mispair, Pol β was observed to adopt a partially open conformation and the Watson-Crick edges of the mispair adopted a staggered conformation and were unable to form hydrogen bonds [112]. The formation of a partially open ternary complex was also observed using small-angle x-ray scattering and molecular modeling to study the structure of Pol β with a mismatch in the active site [78].

In 2008, a crystal structure of Pol β in complex with a mispair was published [113]. The authors used a non-hydrolyzable dAMPCPP and Mn^{2+} in the structure in order to capture the reaction intermediate and to decrease polymerase fidelity, thereby increasing mismatch binding. The crystal structure showed that the incorrect nucleotide binds in the same conformation as the correct base. However, the presence of the mispair caused the template to shift, leaving an abasic site at the position of the templating base and causing rotation of the primer terminus, resulting in an altered active site geometry that deters misincorporation. The mispaired bases were staggered as in the crystal with the nicked substrate, and Pol β was in the closed conformation. Based on the above mispaired crystal structure, a theoretical investigation was conducted. It identified two stages in the catalytic mechanism of misincorporation: a "ground state," which is the most stable but catalytically unfavorable conformation observed in the crystal, and a "prechemistry state" that involves a subtle reorganization of the active site, allowing for catalysis to follow the same reaction mechanism as used for correct nucleotide insertion [83].

Regulation of Pol β Gene Expression

Pol β is constitutively expressed in most tissues, although increases in mRNA expression levels have been observed before and during DNA replication in certain cases [114, 115]. In general, the most variation in Pol β expression occurs in a tissue-dependent manner with the highest expression observed in testis, brain, thymus and spleen [116]. Expression of Pol β can be induced through use of a DNA damaging agent such as MNNG [117]. Pol β expression is controlled with a series of binding elements in its promoter region, including a Sp1 binding element and a cAMP response element (CRE) [118, 119]. Binding by phospho-CREB1 has been shown to activate Pol β transcription and can account for some of the increased Pol β expression following exposure to the alkylating agent MNNG [120, 121]. Telomerase transcriptional element-interacting factor (TEIF) can also bind to and induce expression from the Pol β promoter [122].

Posttranslational Modification of Pol β

Pol β undergoes several types of posttranslational modifications. It is methylated by protein arginine methyl transferases PRMT1 and PRMT6. PRMT1 methylates Pol β at residue Arg137, inhibiting the enzyme's interaction with PCNA [123]. PRMT6 is capable of

methylating residues Arg83 and Arg152, resulting in an increase in the DNA binding ability of the protein [124]. Pol β can be acetylated by p300, leading to a reduction in the dRP lyase activity [125]. The CHIP E3 ubiquitin ligase can also ubiquitilate Pol β, leading to its degradation [126]. Together, these post-translational modifications work to modulate specific Pol β enzymatic activities and stability in a cell.

How the cell chooses which polymerase to use in a given scenario is still unclear. Both Pol β and Pol λ are capable of contributing to BER in cell extracts, although the role of Pol λ is much less than Pol β [127]. Pol β is capable of interacting with the 9-1-1 complex in cells, and this interaction may work to preferentially direct Pol β to sites of DNA damage [128, 129]. Both Pol β and Pol λ are capable of bypassing both abasic sites and the 8oxoG lesion [95, 130]. In both cases, Pol λ was shown to be more efficient at bypass synthesis. This could be due to interactions with RPA and PCNA, which in steady-state *in vitro* assays were shown to decrease Pol β's efficiency in conducting correct lesion bypass of 8oxoG while having no effect on Pol λ [131].

Germline SNPs of Pol β

Four non-synonymous amino acid substitution variants of Pol β have been identified in healthy individuals (dbSNP Build 129, GeneID:5423). Two of these variants, Arg137Gln and Pro242Arg, are found at low frequency in specific populations (~6% in East Asians and ~2% in Europeans, respectively) [132]. To date, the function of only one germline polymorphism has been characterized. The Arg137Gln variant was shown to have decreased steady-state activity and aberrant interaction with PCNA, impairing its function in BER [133]. In addition to these germline variants, several tumor-specific amino-acid substitution variants have been identified. These variants have been shown to exhibit altered polymerase activity, fidelity, and dRP lyase activity, and the expression of certain variants has been shown to induce transformation in cell culture (reviewed in [134]). Given the work on tumor-associated mutants of Pol β, it is possible that aberrant activity of germline variants could contribute to cancer predisposition.

DNA Polymerase λ

DNA Polymerase λ is a 68 kD Polymerase that is encoded by the *POLL* gene and maps to chromosome 10q23 [135, 136]. Pol λ is a single-subunit protein with a BRCT domain and dRp lyase activity [137]. The dRP lyase activity is likely to proceed via beta elimination using Lys310 as the major catalytic residue. Pol λ catalyzes DNA synthesis usually in a template-dependent manner and has no associated proofreading activity [138].

Pol λ DNA Interactions

Like another Pol X family member, terminal transferase, Pol λ has been reported to exhibit template-independent terminal transferase activity [135, 139]. Like Pol β, Pol λ is processive on small gaps with a 5′ phosphate. It is distributive on large gaps, recessed DNA, and gaps that do not contain a 5′ phosphate. However, unlike Pol β, Pol λ is able to catalyze DNA synthesis with a DNA substrate that has a discontinuous template with a paired primer-terminus [140]. It is tolerant of gaps and base damage near the primer terminus. This is thought to be due to the presence of a flexible loop (loop 1), which is likely to compensatefor the lack of a continuous template strand.

Pol λ Activity

The overall kinetic scheme of Pol λ is similar to that of Pol β, shown in Figure 3. The dissociation rate of Pol λ from the DNA is 0.8 sec^{-1} [141], roughly 3-fold less than the observed rate of polymerization, which is 2.1 sec^{-1}. Under single-turnover conditions, a

truncated version of Pol λ, in which the BRCT and proline rich domains are deleted, catalyzes DNA synthesis with an observed rate of 3-6 sec^{-1} with correct nucleotide substrate and single-nucleotide gapped DNA [141]. Single-turnover analysis with the full-length protein yields a similar rate [138]. This rate is slightly lower than that of Pol β, but significantly less than that of polymerases that function in DNA replication. The use of a DNA substrate with a 5′ tetrahyrofuran group to mimic a 5′dRP results in a significant decrease in polymerase catalytic efficiency resulting from a decrease in the observed rate of polymerization [142]. This indicates that the dRP lyase activity of Pol λ might remove the 5′dRP group before filling the gap during the repair process. Alternatively, there might be some advantage to Pol λ having a low catalytic efficiency *in vivo*.

Pol λ Fidelity

Strikingly, Pol λ has a single-base deletion rate that exceeds the misincorporation rate and is 30-fold higher than that of Pol β in an M13-lacZα-based forward mutation assay [143]. Pol λ even induces single-base deletions in non-iterated DNA, suggesting that only one base pair is required to stabilize the misaligned DNA intermediate. This was the first indication that Pol λ interacted with the DNA in a manner different than that of Pol β, and this will be discussed further (see below). The fidelity of the truncated version of Pol λ is in the range of 10^{-3}-10^{-4}, with the exception of dTMP:G, which is 10^{-2} [141]. These estimates using single-turnover kinetics [141] are in complete agreement with the results of the M13 forward mutation assay [143]. The fidelity of incorporation of the truncated version of Pol λ is one to two orders of magnitude lower than that of Pol β. However, inclusion of the proline-rich domain of Pol λ increases fidelity to levels similar to that of Pol β by an unknown mechanism [144]. Discrimination of the correct from the incorrect dNTP occurs mostly at the level of k_{pol}, which encompasses the rate of conformational changes and nucleotidyl transfer [141]. Interestingly, correct and incorrect dNTPs have similar binding affinities that are quite high, suggesting that this polymerase is able to catalyze DNA synthesis when the levels of nucleotide pools are low. Mispairs are readily extended by Pol λ, likely because this enzyme only requires the terminal base pair for stabilization during nucleotidyl transfer [145].

Tyr505 and Phe506 of Pol λ have structural homology to Tyr271 and Phe272 of Pol β, which are important for nucleotide discrimination [62, 65, 146]. Alteration of Tyr505 to Ala did not change the properties of the polymerase [147]. However, alteration of Phe506 to Arg resulted in a 50-fold loss in catalytic efficiency. These results indicate that Tyr505 and Phe506 are not likely to be important for nucleotide discrimination by Pol λ. Alteration of Tyr-505 and Phe-506 results in enzymes with significantly less terminal transferase activity, suggesting that these residues may be critical for DNA synthesis when there are discontinuities in the DNA [147].

Pol λ Structure

Comparison of a binary to a ternary complex of Pol λ by crystallography provides evidence for the absence of a large subdomain movement that precedes chemistry [148]. Upon dNTP binding, Tyr505 moves in order to permit entry of the dNTP into the active site, as beta strands 3 and 4 of Pol λ move. This movement results in the repositioning of the DNA templating base into the active site. Arg517 assesses proper nascent base pair geometry in response to template movement by monitoring the minor groove of the DNA. This amino acid residue likely has a role similar to that of Arg283 of Pol β. Strong proof for the lack of large subdomain movement in Pol λ came from work in which metal ions were soaked into crystals of Pol λ containing DNA and dNTP. Catalysis occurred within the crystal, but no large subdomain movements were observed [149]. This structure of Pol λ also provided evidence for an inline mechanism of DNA synthesis.

Remarkably, utilization of a DNA template with a pair of iterated Ts yielded a crystal structure with one of the Ts in an extrahelical position, providing direct evidence for the mechanism of Streisinger slippage [150]. This structure consisted of a postcatalytic intermediate showing incorporation of the base opposite the non-slipped T. The extrahelical T is stabilized by Lys544 in a loop that is not conserved in other polymerases, which may explain the propensity for Pol λ to induce single base deletions at rates significantly increased over those of other DNA polymerases. Strikingly, correct geometry of the base pair is observed within the active site even in the presence of the extrahelical T and the distortion is accommodated by the upstream DNA template strand. When Arg517 is altered to Lys, they observe conformational flexibility of the DNA within the crystal [151]. In some cases the residues of the polymerase are in a precatalytic conformation but the template has assumed its position for catalysis. This suggests that Arg517 is important for coupling DNA template movement to the binding of dNTP.

Cellular Roles of Pol λ

The results of several studies suggest that Pol λ functions in nonhomologous end-joining (NHEJ). First, Pol λ has a BRCT domain, which is important for interaction with proteins that function in NHEJ [152]. Second, using immunodepletion of cellular extracts, Povirk and colleagues found that the filling of gaps with partially complementary ends was dependent upon Pol λ and not Pol μ [153]. Gap-filling in this *in vitro* system was dependent on the presence of XRCC4 and Ligase IV, proteins that function exclusively in NHEJ. Importantly, gap-filling was accurate, suggesting that Pol λ functions in an error-free mode of NHEJ. Third, in an *in vitro* system in which the joining of incompatible ends is dependent on Ku, Artemis, DNA-Pkcs, XRCC4, and DNA ligase IV, addition of Pol λ preserves the 5′ overhang and increases the size of the junctions [152]. Fourth, Pol λ deficient mouse embryo fibroblasts are sensitive to hydrogen peroxide, camptothecin, and etoposide, but they are not sensitive to ionizing radiation (IR) [154, 155]. All of these agents produce breaks in DNA, so the sensitivity of Pol λ-deficient cells suggests that it functions during the repair of double- and single-strand breaks. The lack of sensitivity to IR and the finding that Pol λ is induced in response to this damaging agent appears to be a paradox. However, Vermeulen and colleagues suggest that Pol λ is unlikely to participate in the base excision repair of lesions induced by oxidative damage, which occurs when cells are treated with IR. Instead, it may function as an end remodeling protein and polymerase during break repair [153, 154]. Importantly, Pol λ-deficient cells are not sensitive to alkylating agents [155], even though it catalyzes the removal of dRP groups from the 5′ end of the DNA break [137].

Pol λ can function in a backup BER pathway, because cells which are Pol β-deficient and immunodepleted for Pol λ cannot perform BER [127]. Pol λ also functions in the joining of IgG heavy chain junctions in B-cells [156] based upon the finding that mice deleted of Pol λ have shorter junctions. This group also suggested that Pol λ is recruited at a step that precedes terminal transferase.

Lesion Bypass by Pol λ

A summary of lesion bypass by Pol λ is presented in Figure 7. Pol λ is able to bypass 2-hydroxyadenine in an error-free manner. However, this depended upon Tyr505 because when altered to Ala, the enzyme became less accurate upon bypass of 2-hydroxyadenine [157]. This might indicate a role for Pol λ in bypass of this common lesion produced by endogenous reactive oxygen species. 8-oxoG is also a common lesion induced by reactive oxygen species, and, unlike Pol β, Pol λ incorporates C and A opposite this lesion with nearly equal catalytic efficiencies [95, 158]. Interestingly, incorporation of 8-oxodGTP opposite A was more efficient than incorporation opposite C [95]. However, Pol λ readily extends a C:8-oxodGTP pair but does not appear to extend an A:8-oxodGTP base pair [158].

This suggests that in the absence of lesion removal by a DNA glycosylase there could be a mechanism to prevent the accumulation of mutations resulting from incorrect incorporation opposite 8-oxoG or from incorporation of 8-oxodGTP opposite A. In addition, RPA reduces the rate of incorporation of dCTP opposite 8-oxoG and PCNA increases the efficiency of free bypass of Pol λ opposite this lesion [131], which is much higher than that of Pol β. Thus, when the MutY DNA glycosylase removes A from an A:8-oxoG mispair, Pol λ might have a specialized role in inserting C opposite the 8-oxoG in the template, especially during replication, given the increase in efficiency in the presence of PCNA.

Pol λ Protein Partners

PCNA interacts directly with Pol λ, stabilizing its interaction with the DNA primer [159, 160]. Interaction of Pol λ with PCNA stimulates bypass of an abasic site and continued extension. Pol λ exists mainly in a hypophosphorylated state during S phase but is exclusively hyperphosphorylated during G2/M [161]. CDK2/CyclinA interacts directly with and is able to phosphorylate Pol λ, and this results in decreased association with PCNA. This suggests that Pol λ is likely to interact with PCNA during S-phase, where it could participate in lesion bypass, perhaps at the replication fork. However, its lack of association with PCNA during G2/M when it is hyperphosphorylated indicates that it is likely to function in the repair, perhaps NHEJ, of non-replicating DNA at this time.

Pol λ Germline Variants

There are two germline single nucleotide polymorphisms (SNPs) in the gene encoding Pol λ in 2-14% of the population studied in the Environmental Genome Project that result in nonsynonymous amino acid substitutions. Arg438Trp maps to the catalytic palm domain, which could result in decreased polymerase activity or fidelity. Thr221Pro maps to the BRCT domain and could result in aberrant interactions with its protein partners or chromatin, leading to an altered ability to function in NHEJ [162].

DNA Polymerase μ

DNA polymerase μ is a 55 kDa enzyme that is encoded by the *Pol M* gene, which maps to chromosome 7p13 [136, 163]. Pol μ is a single-subunit polymerase with a BRCT domain.

Pol μ DNA Interactions

Pol μ catalyzes DNA synthesis in a template-dependent and independent manner with no proofreading activity [140, 163]. Pol μ exhibits terminal transferase activity that is dependent upon loop I, which is highly conserved in terminal transferase and Pol μ, but not the other members of family X [164]. Pol μ is also able to catalyze DNA synthesis where there is no complementarity at the ends of the DNA [140]. Remarkably, this type of synthesis is template-dependent and depends upon an end-bridging activity that is specific to Pol μ [165].

Pol μ Activity

Pol μ is a distributive polymerase [136, 163, 166] with a much higher DNA dissociation rate constant than its incorporation rate constant [167]. Under single-turnover conditions, the polymerization rate for correct dNTP substrate is 0.006-0.076 sec^{-1}, which is considerably slower than what is observed for Pol β and Pol λ and similar to their rates of misincorporation. Pol μ does not have dRP lyase activity.

Pol μ Fidelity

The fidelity of incorporation by Pol μ has been studied under steady-state and presteady-state conditions. In both cases, Pol μ prefers to incorporate the correct dNTP. However, in steady-state Pol μ appears to be less accurate, with fidelity values (f_{inc}) ranging from 10^{-2} to 10^{-4} for incorrect substrate [163, 166]. The insertion of dGMP opposite template A is the least accurate under steady-state conditions. The fidelity values under presteady-state conditions are approximately an order of magnitude lower [167]. In both cases, recessed DNA substrates were used. Correctly matched rNTPs were also incorporated by Pol μ, suggesting that this polymerase has RNA polymerase activity [167, 168]. Of note, Pol μ induces one-base deletion frameshifts at a very high frequency. This is the predominant mechanism of DNA synthesis when dinucleotide repeats are present and the 3′ end of the primer is complimentary to the next templating base [166]. Pol μ also uses template misalignment to extend mispaired primer-termini. Using a fluorescence-based approach, Goodman and colleagues provided evidence that Pol μ employs a Streisinger slippage mechanism in reiterative sequences and uses dNTP-stabilized misalignment in non-reiterative sequences to induce frameshift mutations [169].

Pol μ Structure

Recently, a ternary complex of Pol μ with DNA and dNTP was obtained [170]. There is a high density of interactions of the protein with the DNA along the upstream template region and another region surrounding the templating base. The DNA is bent 90 degrees in the active site of Pol μ, as with other members of the X family. The crystal structure of a ternary complex of Pol μ reveals several differences when compared to the structures of Polβ and μ [170].

Of note is that Pol μ contains several flexible loops. One of these is in the C-terminal domain and consists of residues 465-471. This loop in Pol μ is much shorter than the homologous loop in Pol λ, which is responsible for stabilization of the extrahelical nucleotide that is present in frameshift intermediates in that enzyme. This loop in Pol μ is also much farther from the DNA binding cleft and resembles the conformation present in terminal transferase, so it may have biological relevance. Loop 1, which lies between β strands 3 and 4, is long in Pol μ when compared to Pols β and λ. It is well ordered and assumes multiple conformations in the crystal. In terminal transferase, this loop is positioned within the substrate binding cleft and is suggested to facilitate the template independent synthesis that is catalyzed by this enzyme. In Pol μ, deletion of this loop reduces template-independent activity [164]. His329 likely facilitates template-independent synthesis by stabilization of the primer strand. Pol μ has fewer interactions with the incoming dNTP than Pols β and λ, which may in part explain its ability to incorporate ribonucleotides. The 8 kD domain appears to interact with the downstream DNA. This interaction is likely required for the end-bridging activity of Pol μ. However, the interacting residues have fewer positive charges than what is observed in Pols β and λ, likely explaining its dRP lyase deficiency.

Lesion Bypass by Pol μ

Pol μ functions in lesion bypass using the 1 base deletion mechanism as described above in the mutagenesis studies [171]. Specifically, Pol μ bypasses lesions including 8oxoG, AP sites, and 1,N^6-ethenoadenine by realigning the template such that the lesion moves to an extrahelical position, as summarized in Figure 7. Pol μ then searches for microhomology, anneals, and synthesizes DNA. Importantly, mutation spectra of targets with these lesions consist predominantly of base substitution mutations, so it is suggested that the net result is not a deletion. Rather Pol μ may first bypass these lesions, which are then repaired by the BER machinery [171]. Alternatively, Pol μ may not play a major role in the bypass of these lesions in cells. Strikingly, Pol μ is able to bypass thymidine dimers in an error-free manner,

and this could be a biologically relevant role for this polymerase. Pol μ also readily bypasses cisplatin and oxaliplatin-GG adducts using the misalignment mechanism [172], because it is dependent on the presence of a downstream piece of DNA with which to anneal. Pol μ bypasses these lesions with an efficiency second only to Pol η, suggesting that Pol μ may function in this process *in vivo*. Livneh and colleagues provide evidence that Pol μ has a terminal transferase-like activity when the 3′ end of the primer is opposite an abasic site [173]. This activity could be employed when there is damage present near to or at the 3′ end of the primer strand [140].

Cellular Roles of Pol μ

Pol μ is preferentially expressed in lymphoid tissue [136, 163]. This, together with the finding that Pol μ-deficient mice exhibit abnormal B-cell differentiation, suggests that this polymerase is important for VDJ recombination. In fact, Pol μ-deficient mice display impaired IgG light chain rearrangement [174]. This polymerase likely functions at a time when terminal transferase is no longer present. Pol μ promotes accurate joining during junction formation, and this correlates with the ability of this polymerase to utilize primer-termini that lack complimentary bases and end-bridging during DNA synthesis [140, 165]. Pol μ associates with XRCC4 and DNA ligase IV, suggesting that this polymerase in fact functions in end-joining during VDJ recombination [175]. Whether Pol μ functions in lesion bypass in cells remains to be seen, but given its expression profile, its lesion bypass activity might be confined to lymphoid tissues.

Examination of protein levels suggests that Pol μ is induced in response to ionizing radiation [175]. However, quantification of RNA levels suggests that this is not the case [136]. Thus the induction of Pol μ in response to ionizing radiation likely occurs by a posttranscriptional mechanism, that could include alteration in stability that might result from protein modification. Pol μ forms foci after ionizing radiation that colocalize with γ H2AX, a marker of double-strand breaks (DSB) [175], suggesting it could function in NHEJ in response to DSB formation. This may be especially important during the treatment of lymphoid disorders by agents that induce double-strand breaks. *In vitro* studies of Pol μ synthesis in the presence of a pool bias of ribonucleotides shows that it can incorporate them into DNA [168]. The authors of this study note that dNTP pools are lower during G1 of the cell cycle when NHEJ occurs and suggest that Pol μ may incorporate ribonucleotides at this time. In this way, ribonucleotides can subsequently be used as a "flag" for sites of DNA that need to be repaired or to induce a checkpoint response, especially as a consequence of stalling by the replication fork. Pol μ is highly expressed in B-cell non-Hodgkin's lymphomas [176]. Its expression level is unrelated to the proliferative index, so it could be associated with pathogenesis by increasing genomic instability, particularly during NHEJ.

Pol μ Germline Variants

Resequencing of the Pol μ gene indicates that there are five SNPs in the germline that are present in 5-10% of the population studied in the Environmental Genome Project and that result in nonsynonymous amino acid substitutions within the protein. Asp107Glu maps to the BRCT domain and could result in aberrant interactions with protein partners and aberrant NHEJ. It is possible that the other SNPs could alter the ability of Pol μ to participate in NHEJ in other ways, including compromised end-bridging or misalignment of the template. This could manifest itself as altered genomic stability or impact the responses of cells to cancer therapies.

Summary

Cells have at least four DNA polymerases that are members of Family X. Pols β, λ, and μ are structurally very similar yet participate in different cellular functions, due to subtle

structural differences and tissue-specific expression. These structural differences result in enzymes that interact with DNA in different ways. Whereas Pol β prefers to fill in single nucleotide gaps during BER, Polsλ and μ have the intrinsic ability to catalyze DNA synthesis at discontinuous templates with paired and unpaired termini, respectively, making them both suitable for NHEJ, as shown in Table 1. The presence of BRCT domains in Pols λ and μ enables these enzymes to participate in NHEJ. The tissue-specific expression of Pol μ mainly restricts it to functioning in NHEJ during VDJ recombination. Pol λ also functions in VDJ recombination but is expressed in other tissues where it can catalyze gap filling during end-joining. Like its other family member Tdt, Pol μ is able to catalyze template-independent DNA synthesis, likely due to the presence of a loop I that is longer than its counterparts in other family members.

Deletion of Family X protein members from mice results in specific and non-overlapping phenotypes, as shown in Table 1. Deletion of Pols λ and μ from mice results in subtle immune defects because Pol λ functions in the processing of immunoglobulin heavy chains and Pol μ in light chains. In contrast, deletion of Pol β results in mice that die about an hour after birth likely due to massive apoptosis of postmitotic neurons, suggesting that this polymerase has a specific function that cannot be performed by any other enzyme in neurons, even members of the same family who have quite similar structural portraits. Little is known about the variants of these enzymes that are present in people. It is likely that these variants will confer subtle biochemical and structural alterations to the enzymes. Given that small structural differences in the ancestral members of this family lead to differing cellular roles, their variants may confer interesting phenotypes when examined in a whole organism.

Acknowledgments

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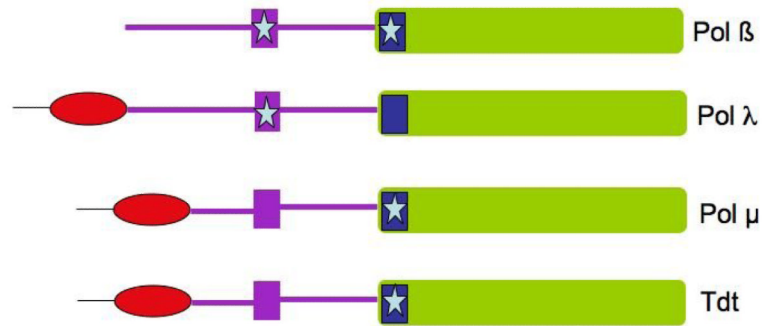
Figure 1. Alignment of Pol X Family Members
In red is the BRCT domain, purple is the 8 kDa domain, and green is the polymerase domain, consisting of thumb, palm, and fingers (not shown). The blue boxes represent Helix-Hairpin-Helix motifs, and the ones with the best conservation contain a star.
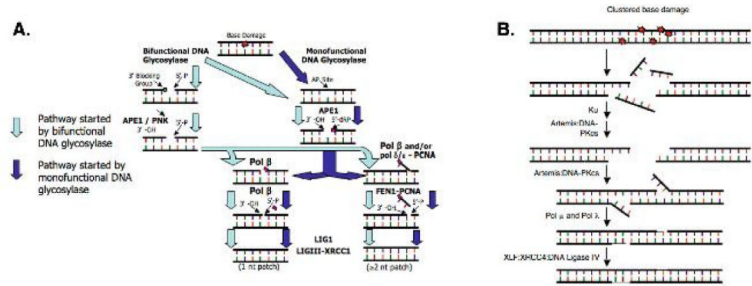
Figure 2. Repair Pathways

A. Base excision repair pathway. Light blue arrows connect steps in pathway started by a monofunctional DNA glycosylase. Dark blue arrows connect steps in pathway started by a binfunctional DNA glycosylase. Adapted from [48]. B. Non-homologous end joining pathway. Adapted from [177]. See text for detailed explanations.
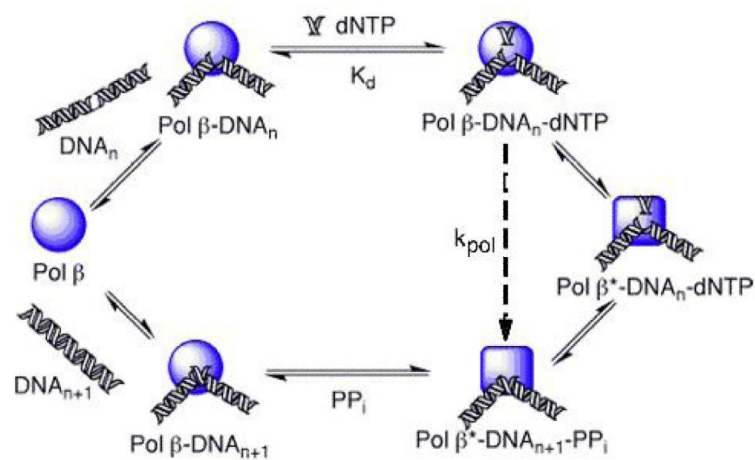
Figure 3. Kinetic Pathway of DNA Polymerases β and λ
Schematic of catalytic scheme of pol β. Abbreviations: DNA$_n$, substrate DNA; DNA$_{n+1}$ extended product; Pol β* pol β in the transition state; PP$_i$ pyrophosphate.
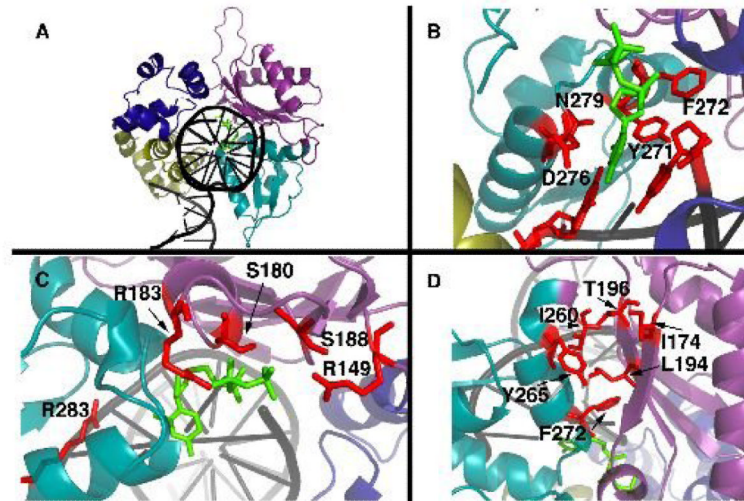
Figure 4. Crystal Structures of DNA Polymerase β
Crystal structures drawn using PDB 1BPY. 8kD domain is yellow, thumb subdomain is dark blue, palm subdomain is purple, fingers subdomain is teal, and the DNA is black. A. Overview of pol β. B. Close up view of the dNTP binding pocket. Residues of the dNTP binding pocket are shown as red sticks. The ddCTP is shown as green sticks. C. Close up view of other important dNTP binding residues. Key residues are shown as red sticks. ddCTP is shown as green sticks. D. Close up view of the hinge region of pol β. Hinge residues are shown as red sticks. ddCTP is shown as green sticks.
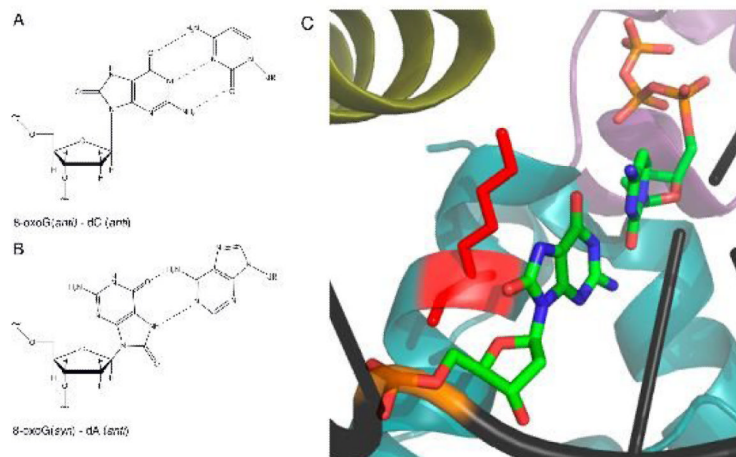
Figure 5. Pol β and 8oxoG
Structure of nucleotide insertions opposite an 8oxoG lesion. A. Chemical structure of an 8oxoG base in the *anti* configuration base paired with a dC. Adapted from [110]. B. Chemical structure of an 8oxoG base in the *syn* configuration base paired with a dA. Adaped from [110]. C. Crystal structure of pol β with an 8oxoG lesion in the active site opposite a dCTP from PDB 1MQ3. The 8oxoG and dCTP are colored by element. Pol β residue Lys280 is shown as red sticks. Subdomains are colored as for Figure 2.
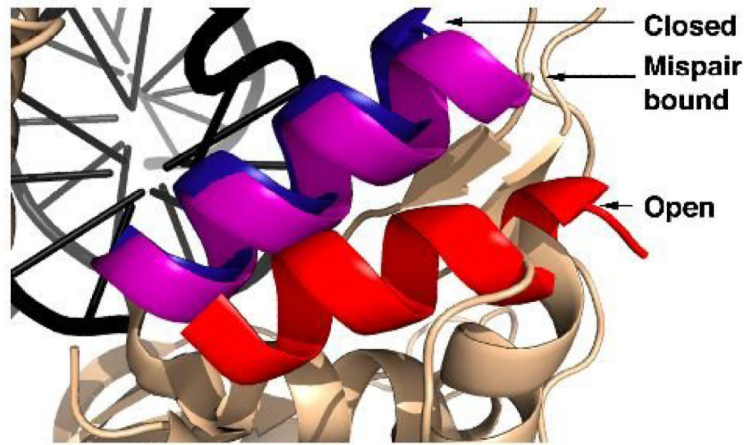
Figure 6. Movement of Helix N upon Binding of a Mispair by Pol β
Cartoon representation of crystal structure of pol β with a mispair in the active site (PDB 3C2M). The mispaired crystal structure was aligned with crystal structures of the binary and correctly paired ternary complexes of pol β (PDB 1BPX and 1BPY, respectively). Only Helix N is shown of the 1BPX and 1BPY structures. The position of Helix N in the closed ternary complex with a correct pair is shown in blue, in the partially open ternary complex with a mispair is shown in purple, and in the open binary complex is shown in red.

Figure 7. Lesion Bypass by X Family Polymerases
The chemical structures of DNA lesions are shown along with X family polymerases capable of conducting bypass synthesis past them.

Table 1

Summary of characteristics of Pols β, λ, and μ.

	Pol β	**Pol λ**	**Pol μ**
Chromosomal Location	8p11	10q23	7p13
Gene Name	*POLB*	*POLL*	*POLM*
Gene Size	~33kb	~9kb	~4kb
Protein Size	39 kDa	68 kDa	55 kDa
Polymerization Rate	10-14 sec^{-1}	2 sec^{-1}	0.006-0.076 sec^{-1}
DNA Substrates	1-bp gap with $3'$-OH and $5'$-PO$_4$	Gap with $3'$-OH and $5'$-PO$_4$; discontinuous template with paired terminus	Recessed DNA; end-bridging where there is no complementarity; template independent DNA synthesis
Cellular Role	BER	NHEJ/VDJ recombination	NHEJ/VDJ recombination
Deletion in Mice	Lethal due to massive apoptosis of postmitotic neurons	Alive; defect in processing of immunoglobulin heavy chains	Alive; defect in processing immunoglobulin light chains