HHS Public Access

Author manuscript

Annu Rev Biochem. Author manuscript; available in PMC 2018 August 18.

Published in final edited form as:

Annu Rev Biochem. 2018 June 20; 87: 239–261. doi:10.1146/annurev-biochem-062917-012405.

Translesion and repair DNA polymerases: diverse structure and mechanism

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Abstract

The number of DNA polymerases identified in each organism has mushroomed in last two decades. Most newly found DNA polymerases specialize in translesion synthesis and DNA repair instead of replication. Although intrinsic error rates are higher for translesion and repair polymerases than for replicative polymerases, the specialized polymerases increase genome stability and reduce tumorigenesis. Reflecting the numerous types of DNA lesions and variations of broken DNA ends, translesion and repair polymerases differ in structure, mechanism and function. Here we review the unique and general features of polymerases specialized in lesion-bypass, gap-filling and end-joining synthesis.

Keywords

A-, B-, X- and Y-family; proofreading; DSBs; TLS; sGRS (small gap-filling repair synthesis); NHEJ; TMEJ

1. INTRODUCTION

DNA must be replicated accurately and repaired properly upon damage to maintain life. Shortly after the discovery of the double-helical structure of DNA and unveiling of its copying mechanism by Watson and Crick, Arthur Kornberg and his team isolated DNA polymerase I (Pol I) from *E. coli* in 1956 and demonstrated its ability to copy DNA accurately and processively (1, 2). The first crystal structure of a DNA polymerase, fittingly the large fragment of *E. coli* Pol I, was determined in 1985 by Tom Steitz's group (3) and ushered in an era of mechanistic understanding of the intricacies of DNA replication. In the last sixty years, the number of known DNA polymerases has proliferated. By latest account there are seventeen DNA polymerases in humans (Table 1), of which Pol α, γ, δ, ε and telomerase are responsible for replicating the bulk of nuclear and mitochondrial DNA. The rest specialize in translesion and repair synthesis.

DNA base lesions arise both endogenously, for example, from oxidation, deamination, alkylation and spontaneous loss of bases (forming abasic lesion, AP) (4), and from environmental assaults, including ultraviolet light from the sun (5, 6), natural and manmade chemicals, such as benzo[a]pyrene and cisplatin (7, 8). These lesions destabilize DNA

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helical structure and obstruct replication and transcription (9). Most base lesions are removed by base excision (BER) or nucleotide excision repair (NER) (10), but residual lesions in the S phase are bypassed by translesion synthesis (TLS) (11–13).

DNA double-strand breaks (DSBs) are programmed and necessary for meiosis, immunoglobulin gene rearrangement and class switching or induced by damaging agents including ionization radiation (14). DSBs are repaired by homologous recombination, non-homologous end joining (NHEJ), or Pol θ-mediated end joining (TMEJ) (15–18). Both NHEJ and TMEJ depend on specialized polymerases to bridge DNA ends and fill small gaps before ligation.

In contrast to the high speed, high fidelity and high processivity of DNA replication, translesion and small gap-filling repair synthesis (sGRS) are characterized by low efficiency, low accuracy and low processivity as trade-offs for tolerance of damaged bases and broken DNA. The mutagenic feature of specialized DNA polymerases is also used to increase diversity in antigen receptors (19) and their specificity through somatic hypermutation (20). Aside from different accuracies, which are inversely related to error rates (21), replicative and specialized polymerases are not fundamentally different. With subtle changes, homologues of replicative polymerases are able to carry out low-fidelity TLS and repair (22, 23). Discovery and characterization of new DNA polymerases specialized in TLS and sGRS in recent years have expanded our understanding of genome maintenance and provide new targets for therapeutic treatment.

Here we review general properties of all DNA polymerases and summarize unique features and mechanisms of TLS and sGRS. For comprehensive information of all DNA polymerases and for replication fork restart, readers are referred to the book “DNA polymerases” by Hübscher et al. (24) and the review by K. Marians in this volume, respectively. Although beyond the scope of this review, resolving conflicts between replication and transcription (25) and removing ribonucleotides incorporated during replication and repair (26) are highly relevant to TLS and sGRS and should not be overlooked.

2. DNA POLYMERASES FOR REPLICATION AND REPAIR

Based on protein sequence, DNA polymerases are divided into A, B, C, D, X, Y, RT (reverse transcriptase, including telomerase), and PrimPol (primase and polymerase) families (27–30) (Table 1). Most members of families A-D are replicative polymerases and are associated with proofreading 3′ −5′ exonuclease activities (31), but a few with inactivated 3′−5′ exonucleases are involved in TLS and sGRS (Table 1) (16, 32–34). X-family polymerases perform small gap-filling synthesis associated with BER and NHEJ (35, 36). Y-family members are specialized in TLS by incorporating nucleotides directly opposite damaged bases (11–13, 37). Telomerase uses its own RNA subunit to template DNA synthesis of many telomere repeats (38). PrimPol is unique in that it can synthesize DNA primers and continue DNA synthesis without an RNA primer (30). A common feature among X, Y, RT and PrimPol families is the absence of a proofreading exonuclease. Except for C and D families, which exist in bacteria and archaea (39, 40), respectively, human DNA polymerases are present in all six other families.

Each DNA polymerase may consist of a single polypeptide chain as is observed for X and Y family members (35, 37), or along with 1–3 accessary subunits as many A- and B-family members (41–47). Telomerase (RT family) is composed of a catalytic subunit (TERT), RNA (TER) and multiple other protein subunits (29, 48). Occasionally accessary subunits may be shared among different DNA polymerases, for example, Pol δ and ζ (43–46). Although the main function of Pol δ is lagging strand synthesis, it performs high-fidelity large gap-filling synthesis during replication and repair (49).

Atomic resolution structures of catalytic subunits of DNA polymerases from each family (except for D) have been determined. They are characterized most often as a right hand with palm, thumb and finger domains (Fig. 1a). The exceptions are Y-family polymerases, which have an additional Little-Finger domain (LF, also known as PAD) essential for DNA binding (12, 50), and PrimPol members, which lack a distinct thumb domain (51). The catalytic center of all DNA polymerases resides in the palm domain and usually contains three carboxylates. Despite drastically different sequences, topologies and structures, the thumb domain always binds DNA substrate and the finger domain contacts the nascent base pair between a template and incoming nucleotide (dNTP) (Fig. 1a). The palm domains of A, B, Y and RT family share a conserved "polymerase" fold (Fig. 1b, 1c), while those of C and X family share a nucleotidyltransferase (NT) fold (Fig. 1d). The palm domain of PrimPol has a polymerase-like fold, but is turned roughly 180° when approaching DNA substrate (Fig. 1e). Despite these many differences, the catalytic centers of all DNA polymerases including DNA primer, incoming dNTP, and Mg^{2+} ions are superimposable (Fig. 1f).

3. Determinants of accuracies of DNA POLYMERASES

DNA replication occurs in the context of replisomes, which enhance the processivity and efficiency of fork movement, but the ability to discriminate against ribonucleotides (NTPs), mismatched, damaged or non-native dNTPs is intrinsic to replicative polymerases. Owing to conformational and chemical selection (see below), the error rate of a replicative polymerase (defined as the ratio of incorporation efficiencies (k_{pol}/K_d in pre-steady state or k_{cat}/K_M in steady state) of incorrect over correct dNTPs) can be as low as 10^{-5} to 10^{-6}, and proofreading and the context of replisome machineries reduces it by 100 fold to ~10^{-7} to 10^{-8} (21) (Fig. 2a). Post-replication mismatch repair further lessens the error rate of replication to 10^{-10} (52).

The DNA synthesis reaction makes a phosphodiester bond between the 3′-OH of a DNA primer and the α-phosphate of an incoming dNTP. DNA polymerases bind Watson-Crick (WC) paired DNA template/primer duplexes with a dissociation constant (K_d) in the range of 5–20 nM and bind a correct dNTP (K_d of low μM) much better than a mismatched one (53–55). Replicative polymerases perform rapid conformational selection of dNTP before the synthesis reaction, which imposes chemical selection (56–59).

3.1. Conformational selection

Conformational selection, which was originally called induced fit (60), is a process of structural changes from a finger-open DNA-bound binary complex to a finger-closed polymerase-DNA-dNTP ternary complex when the dNTP forms a WC pair with the

templating base (61, 62) (Fig. 2b). This change occurs because a nascent WC base pair selectively stabilizes the closed conformation. A damaged templating base, ribonucleotides (rNTPs), and damaged or mismatched dNTPs destabilize the ternary complex and lead to a persistent finger-open state. In a closed ternary complex, the polymerase seamlessly contacts the nascent base pair on the face of bases, in the minor groove, and deoxyribose and triphosphates of dNTP, and DNA and dNTP become perfectly aligned for chemistry to take place (Fig. 2c). Conformational selection is a hallmark of replicative polymerases and increases their accuracies by ~100 fold (Fig. 2a). In Y- and X-families (except for Pol β) the finger is closed without dNTP, so there is no conformational selection.

3.2. DNA synthesis reaction and Mg^{2+}-mediated chemical selection

In DNA synthesis reactions, regardless of error rates, a polymerase-DNA-dNTP ternary complex needs to capture three catalytic Mg^{2+} ions (63), which occurs in three steps (Fig. 2b). The first Mg^{2+} ion occupies the B site and binds DNA polymerase together with an incoming dNTP, but this association is rapidly reversed if the dNTP and the templating base do not match. Only if the dNTP is correct, the second Mg^{2+} ion occupies the A site. With replicative polymerases, binding of the A-site Mg^{2+} occurs after the conformational selection. The polymerase-DNA-dNTP ternary complexes with two Mg^{2+} ions bound are reaction ready, but the new phosphodiester bond does not form until a third Mg^{2+} ion occupying the C site is captured by dNTP (Fig. 2b). Mg^{2+} ions are exquisitely sensitive to coordination environment and ligand geometry. Slight alterations of active site composition, DNA or dNTPs can have a dramatic impact on Mg^{2+} binding and thus DNA synthesis reactions. Without conformational selection misincorporation occurs at a frequency of 10^{-3}-10^{-4}, much lower than the free energy difference between a correct and incorrect base pair (1–2 kcal, which is equivalent to 10 to 100-fold difference in dNTP selection). Mg^{2+}-mediated accuracy enhancement, which significantly reduces error rates of DNA synthesis, forms the chemical selection.

3.3. Proofreading

A proofreading 3′ −5′ exonuclease resides on the same polypeptide chain in the A- and B-family replicative polymerases or exists as a separate subunit, for example the ε subunit of the *E. coli* Pol III holoenzyme (31) (Table 1). A mismatch in a template/primer pair weakens the DNA binding to the polymerase active site and triggers DNA relocation for proofreading (31) (Fig. 2d). The distance between the active sites of polymerase and exonuclease is ~35–50 Å (3, 22), and the DNA distortion necessary for proofreading is dramatic (64). A finely tuned partition of DNA substrate between polymerase and exonuclease enhances fidelity without diminishing processivity and efficiency of DNA synthesis (31). Efficient proofreading inhibits TLS, and in the absence of proofreading replicative polymerases display ability to tolerate and bypass lesions (31, 65).

4. GEneral features of TLS and sGRS

Normal WC base pairs have the same shape, and replicative polymerases are thus one-size-fits-all. DNA lesions occur in different shapes, sizes and chemical types, however, and TLS polymerases usually specialize in bypassing certain type of lesions. Complete TLS requires

polymerases to (1) accommodate and use a damaged template base to direct dNTP incorporation, and (2) accommodate the abnormal and distorted base pair in DNA duplex for primer extension. These two steps are known as insertion and extension of TLS, respectively, and are often accomplished by two different polymerases (66, 67) (Fig. 3a). Y-family DNA polymerases, characterized by an open and uniquely shaped active site that accommodates different DNA lesions, specialize in the insertion step (11–13). The B-family *E. coli* Pol II and eukaryotic Pol ζ are error-prone and perform efficient TLS extension (16, 22, 46).

DNA small gap-filling repair synthesis (sGRS) associated with BER and end joining in DSB repair needs to accommodate discontinuous DNA backbones. At first glance TLS and sGRS have little in common, but both use template or primer looping-out mechanisms to circumvent distorted or discontinuous double helices (Fig. 3b-c). It has also been suggest that DNA primer looping-out enhances processivity of telomere repeat synthesis (68). Alternative alignment of templates and primers may give rise to the appearance of direct lesion bypass, but misaligned templates/primers in TLS and repair synthesis may be followed by realignment and result in complex mutations.

Because Mg^{2+}-mediated chemical selection occur in all polymerases, and the error rate of TLS and sGRS can be as low as 10^{-3}-10^{-4} (69) (Fig. 2a). Higher error rates among specialized polymearases are often due to template-independent dNTP binding, dNTP-independent finger closing, or biased dNTP selection in a modified active site (see below). Lesion accommodation requires reduced polymerase-substrate interactions, which may lead to reduced dNTP selection (70) and flexible DNA and dNTP alignment, and thus results in inefficient capture of Mg^{2+} ions and low catalytic efficiency (71). Because of reduced selectivity, TLS and sGRS polymerases are often shown to bypass more types of lesions *in vitro* than *in vivo*. In the following sections, specific mechanisms for TLS insertion, extension and sGRS in BER, NHEJ and TMEJ are illustrated (Fig. 3).

5. TLS insertion by Y-family polymerases

All Y-family polymerases have a preformed active site, where dNTPs and pyrophosphate diffuse in and out freely because of a small finger and thumb domain (72) (Fig. 4a-b). With no conformational selection or proofreading, accuracy of Y-family polymerases is achieved by chemical selection alone and can deteriorate significantly. The finger domain, which interacts with a nascent base pair, has varied sequences and contributes to different lesion preferences and biased dNTP selection. Despite the lack of finger-domain movement, the Little Finger domain (LF) may be flexible, and a semi-detached LF in the DINB branch of Y polymerases creates a structural gap capable of accommodating bulky aromatic adducts and inducing frameshift mutations (73–77) (Fig. 4b).

5.1. Pol η

Pol η, encoded by the POLH gene, is the only human DNA polymerase known for an anti-cancer role before the gene and protein were characterized (78, 79). Pol η is present in all eukaryotes and performs both the insertion and extension steps of TLS when bypassing a thymine dimer (80), which is the predominant form of cyclobutane pyrimidine dimers (CPD)

caused by ultraviolet radiation (UV) (81). Without active Pol η, humans develop a variant form of xeroderma pigmentousum (XPV), which is a UV sensitive syndrome with increased risk of skin cancers (82, 83). The error rate of human Pol η at 3.5×10^{-2} is relatively high even among Y-family members (70), which likely stems from its ability to bind dNTP without DNA substrate and its tendency to misincorporate dGTP opposite dT. The mutagenic nature of Pol η is involved in somatic hypermutation, which generates highly specific antigen receptors (20, 84).

Crystal structures of the Pol η polymerase catalytic region (residues 1–432) performing TLS insertion and extension reveal several interesting features (72). The first is that its open active site specially accommodates two covalently linked and closely spaced DNA bases (Fig. 4c). Without the covalent linkage, two normal DNA bases are less stably bound and lead to more erroneous dNTP incorporation than a thymine dimer. A second feature are strong electrostatic interactions with four consecutive phosphates of the DNA template strand, which stabilize DNA in the B-form conformation. When the backbone of DNA is fixed by Pol η, the distortion to the DNA helix introduced by the thymine dimer is minimized. Therefore Pol η has been likened to a molecular splint. Missense mutations of Pol η found in XPV patients are located either around the catalytic center or the molecular splint (72).

The widely used chemotherapeutic agent cisplatin and related platinum derivatives kill rapidly dividing cells by crosslinking adjacent purine bases and blocking replicative polymerases (8). Two intrastrand purines crosslinked by platinum resemble a thymine dimer, and human Pol η is able to incorporate dCTPs opposite crosslinked dG bases efficiently (85, 86). Full bypass of a cisplatin crosslinked lesion, however, requires Pol ζ to extend the primer beyond the damaged site (87). Patients undergoing cisplatin or related chemotherapies may develop elevated Pol η expression and chemo-resistance. It has been shown that the survival rates in non-small cell lung cancer, metastatic gastric adenocarcinoma and head and neck squamous cell cancer patients are inversely correlated with Pol η expression levels (8). Targeted inhibition of Pol η, therefore, may increase efficacy of platinum treatment.

5.2. Pol ι

Pol ι is a paralog of Pol η, but mice without Pol ι show no detectible defects and no UV sensitivity (88). Loss of Pol ι in the Pol η deficient mice induces a slight increase in UV sensitivity and mesenchymal tumors in addition to epithelial skin tumors (89). Studies of Pol ι dNTP selectivity reveal a four-order of magnitude range of accuracies depending on the nature of the templating base. Pol ι can accurately and efficiently incorporate dTTP opposite dA (error rate of 10^{-4}), but it prefers to incorporate dGTP opposite dT (13).

Crystallographic analysis of human Pol ι reveals a uniquely narrow pocket for the nascent base pair (90, 91). In the presence of an incoming dNTP, Pol ι favors the high-energy *syn* conformation for a template purine. Since adenine in the *syn* conformation still base pairs preferentially with thymine, dTTP incorporation is specific and efficient (92). Recently, the Prakash and Aggarwal groups showed that the dA(*syn*):dTTP(*anti*) pair may allow Pol ι to accurately bypass N1-methylated deoxyadenosine (93) (Fig. 4d). A similar Hoogsteen

configuration is also adopted by Pol ι when correctly incorporating dCTP opposite 8-oxo-G, a prevalent lesion caused by oxidation (94).

5.3. Pol κ

Pol κ specially bypasses polycyclic aromatic hydrocarbon adducts, such as benzo[a]pyrene diol epoxide (BPDE) covalently attached to N2 of guanine in the minor groove (95, 96). Although incapable of accommodating or bypassing UV lesions, Pol κ is reported to facilitate repair synthesis after excision of UV damage (97). Pol κ deficient mice exhibit a spontaneous mutator phenotype, and the mutation rate increases when dietary cholesterol is increased (98). Therefore it has been suggested that Pol κ may accurately bypass naturally occurring steroid adducts (99).

Pol κ, *E. coli* Pol IV (DinB) and archaeal Dpo4 are homologs, and they all contain a structural gap to accommodate a bulky lesion in the minor groove (Fig. 4b). *E. coli* Pol IV is found to efficiently bypass N2-furfuryl-dG adducts (100). The structural gap in Pol κ is particularly large, and perhaps as a result Pol κ is most efficient and accurate in bypassing minor-groove adducts (76, 95, 96, 101, 102). The structural gap, however, is implicated in template slippage / misalignment, which leads to deletional mutations (73, 75, 103). The other unique feature of Pol κ is a 75-aa N-terminal extension, called the N-clasp, which stabilizes the otherwise detached LF (74) (Fig. 4b). The flexible region N-terminal to the N-clasp, which is not yet characterized structurally, also enhances lesion bypass capability (76).

5.4. Rev1

Rev1 was the first member of the Y-family shown to have DNA synthesis activity (104, 105), although it incorporates dCTP only. The deoxycytidyl (dCMP) transferase activity of Rev1 is highest when the template is abasic, followed by dG and dA (104), and later Rev1 is confirmed to bypass abasic lesions *in vivo* (106). Like Pol η, Rev1 has a role in somatic hypermutation (107). Initially identified in *Sacharomyces cerevisiae* for its role in UV-induced mutagenesis (108), Rev1 is ubiquitous in eukaryotes. The ~700 residues outside of the dCMP transferase region (Fig. 4a) serve as a ‘hub” for coordinating TLS process (37) (see below).

Structurally, Rev1 has a detached LF like Pol κ and an N-terminal extension, called the N-digit, attaches LF to the catalytic core (CC) (109, 110). The N-clasp in Pol κ traverses the front of the active site and interacts with the DNA major groove (Fig. 4b), while the N-digit inserts itself between CC and LF in the back of Rev1 and interacts with the DNA minor groove (Fig. 4e). The most striking feature of Rev1 is that it flips out the templating nucleotide and supplies an Arg sidechain from the N-digit as a surrogate, which forms two hydrogen bonds with an incoming dCTP (Fig. 4e) and results in dCMP transferase activity regardless of whether the template is abasic (AP), dG, dA or dU (104).

6. TLS extension by B-family DNA polymeraseS

All A-D family polymerases undergo conformational selection to produce a snug active site and are thus less suited for TLS insertion. With intermittent interactions with DNA template/

primer, Pol II and Pol ζ of the B-family carry out TLS extension by accommodating non-WC base pairs at the primer end and alternative DNA alignment to avoid lesions near the active site (Fig. 3b).

6.1. Pol ζ

Pol ζ is composed of the catalytic subunit Rev3, Rev7 and two other accessary subunits (p50 and p66), which are shared with Pol δ (43–46). Chris Lawrence and colleagues isolated the yeast Rev3/Rev7 heterodimer and showed it to have translesion synthesis activity in 1996 (111). Pol ζ was the sixth DNA polymerase found in eukaryotes after α, β, γ, δ and ε and the first to exhibit TLS activity. Pol ζ is ubiquitous in eukaryotes. Mammalian Rev3 at over 3000 residues is twice as large as its yeast homolog (112) (Fig. 5a). The N-terminal 250 aa and C-terminal 800 aa of Rev3 are homologous to Pol α, δ and ε, but the 3′–5′exonuclease is inactivated by mutations. The very C-terminal Cys-rich region, which binds Zn^{2+} and an iron-sulfur cluster and is sensitive to oxidation (46), interacts with the two subunits shared with Pol δ (43–45). Except for a positively charged domain (PCD) for DNA binding and two Rev7 interaction regions, most of the inserted regions in Rev3 have unknown function.

REV3 is a non-essential gene in yeast. But Rev3 knockout in mice is embryonic lethal and mammalian cells cannot proliferate without Pol ζ even in the absence of external mutagens (16, 113). Pol ζ increases genomic stability by increasing DNA-damage tolerance and reducing spontaneous tumorigenesis. Given a perfect primer and template pair, the error rate of Pol ζ is 10^{-3}–10^{-4}, but Pol ζ is exceptionally efficient in extending primer strands beyond a mismatched base pair (66). In the early days of its characterization, Pol ζ was reported to bypass all sorts of DNA lesions and incorporate mismatched dNTPs, but its central function in TLS is primer extension emerged in subsequent years (46, 66, 67). In the absence of an atomic structure of Pol ζ, we use the structure of *E. coli* DNA Pol II as a basis to illustrate possible mechanisms for Pol ζ-mediated TLS extension.

6.2. *E. coli* DNA Pol II

Like Pol ζ, *E. coli* Pol II is induced upon DNA damage and carries out TLS extension (22, 114, 115). Although equipped with a functional 3′–5′exonuclease, Pol II has a much reduced proofreading activity because substrate partitioning is shifted toward DNA synthesis (22). Expression of *E. coli* Pol II leads to a mutator phenotype. *E. coli* cells without DNA Pol II are viable but less fit in competition with WT strains (116). Similar to Pol ζ, *E. coli* Pol II is efficient in extending primer strands beyond an abasic lesion or mismatched base pair (22).

Initial crystal structures of *E. coli* Pol II are identical to those of B-family replicative polymerases (Fig. 2c). Further structural and biochemical analyses of Pol II reveal a mechanism for TLS extension that involves looping out of the template strand where the lesion resides and using an undamaged base downstream to template primer synthesis (22) (Fig. 3b, 5b). Looping out is facilitated by a dinucleotide repeat (117, 118). Our analyses of human Pol ζ indicate that Pol ζ is more flexible and more efficient than Pol II in accommodating a looped-out template strand (He and Yang, unpublished data). An increased dwell time for DNA substrate in the polymerase active site and a tendency for the finger

domain to close may give Pol II and Pol ζ the ability to mediate primer extension after AP lesions and mismatches.

7. The A-family Pol ν and θ in DNA end-joining

Pol ν and θ are different from most A-family replicative polymerases in that they lack a 3´–5´ exonuclease activity, and Pol θ is the only human DNA polymerase that has an ATP-dependent DNA helicase activity (33). The N-terminal helicase and C-terminal polymerase of Pol θ are separated by a large unstructured central region (Fig. 6a). The Pol θ helicase activity is necessary for interstrand crosslinking repair in Drosophila (119). Without the Pol θ polymerase activity, all eukaryotic cells have increased micronuclei and are hypersensitive to DSB agents (120, 121). In contrast, mice without Pol ν show no difference from WT in life span or lesion tolerance, but they exhibit slightly reduced meiotic crossover frequencies at certain sites (34).

Pol θ has a unique role in DNA end-joining. Its polymerase activity can preserve 3´ overhangs on DSBs and use minimal base pairs to bridge broken DNA ends for 3´ extension (17, 18, 121) (Fig. 3c). With such flexibility, Pol θ seemingly can extend ssDNA without a template. The reported TLS activities of Pol θ on AP and thymine glycol (33) may be a result of alternative template usage. TMEJ is independent of recruitment by Ku70/80 and DNA PK and thus different from and complementary to NHEJ in DSB repair (121–124). Removal of both NHEJ and TMEJ abolishes random integration and increases DNA gene targeting specificity to 100% homologous recombination dependent (125, 126).

The DNA polymerase regions of Pol ν and Pol θ are homologous and share three insertions (Fig. 6a), which contribute to their TLS activities. The polymerase region of Pol θ is much more active than Pol ν (23, 33). Given a normal DNA template/primer pair, the error rate of Pol θ is 10^{-3}-10^{-4} (33). In contrast, Pol ν has a tendency to misincorporate dTTP opposite a dG template, and the error rate is DNA sequence dependent and varies by two orders of magnitude (23).

Structural analyses of Pol ν and Pol θ complexed with DNA substrate reveal potential mechanisms for low-fidelity TLS DNA synthesis and end-joining activities (23, 127). The overall structures of Pol ν and Pol θ ternary complexes are essentially identical to those of high-fidelity A-family members (as shown in Fig. 1a). Close examination reveals that (1) the finger domain of Pol ν can close without an incoming dNTP, (2) a positively charged residue in the Pol ν finger domain favors misincorporation of dTTP opposite dG, and (3) the second insertion loop (Ins2) near the thumb and palm domain interface gives rise to a flexible thumb and a cavity for the primer strand to slip out in Pol ν (23) (Fig. 6b). In solution Pol θ is more flexible and efficient in accommodating primer looping-out than Pol ν (23). Pol θ also has a series of positively charged residues in the thumb domain that strengthens its interactions with the primer strand (127). A combination of tight primer-strand binding and alternative template/primer alignment may underlie Pol θ-mediated end-joining.

8. Mechanisms for sGRS by X-family polymerases

All X-family polymerases carry out small gap-filling repair synthesis (sGRS) (35). Terminal deoxynucleotidyl transferase (TdT) was the first member of X-family isolated and shown to synthesize DNA without a template (128). Two decades later the role of TdT in antigen-receptor gene diversification is established where it acts on DSBs made by the RAG1/2 recombinase (129). Pol β was the second member of X-family to be identified and also the second *bona fide* DNA polymerase found in mammals (130). Crystal structures of Pol β determined in 1994 revealed for the first time how dNTP binding induces finger closing (61). The finger and thumb domains in Pol β are often swapped because a "left-hand" instead of the "right-hand" analogy is used for the X family (35, 61) (Fig. 7b). In this review, we adhere to the "right hand" convention, so that finger domain always interacts with a nascent base pair and undergoes open-to-closed change upon dNTP binding (Fig. 1). In early 2000, two new members of X-family, Pol λ and Pol μ, were discovered (35, 131). Currently mammals have four X-family polymerases (Fig 7a), while yeast has one and bacteria have few (132, 133) (Table 1).

Pol β fills the one-nucleotide gaps generated during BER, and utilizes an intrinsic lyase activity to remove deoxyribose phosphate (dRP) at the 5′ end of the gap for DNA ligation to complete BER (134) (Fig. 7c). Pol μ was the first DNA polymerase shown to play a role in NHEJ (135, 136). Pol μ can bridge non-complementary DNA ends and extend DNA for end-joining. This ability is attributed to an insertion in Loop1 present in Pol μ and TdT but not in Pol β (Fig. 7a) (36). Pol μ and TdT are also devoid of dRP lyase activity. In contrast, Pol λ, which contains dPR lyase activity and a shorter Loop1 than Pol μ, functions in both BER and NHEJ (137). When filling a gap between two DNA ends, Pol λ requires at least one WC base pair between 3′-overhangs and uses one end as template for dNTP incorporation on the opposite end (Fig. 7d).

Structures of all four mammalian X-family polymerases have been determined, and the protein components of the ternary complexes are superimposable. Only Pol β (both the finger and lyase domains) undergoes conformational changes upon substrate binding. Pol λ, Pol μ and TdT are increasingly more rigid, and the increased rigidity correlates with end-joining ability (138). In the ternary complex structures of Pol β, λ and μ, all bound DNAs contain a small gap and are segregated into two duplexes (137, 138) (Fig. 7b-c). The upstream duplex from the replicating base pair is bound like all other polyemerases, and the downstream duplex contacts the 8-kDa lyase (β and λ) or lyase-like (μ and TdT) domain. These structures reveal how Pol β and λ perform gap filling associated with BER.

Recently reported structures of TdT in complex with two DNA fragments reveal how DNA ends are connected without any complementary base pair (139). A 3′-primer end of a DNA fragment and an incoming dNTP can be aligned in the active site of TdT for the synthesis reaction when a mismatched template DNA is provided in trans (Fig. 7e). The two pseudo base pairs in the active site are isolated from the upstream DNA and stabilized by the elongated Loop1. Even though the sequence of the "template" strand has no influence on dNTP selection, TdT and the pseudo base pairs stabilize each other leading to nucleotide incorporation. Without base pairing the two DNA fragments likely dissociate after each

nucleotide incorporation and re-associate for the next round of the reaction, which results in TdT appearing to not use any template at all.

Pol μ probably uses a mechanism similar to TdT for bridging two DNA ends, but the template end supplies one 3´-overhang base to form a WC pair with an incoming dNTP for incorporation (140). A second templating base opposite the primer end could be absence or mismatched (Fig. 7f). Loop1 of Pol μ likely segregates the two base pairs in the active site from the upstream DNA as in TdT. Even when filling a 2-nt gap, Pol μ skips the first nucleotide and uses the second, which is juxtaposed to the downstream duplex, as a template (141). The first nucleotide probably forms a mismatched pair with the 3´-primer end, while the original partner of the 3´ end is flipped out by Loop1 (Fig. 7f). Both TdT and Pol μ can efficiently incorporate ribonucleotides (142–144). Because duplex stability is increased in presence of ribonucleotides, their incorporation may stabilize non-complementary DNA ends.

9. Regulation of TLS and sGRS synthesis

Given their mutagenic nature, TLS and repair synthesis must be tightly regulated. Mono-ubiquitylation of the sliding clamp PCNA by Rad6-Rad18, binding of PCNA by PIP (PCNA interaction peptide) of Pol η, ι and κ or by the BRCT domain of Rev1, and recognition of ubiquitin by UBM and UBZ domains of Y-family polymerases (Fig. 4a) play key roles in TLS regulation (13, 145). Furthermore, post-translational modifications of PCNA and polymerases have been shown to influence cell cycle, replication fork movement, polymerase switch, and TLS activities (146, 147). Rev1 serves as a TLS hub and binds both TLS insertion polymerase (η, ι or κ) via RIR (Rev1 interacting region) (Fig. 4a) and TLS extension polymerase ζ via the Rev7 subunit (37). Yet after 15 years of probing, our knowledge of how different DNA polymerases are coordinated and juggled to complete genome replication remains sketchy.

Pol λ, Pol μ and TdT each contain an N-terminal BRCT domain, which interacts with Ku protein and XRCC4-LigIV to coordinate their participation in NHEJ. The Ser-Pro rich region between BRCT and polymerase is also subject to post-translational modification to modulate the polymerase activities and restrict proteasome degradation (137, 148).

In summary, molecular mechanisms for many TLS and sGRS polymerases have been established. Functional and mechanistic details of Pol ν, θ, ζ and PrimPol are still being investigated. Particularly, where on genome Pol ζ is needed for replication without exogenous damage and how it is recruited and modulated are yet to be revealed. In general, regulation of specialized polymerases and coordination of bypass, repair and replication for the ultimate purpose of genome stability remains an important topic for future research.

Acknowledgements

We thank D. J. Leahy and R. Craigie for critical reading and editing the manuscript. This work is funded by NIH intramural program (DK036146–08, W.Y.).

References

1. Bessman MJ , Kornberg A , Lehman IR , Simms ES . 1956 Enzymic synthesis of deoxyribonucleic acid. Biochim Biophys Acta 21: 197–8 [PubMed: 13363894]
2. Englund PT , Deutscher MP , Jovin TM , Kelly RB , Cozzarelli NR , Kornberg A . 1968 Structural and functional properties of Escherichia coli DNA polymerase. Cold Spring Harb Symp Quant Biol 33: 1–9 [PubMed: 4306813]
3. Ollis DL , Brick P , Hamlin R , Xuong NG , Steitz TA . 1985 Structure of large fragment of Escherichia coli DNA polymerase I complexed with dTMP. Nature 313: 762–6 [PubMed: 3883192]
4. Lindahl T 2001 Keynote: past, present, and future aspects of base excision repair. Prog Nucleic Acid Res Mol Biol 68: xvii–xxx [PubMed: 11554316]
5. Setlow RB . 1966 Cyclobutane-type pyrimidine dimers in polynucleotides. Science 153: 379–86 [PubMed: 5328566]
6. Witkin EM . 1966 Mutation and the repair of radiation damage in bacteria. Radiat Res: Suppl 6:30–53
7. Boysen G , Hecht SS . 2003 Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. Mutat Res 543: 17–30 [PubMed: 12510015]
8. Dasari S , Tchounwou PB . 2014 Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 740: 364–78 [PubMed: 25058905]
9. Yang W 2008 Structure and mechanism for DNA lesion recognition. Cell Res 18: 184–97 [PubMed: 18157156]
10. Lindahl T , Karran P , Wood RD . 1997 DNA excision repair pathways. Curr Opin Genet Dev 7: 158–69 [PubMed: 9115419]
11. Ohmori H , Friedberg EC , Fuchs RP , Goodman MF , Hanaoka F , et al. 2001 The Y-family of DNA polymerases. Mol Cell 8: 7–8 [PubMed: 11515498]
12. Yang W , Woodgate R . 2007 What a difference a decade makes: insights into translesion DNA synthesis. Proc Natl Acad Sci U S A 104: 15591–8 [PubMed: 17898175]
13. Vaisman A , Woodgate R . 2017 Translesion DNA polymerases in eukaryotes: what makes them tick? Crit Rev Biochem Mol Biol 52: 274–303 [PubMed: 28279077]
14. Franco S , Alt FW , Manis JP . 2006 Pathways that suppress programmed DNA breaks from progressing to chromosomal breaks and translocations. DNA Repair (Amst) 5: 1030–41 [PubMed: 16934538]
15. Ceccaldi R , Rondinelli B , D'Andrea AD . 2016 Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol 26: 52–64 [PubMed: 26437586]
16. Lange SS , Tomida J , Boulware KS , Bhetawal S , Wood RD . 2016 The Polymerase Activity of Mammalian DNA Pol zeta Is Specifically Required for Cell and Embryonic Viability. PLoS Genet 12: e1005759 [PubMed: 26727495]
17. van Schendel R , van Heteren J , Welten R , Tijsterman M . 2016 Genomic Scars Generated by Polymerase Theta Reveal the Versatile Mechanism of Alternative End-Joining. PLoS Genet 12: e1006368 [PubMed: 27755535]
18. Wyatt DW , Feng W , Conlin MP , Yousefzadeh MJ , Roberts SA , et al. 2016 Essential Roles for Polymerase theta-Mediated End Joining in the Repair of Chromosome Breaks. Mol Cell 63: 662–73 [PubMed: 27453047]
19. Dudley DD , Chaudhuri J , Bassing CH , Alt FW . 2005 Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. Adv Immunol 86: 43–112 [PubMed: 15705419]
20. Zeng X , Winter DB , Kasmer C , Kraemer KH , Lehmann AR , Gearhart PJ . 2001 DNA polymerase eta is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nat Immunol 2: 537–41 [PubMed: 11376341]
21. Wu WJ , Yang W , Tsai MD . 2017 How DNA polymerase catalyze replication and repair with constrasting fidelity. Nature Reviews Chemistry
22. Wang F , Yang W . 2009 Structural insight into translesion synthesis by DNA Pol II. Cell 139: 1279–89 [PubMed: 20064374]

23. Lee YS , Gao Y , Yang W . 2015 How a homolog of high-fidelity replicases conducts mutagenic DNA synthesis. Nat Struct Mol Biol 22: 298–303 [PubMed: 25775266]
24. Hübscher U , Spadari S , Villani G , Maga G . 2010 DNA polymerases. Singapore: World Scientific Publishing Co. Pte. Ltd.
25. Oestergaard VH , Lisby M . 2017 Transcription-replication conflicts at chromosomal fragile sites-consequences in M phase and beyond. Chromosoma 126: 213–22 [PubMed: 27796495]
26. Williams JS , Lujan SA , Kunkel TA . 2016 Processing ribonucleotides incorporated during eukaryotic DNA replication. Nat Rev Mol Cell Biol 17: 350–63 [PubMed: 27093943]
27. Ito J , Braithwaite DK . 1991 Compilation and alignment of DNA polymerase sequences. Nucleic Acids Res 19: 4045–57 [PubMed: 1870963]
28. Filee J , Forterre P , Sen-Lin T , Laurent J . 2002 Evolution of DNA polymerase families: evidences for multiple gene exchange between cellular and viral proteins. J Mol Evol 54: 763–73 [PubMed: 12029358]
29. Nakamura TM , Cech TR . 1998 Reversing time: origin of telomerase. Cell 92: 587–90 [PubMed: 9506510]
30. Rudd SG , Bianchi J , Doherty AJ . 2014 PrimPol-A new polymerase on the block. Mol Cell Oncol 1: e960754 [PubMed: 27308331]
31. Reha-Krantz LJ . 2010 DNA polymerase proofreading: Multiple roles maintain genome stability. Biochim Biophys Acta 1804: 1049–63 [PubMed: 19545649]
32. Bruck I , Goodman MF , O'Donnell M . 2003 The essential C family DnaE polymerase is error-prone and efficient at lesion bypass. J Biol Chem 278: 44361–8 [PubMed: 12949067]
33. Wood RD , Doublie S . 2016 DNA polymerase theta (POLQ), double-strand break repair, and cancer. DNA Repair (Amst) 44: 22–32 [PubMed: 27264557]
34. Takata KI , Reh S , Yousefzadeh MJ , Zelazowski MJ , Bhetawal S , et al. 2017 Analysis of DNA polymerase nu function in meiotic recombination, immunoglobulin class-switching, and DNA damage tolerance. PLoS Genet 13: e1006818 [PubMed: 28570559]
35. Moon AF , Garcia-Diaz M , Batra VK , Beard WA , Bebenek K , et al. 2007 The X family portrait: structural insights into biological functions of X family polymerases. DNA Repair (Amst) 6: 1709–25 [PubMed: 17631059]
36. Nick McElhinny SA , Havener JM , Garcia-Diaz M , Juarez R , Bebenek K , et al. 2005 A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. Mol Cell 19: 357–66 [PubMed: 16061182]
37. Yang W 2014 An overview of Y-Family DNA polymerases and a case study of human DNA polymerase eta. Biochemistry 53: 2793–803 [PubMed: 24716551]
38. Blackburn EH , Greider CW , Szostak JW . 2006 Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat Med 12: 1133–8 [PubMed: 17024208]
39. Timinskas K , Balvociute M , Timinskas A , Venclovas C . 2014 Comprehensive analysis of DNA polymerase III alpha subunits and their homologs in bacterial genomes. Nucleic Acids Res 42: 1393–413 [PubMed: 24106089]
40. Matsui I , Matsui E , Yamasaki K , Yokoyama H . 2013 Domain structures and inter-domain interactions defining the holoenzyme architecture of archaeal d-family DNA polymerase. Life (Basel) 3: 375–85 [PubMed: 25369811]
41. Yin YW . 2011 Structural insight on processivity, human disease and antiviral drug toxicity. Curr Opin Struct Biol 21: 83–91 [PubMed: 21185718]
42. Klinge S , Nunez-Ramirez R , Llorca O , Pellegrini L . 2009 3D architecture of DNA Pol alpha reveals the functional core of multi-subunit replicative polymerases. EMBO J 28: 1978–87 [PubMed: 19494830]
43. Baranovskiy AG , Lada AG , Siebler HM , Zhang Y , Pavlov YI , Tahirov TH . 2012 DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta. J Biol Chem 287: 17281–7 [PubMed: 22465957]
44. Makarova AV , Stodola JL , Burgers PM . 2012 A four-subunit DNA polymerase zeta complex containing Pol delta accessory subunits is essential for PCNA-mediated mutagenesis. Nucleic Acids Res 40: 11618–26 [PubMed: 23066099]

45. Johnson RE , Prakash L , Prakash S . 2012 Pol31 and Pol32 subunits of yeast DNA polymerase delta are also essential subunits of DNA polymerase zeta. Proc Natl Acad Sci U S A 109: 12455–60 [PubMed: 22711820]
46. Lee YS , Gregory MT , Yang W . 2014 Human Pol zeta purified with accessory subunits is active in translesion DNA synthesis and complements Pol eta in cisplatin bypass. Proc Natl Acad Sci U S A 111: 2954–9 [PubMed: 24449906]
47. Yan J , Beattie TR , Rojas AL , Schermerhorn K , Gristwood T , et al. 2017 Identification and characterization of a heterotrimeric archaeal DNA polymerase holoenzyme. Nat Commun 8: 15075 [PubMed: 28462924]
48. Jiang J , Chan H , Cash DD , Miracco EJ , Ogorzalek Loo RR , et al. 2015 Structure of Tetrahymena telomerase reveals previously unknown subunits, functions, and interactions. Science 350: aab4070 [PubMed: 26472759]
49. Yeeles JT , Janska A , Early A , Diffley JF . 2017 How the Eukaryotic Replisome Achieves Rapid and Efficient DNA Replication. Mol Cell 65: 105–16 [PubMed: 27989442]
50. Ling H , Boudsocq F , Woodgate R , Yang W . 2001 Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. Cell 107: 91–102 [PubMed: 11595188]
51. Rechkoblit O , Gupta YK , Malik R , Rajashankar KR , Johnson RE , et al. 2016 Structure and mechanism of human PrimPol, a DNA polymerase with primase activity. Sci Adv 2: e1601317 [PubMed: 27819052]
52. Li GM . 2008 Mechanisms and functions of DNA mismatch repair. Cell Res 18: 85–98 [PubMed: 18157157]
53. Patel SS , Wong I , Johnson KA . 1991 Pre-steady-state kinetic analysis of processive DNA replication including complete characterization of an exonuclease-deficient mutant. Biochemistry 30: 511–25 [PubMed: 1846298]
54. Kati WM , Johnson KA , Jerva LF , Anderson KS . 1992 Mechanism and fidelity of HIV reverse transcriptase. J Biol Chem 267: 25988–97 [PubMed: 1281479]
55. Fiala KA , Suo Z . 2004 Mechanism of DNA polymerization catalyzed by Sulfolobus solfataricus P2 DNA polymerase IV. Biochemistry 43: 2116–25 [PubMed: 14967051]
56. Shah AM , Li SX , Anderson KS , Sweasy JB . 2001 Y265H mutator mutant of DNA polymerase beta. Proper teometric alignment is critical for fidelity. J Biol Chem 276: 10824–31 [PubMed: 11154692]
57. Showalter AK , Tsai MD . 2002 A reexamination of the nucleotide incorporation fidelity of DNA polymerases. Biochemistry 41: 10571–6 [PubMed: 12186540]
58. Rothwell PJ , Mitaksov V , Waksman G . 2005 Motions of the fingers subdomain of klentaq1 are fast and not rate limiting: implications for the molecular basis of fidelity in DNA polymerases. Mol Cell 19: 345–55 [PubMed: 16061181]
59. Zhang H , Cao W , Zakharova E , Konigsberg W , De La Cruz EM . 2007 Fluorescence of 2-aminopurine reveals rapid conformational changes in the RB69 DNA polymerase-primer/template complexes upon binding and incorporation of matched deoxynucleoside triphosphates. Nucleic Acids Res 35: 6052–62 [PubMed: 17766250]
60. Wong I , Patel SS , Johnson KA . 1991 An induced-fit kinetic mechanism for DNA replication fidelity: direct measurement by single-turnover kinetics. Biochemistry 30: 526–37 [PubMed: 1846299]
61. Pelletier H , Sawaya MR , Kumar A , Wilson SH , Kraut J . 1994 Structures of ternary complexes of rat DNA polymerase beta, a DNA template-primer, and ddCTP. Science 264: 1891–903 [PubMed: 7516580]
62. Doublie S , Sawaya MR , Ellenberger T . 1999 An open and closed case for all polymerases. Structure 7: R31–5 [PubMed: 10368292]
63. Gao Y , Yang W . 2016 Capture of a third Mg(2)(+) is essential for catalyzing DNA synthesis. Science 352: 1334–7 [PubMed: 27284197]
64. Fernandez-Leiro R , Conrad J , Yang JC , Freund SM , Scheres SH , Lamers MH . 2017 Self-correcting mismatches during high-fidelity DNA replication. Nat Struct Mol Biol 24: 140–3 [PubMed: 28067916]

65. Vandewiele D , Borden A , O'Grady PI , Woodgate R , Lawrence CW . 1998 Efficient translesion replication in the absence of Escherichia coli Umu proteins and 3'−5' exonuclease proofreading function. Proc Natl Acad Sci U S A 95: 15519–24 [PubMed: 9861001]
66. Johnson RE , Washington MT , Haracska L , Prakash S , Prakash L . 2000 Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. Nature 406: 1015–9 [PubMed: 10984059]
67. Livneh Z , Ziv O , Shachar S . 2010 Multiple two-polymerase mechanisms in mammalian translesion DNA synthesis. Cell Cycle 9: 729–35 [PubMed: 20139724]
68. Yang W , Lee YS . 2015 A DNA-hairpin model for repeat-addition processivity in telomere synthesis. Nat Struct Mol Biol 22: 844–7 [PubMed: 26581517]
69. Fiala KA , Suo Z . 2004 Pre-steady-state kinetic studies of the fidelity of Sulfolobus solfataricus P2 DNA polymerase IV. Biochemistry 43: 2106–15 [PubMed: 14967050]
70. Matsuda T , Bebenek K , Masutani C , Rogozin IB , Hanaoka F , Kunkel TA . 2001 Error rate and specificity of human and murine DNA polymerase eta. J Mol Biol 312: 335–46 [PubMed: 11554790]
71. Beard WA , Shock DD , Vande Berg BJ , Wilson SH . 2002 Efficiency of correct nucleotide insertion governs DNA polymerase fidelity. J Biol Chem 277: 47393–8 [PubMed: 12370169]
72. Biertumpfel C , Zhao Y , Kondo Y , Ramon-Maiques S , Gregory M , et al. 2010 Structure and mechanism of human DNA polymerase eta. Nature 465: 1044–8 [PubMed: 20577208]
73. Wilson RC , Pata JD . 2008 Structural insights into the generation of single-base deletions by the Y family DNA polymerase dbh. Mol Cell 29: 767–79 [PubMed: 18374650]
74. Lone S , Townson SA , Uljon SN , Johnson RE , Brahma A , et al. 2007 Human DNA polymerase kappa encircles DNA: implications for mismatch extension and lesion bypass. Mol Cell 25: 601–14 [PubMed: 17317631]
75. Bauer J , Xing G , Yagi H , Sayer JM , Jerina DM , Ling H . 2007 A structural gap in Dpo4 supports mutagenic bypass of a major benzo[a]pyrene dG adduct in DNA through template misalignment. Proc Natl Acad Sci U S A 104: 14905–10 [PubMed: 17848527]
76. Liu Y , Yang Y , Tang T-S , Zhang H , Wang Z , et al. 2014 Variants of Mouse DNA Polymerase κ Reveal a Mechanism of Efficient and Accurate Translesion Synthesis Past a Benzo[a]pyrene dG Adduct. Proc Natl Acad Sci U S A in press
77. Burgers PMJ , Kunkel TA . 2017 Eukaryotic DNA Replication Fork. Annu Rev Biochem 86: 417–38 [PubMed: 28301743]
78. Masutani C , Kusumoto R , Yamada A , Dohmae N , Yokoi M , et al. 1999 The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. Nature 399: 700–4 [PubMed: 10385124]
79. Johnson RE , Kondratick CM , Prakash S , Prakash L . 1999 hRAD30 mutations in the variant form of xeroderma pigmentosum. Science 285: 263–5 [PubMed: 10398605]
80. McCulloch SD , Kokoska RJ , Masutani C , Iwai S , Hanaoka F , Kunkel TA . 2004 Preferential cis-syn thymine dimer bypass by DNA polymerase eta occurs with biased fidelity. Nature 428: 97–100 [PubMed: 14999287]
81. Cadet J , Sage E , Douki T . 2005 Ultraviolet radiation-mediated damage to cellular DNA. Mutat Res 571: 3–17 [PubMed: 15748634]
82. Cleaver JE . 1972 Xeroderma pigmentosum: variants with normal DNA repair and normal sensitivity to ultraviolet light. J Invest Dermatol 58: 124–8 [PubMed: 5013606]
83. Lehmann AR , Kirk-Bell S , Arlett CF , Paterson MC , Lohman PH , et al. 1975 Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. Proc Natl Acad Sci U S A 72: 219–23 [PubMed: 1054497]
84. Zhao Y , Gregory MT , Biertumpfel C , Hua YJ , Hanaoka F , Yang W . 2013 Mechanism of somatic hypermutation at the WA motif by human DNA polymerase eta. Proc Natl Acad Sci U S A 110: 8146–51 [PubMed: 23630267]
85. Masutani C , Kusumoto R , Iwai S , Hanaoka F . 2000 Mechanisms of accurate translesion synthesis by human DNA polymerase h. EMBO J 19: 3100–9 [PubMed: 10856253]
86. Zhao Y , Biertumpfel C , Gregory MT , Hua YJ , Hanaoka F , Yang W . 2012 Structural basis of human DNA polymerase eta-mediated chemoresistance to cisplatin. Proc Natl Acad Sci U S A 109: 7269–74 [PubMed: 22529383]

87. Shachar S , Ziv O , Avkin S , Adar S , Wittschieben J , et al. 2009 Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. Embo J 28: 383–93 [PubMed: 19153606]

88. McDonald JP , Frank EG , Plosky BS , Rogozin IB , Masutani C , et al. 2003 129-derived strains of mice are deficient in DNA polymerase iota and have normal immunoglobulin hypermutation. J Exp Med 198: 635–43 [PubMed: 12925679]

89. Ohkumo T , Kondo Y , Yokoi M , Tsukamoto T , Yamada A , et al. 2006 UV-B radiation induces epithelial tumors in mice lacking DNA polymerase eta and mesenchymal tumors in mice deficient for DNA polymerase iota. Mol Cell Biol 26: 7696–706 [PubMed: 17015482]

90. Nair DT , Johnson RE , Prakash S , Prakash L , Aggarwal AK . 2004 Replication by human DNA polymerase-iota occurs by Hoogsteen base-pairing. Nature 430: 377–80 [PubMed: 15254543]

91. Kirouac KN , Ling H . 2009 Structural basis of error-prone replication and stalling at a thymine base by human DNA polymerase iota. EMBO J 28: 1644–54 [PubMed: 19440206]

92. Nair DT , Johnson RE , Prakash L , Prakash S , Aggarwal AK . 2006 An incoming nucleotide imposes an anti to syn conformational change on the templating purine in the human DNA polymerase-iota active site. Structure 14: 749–55 [PubMed: 16615915]

93. Jain R , Choudhury JR , Buku A , Johnson RE , Prakash L , et al. 2017 Mechanism of error-free DNA synthesis across N1-methyl-deoxyadenosine by human DNA polymerase-iota. Sci Rep 7: 43904 [PubMed: 28272441]

94. Kirouac KN , Ling H . 2011 Unique active site promotes error-free replication opposite an 8-oxo-guanine lesion by human DNA polymerase iota. Proc Natl Acad Sci U S A 108: 3210–5 [PubMed: 21300901]

95. Suzuki N , Ohashi E , Kolbanovskiy A , Geacintov NE , Grollman AP , et al. 2002 Translesion synthesis by human DNA polymerase kappa on a DNA template containing a single stereoisomer of dG-(+)- or dG-(−)-anti-N(2)-BPDE (7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene). Biochemistry 41: 6100–6 [PubMed: 11994005]

96. Rechkoblit O , Zhang Y , Guo D , Wang Z , Amin S , et al. 2002 trans-Lesion synthesis past bulky benzo[a]pyrene diol epoxide N2-dG and N6-dA lesions catalyzed by DNA bypass polymerases. J Biol Chem 277: 30488–94 [PubMed: 12063247]

97. Ogi T , Limsirichaikul S , Overmeer RM , Volker M , Takenaka K , et al. 2010 Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. Mol Cell 37: 714–27 [PubMed: 20227374]

98. Singer WD , Osimiri LC , Friedberg EC . 2013 Increased dietary cholesterol promotes enhanced mutagenesis in DNA polymerase kappa-deficient mice. DNA Repair (Amst) 12: 817–23 [PubMed: 23948094]

99. Stancel JN , McDaniel LD , Velasco S , Richardson J , Guo C , Friedberg EC . 2009 Polk mutant mice have a spontaneous mutator phenotype. DNA Repair (Amst) 8: 1355–62 [PubMed: 19783230]

100. Jarosz DF , Godoy VG , Delaney JC , Essigmann JM , Walker GC . 2006 A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. Nature 439: 225–8 [PubMed: 16407906]

101. Jha V , Bian C , Xing G , Ling H . 2016 Structure and mechanism of error-free replication past the major benzo[a]pyrene adduct by human DNA polymerase kappa. Nucleic Acids Res 44: 4957–67 [PubMed: 27034468]

102. Jha V , Ling H . 2017 Structural basis of accurate replication beyond a bulky major benzo[a]pyrene adduct by human DNA polymerase kappa. DNA Repair (Amst) 49: 43–50 [PubMed: 27894903]

103. Godoy VG , Jarosz DF , Simon SM , Abyzov A , Ilyin V , Walker GC . 2007 UmuD and RecA directly modulate the mutagenic potential of the Y family DNA polymerase DinB. Mol Cell 28: 1058–70 [PubMed: 18158902]

104. Nelson JR , Lawrence CW , Hinkle DC . 1996 Deoxycytidyl transferase activity of yeast REV1 protein. Nature 382: 729–31 [PubMed: 8751446]

105. Larimer FW , Perry JR , Hardigree AA . 1989 The REV1 gene of Saccharomyces cerevisiae: isolation, sequence, and functional analysis. J Bacteriol 171: 230–7 [PubMed: 2492497]

106. Kim N , Mudrak SV , Jinks-Robertson S . 2011 The dCMP transferase activity of yeast Rev1 is biologically relevant during the bypass of endogenously generated AP sites. DNA Repair (Amst) 10: 1262–71 [PubMed: 22024240]

107. Jansen JG , Langerak P , Tsaalbi-Shtylik A , van den Berk P , Jacobs H , de Wind N . 2006 Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. J Exp Med 203: 319–23 [PubMed: 16476771]

108. Lawrence CW , Christensen RB . 1978 Ultraviolet-induced reversion of cyc1 alleles in radiation-sensitive strains of yeast. I. rev1 Mutant strains. J Mol Biol 122: 1–21 [PubMed: 209193]

109. Nair DT , Johnson RE , Prakash L , Prakash S , Aggarwal AK . 2005 Rev1 employs a novel mechanism of DNA synthesis using a protein template. Science 309: 2219–22 [PubMed: 16195463]

110. Swan MK , Johnson RE , Prakash L , Prakash S , Aggarwal AK . 2009 Structure of the human Rev1-DNA-dNTP ternary complex. J Mol Biol 390: 699–709 [PubMed: 19464298]

111. Nelson JR , Lawrence CW , Hinkle DC . 1996 Thymine-thymine dimer bypass by yeast DNA polymerase zeta. Science 272: 1646–9 [PubMed: 8658138]

112. Gibbs PE , McGregor WG , Maher VM , Nisson P , Lawrence CW . 1998 A human homolog of the Saccharomyces cerevisiae REV3 gene, which encodes the catalytic subunit of DNA polymerase zeta. Proc Natl Acad Sci U S A 95: 6876–80 [PubMed: 9618506]

113. Gan GN , Wittschieben JP , Wittschieben BO , Wood RD . 2008 DNA polymerase zeta (pol zeta) in higher eukaryotes. Cell Res 18: 174–83 [PubMed: 18157155]

114. Bonner CA , Hays S , McEntee K , Goodman MF . 1990 DNA polymerase II is encoded by the DNA damage-inducible dinA gene of Escherichia coli. Proc Natl Acad Sci U S A 87: 7663–7 [PubMed: 2217198]

115. Iwasaki H , Ishino Y , Toh H , Nakata A , Shinagawa H . 1991 Escherichia coli DNA polymerase II is homologous to alpha-like DNA polymerases. Mol Gen Genet 226: 24–33 [PubMed: 2034216]

116. Yeiser B , Pepper ED , Goodman MF , Finkel SE . 2002 SOS-induced DNA polymerases enhance long-term survival and evolutionary fitness. Proc Natl Acad Sci U S A 99: 8737–41 [PubMed: 12060704]

117. Napolitano R , Janel-Bintz R , Wagner J , Fuchs RP . 2000 All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. Embo J 19: 6259–65 [PubMed: 11080171]

118. Becherel OJ , Fuchs RP . 2001 Mechanism of DNA polymerase II-mediated frameshift mutagenesis. Proc Natl Acad Sci U S A 98: 8566–71 [PubMed: 11447256]

119. Beagan K , Armstrong RL , Witsell A , Roy U , Renedo N , et al. 2017 Drosophila DNA polymerase theta utilizes both helicase-like and polymerase domains during microhomology-mediated end joining and interstrand crosslink repair. PLoS Genet 13: e1006813 [PubMed: 28542210]

120. Shima N , Hartford SA , Duffy T , Wilson LA , Schimenti KJ , Schimenti JC . 2003 Phenotype-based identification of mouse chromosome instability mutants. Genetics 163: 1031–40 [PubMed: 12663541]

121. Yousefzadeh MJ , Wyatt DW , Takata K , Mu Y , Hensley SC , et al. 2014 Mechanism of suppression of chromosomal instability by DNA polymerase POLQ. PLoS Genet 10: e1004654 [PubMed: 25275444]

122. Chan SH , Yu AM , McVey M . 2010 Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in Drosophila. PLoS Genet 6: e1001005 [PubMed: 20617203]

123. Mateos-Gomez PA , Gong F , Nair N , Miller KM , Lazzerini-Denchi E , Sfeir A . 2015 Mammalian polymerase theta promotes alternative NHEJ and suppresses recombination. Nature 518: 254–7 [PubMed: 25642960]

124. Ceccaldi R , Liu JC , Amunugama R , Hajdu I , Primack B , et al. 2015 Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. Nature 518: 258–62 [PubMed: 25642963]

125. Zelensky AN , Schimmel J , Kool H , Kanaar R , Tijsterman M . 2017 Inactivation of Pol theta and C-NHEJ eliminates off-target integration of exogenous DNA. Nat Commun 8: 66 [PubMed: 28687761]

126. Saito S , Maeda R , Adachi N . 2017 Dual loss of human POLQ and LIG4 abolishes random integration. Nat Commun 8: 16112 [PubMed: 28695890]

127. Zahn KE , Averill AM , Aller P , Wood RD , Doublie S . 2015 Human DNA polymerase theta grasps the primer terminus to mediate DNA repair. Nat Struct Mol Biol 22: 304–11 [PubMed: 25775267]

128. Bollum FJ , Groeniger E , Yoneda M . 1964 Polydeoxyadenylic Acid. Proc Natl Acad Sci U S A 51: 853–9 [PubMed: 14173002]

129. Foa R , Casorati G , Giubellino MC , Basso G , Schiro R , et al. 1987 Rearrangements of immunoglobulin and T cell receptor beta and gamma genes are associated with terminal deoxynucleotidyl transferase expression in acute myeloid leukemia. J Exp Med 165: 879–90 [PubMed: 3102679]

130. Tanabe K , Bohn EW , Wilson SH . 1979 Steady-state kinetics of mouse DNA polymerase beta. Biochemistry 18: 3401–6 [PubMed: 465481]

131. Uchiyama Y , Takeuchi R , Kodera H , Sakaguchi K . 2009 Distribution and roles of X-family DNA polymerases in eukaryotes. Biochimie 91: 165–70 [PubMed: 18706967]

132. Lecointe F , Shevelev IV , Bailone A , Sommer S , Hübscher U . 2004 Involvement of an X family DNA polymerase in double-stranded break repair in the radioresistant organism Deinococcus radiodurans. Mol Microbiol 53: 1721–30 [PubMed: 15341650]

133. Leulliot N , Cladiere L , Lecointe F , Durand D , Hübscher U , van Tilbeurgh H . 2009 The family X DNA polymerase from Deinococcus radiodurans adopts a non-standard extended conformation. J Biol Chem 284: 11992–9 [PubMed: 19251692]

134. Beard WA , Wilson SH . 2000 Structural design of a eukaryotic DNA repair polymerase: DNA polymerase beta. Mutat Res 460: 231–44 [PubMed: 10946231]

135. Ruiz JF , Dominguez O , Lain de Lera T , Garcia-Diaz M , Bernad A , Blanco L . 2001 DNA polymerase mu, a candidate hypermutase? Philos Trans R Soc Lond B Biol Sci 356: 99–109 [PubMed: 11205337]

136. Bertocci B , De Smet A , Berek C , Weill JC , Reynaud CA . 2003 Immunoglobulin kappa light chain gene rearrangement is impaired in mice deficient for DNA polymerase mu. Immunity 19: 203–11 [PubMed: 12932354]

137. Bebenek K , Pedersen LC , Kunkel TA . 2014 Structure-function studies of DNA polymerase lambda. Biochemistry 53: 2781–92 [PubMed: 24716527]

138. Moon AF , Pryor JM , Ramsden DA , Kunkel TA , Bebenek K , Pedersen LC . 2014 Sustained active site rigidity during synthesis by human DNA polymerase mu. Nat Struct Mol Biol 21: 253–60 [PubMed: 24487959]

139. Gouge J , Rosario S , Romain F , Poitevin F , Beguin P , Delarue M . 2015 Structural basis for a novel mechanism of DNA bridging and alignment in eukaryotic DSB DNA repair. EMBO J 34: 1126–42 [PubMed: 25762590]

140. Pryor JM , Waters CA , Aza A , Asagoshi K , Strom C , et al. 2015 Essential role for polymerase specialization in cellular nonhomologous end joining. Proc Natl Acad Sci U S A 112: E4537–45 [PubMed: 26240371]

141. Moon AF , Gosavi RA , Kunkel TA , Pedersen LC , Bebenek K . 2015 Creative template-dependent synthesis by human polymerase mu. Proc Natl Acad Sci U S A 112: E4530–6 [PubMed: 26240373]

142. Boule JB , Rougeon F , Papanicolaou C . 2001 Terminal deoxynucleotidyl transferase indiscriminately incorporates ribonucleotides and deoxyribonucleotides. J Biol Chem 276: 31388–93 [PubMed: 11406636]

143. Nick McElhinny SA , Ramsden DA . 2003 Polymerase mu is a DNA-directed DNA/RNA polymerase. Mol Cell Biol 23: 2309–15 [PubMed: 12640116]

144. Ruiz JF , Juarez R , Garcia-Diaz M , Terrados G , Picher AJ , et al. 2003 Lack of sugar discrimination by human Pol mu requires a single glycine residue. Nucleic Acids Res 31: 4441–9 [PubMed: 12888504]

145. Masuda Y , Kanao R , Kaji K , Ohmori H , Hanaoka F , Masutani C . 2015 Different types of interaction between PCNA and PIP boxes contribute to distinct cellular functions of Y-family DNA polymerases. Nucleic Acids Res 43: 7898–910 [PubMed: 26170230]

146. Choe KN , Moldovan GL . 2017 Forging Ahead through Darkness: PCNA, Still the Principal Conductor at the Replication Fork. Mol Cell 65: 380–92 [PubMed: 28157503]

147. Hedglin M , Pandey B , Benkovic SJ . 2016 Characterization of human translesion DNA synthesis across a UV-induced DNA lesion. Elife 5

148. Wimmer U , Ferrari E , Hunziker P , Hübscher U . 2008 Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. EMBO Rep 9: 1027–33 [PubMed: 18688254]

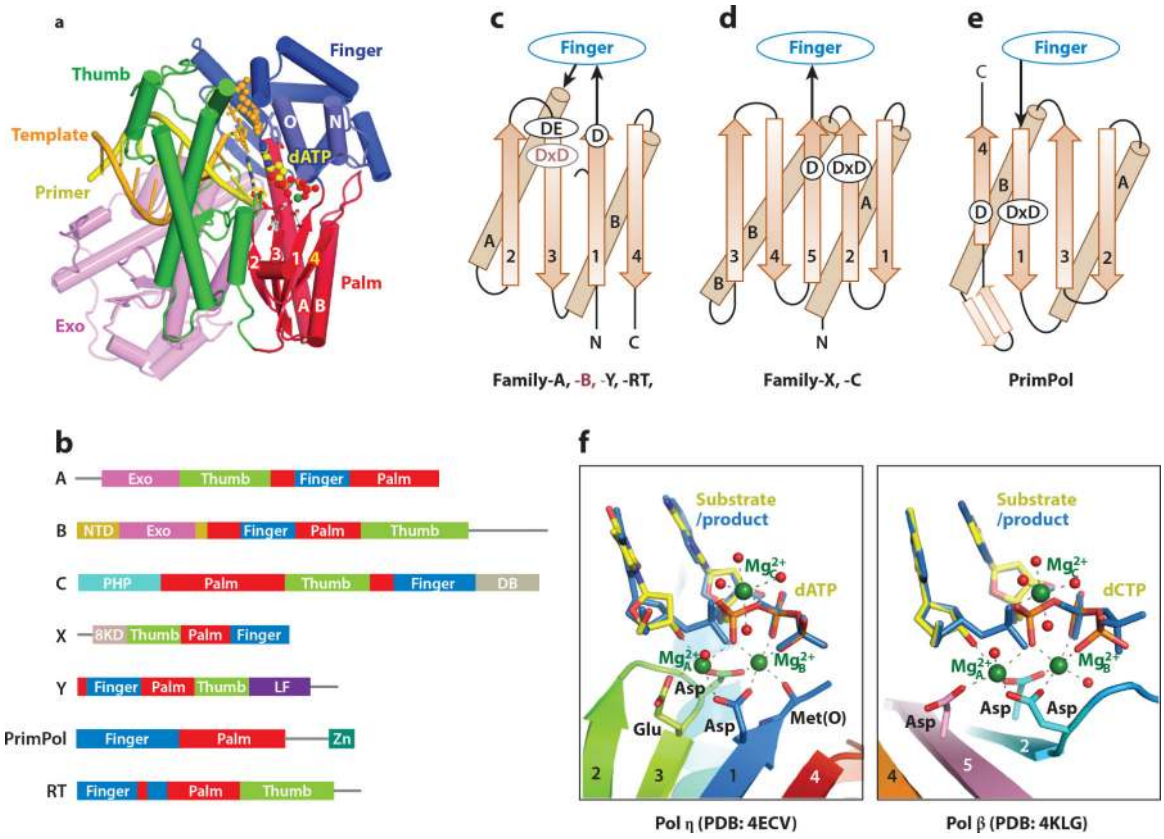

Figure 1.

Architecture of DNA polymerases. (a) A DNA polymerase is composed of thumb (green), finger (blue) and palm domain (red). Replicative polymerases often include an intrinsic proofreading 3′–5′ exonuclease (light purple). The ternary complex of an A-family DNA polymerase (PDB: 1LV5) is shown as an example. (b) Primary structures of seven DNA polymerase families. (c-e) Topology diagrams of the palm domain in A, B, Y, and RT families, C and X families, and PrimPol. (f) Side-by-side view of the catalytic centers of Pol η and Pol β.

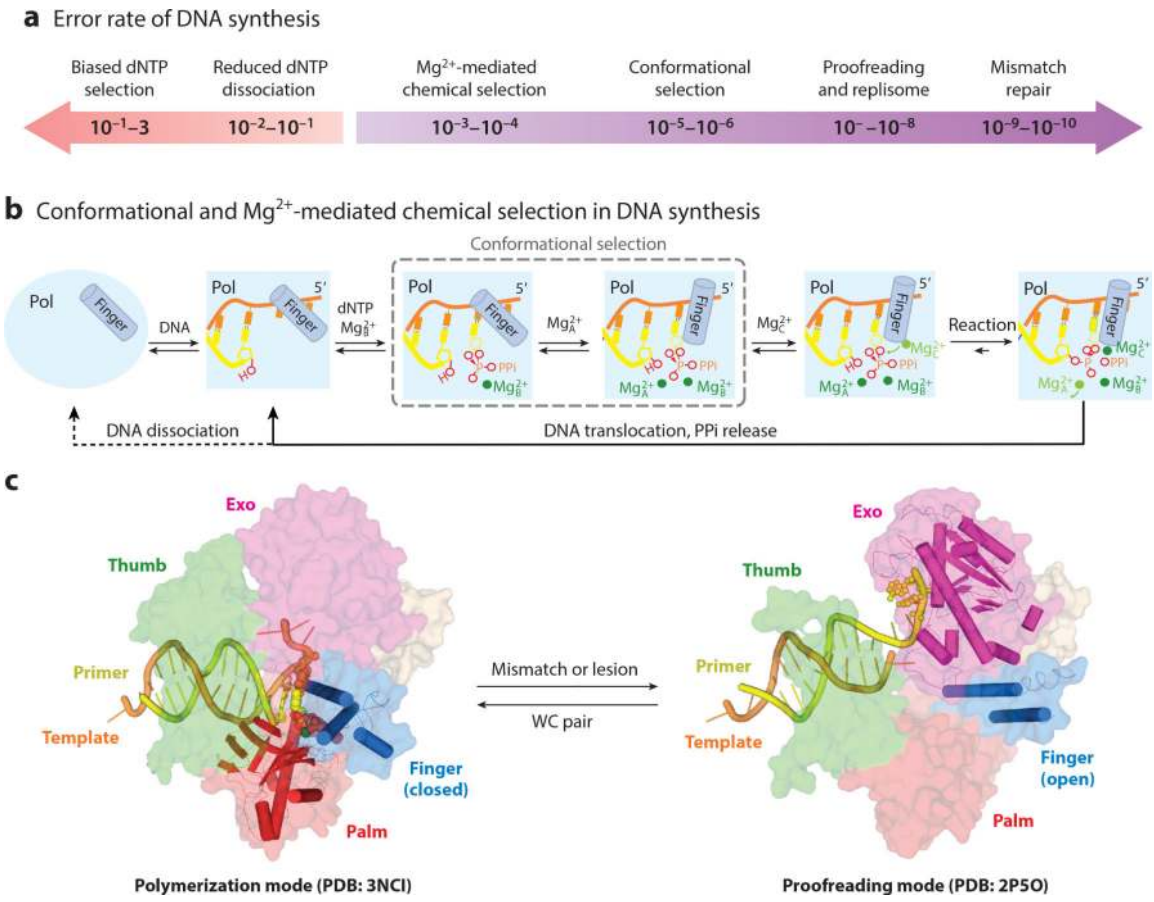

Figure 2.

Basis for DNA polymerase fidelity. (a) Determinants of the error rates of DNA polymerases. (b) Conformational and Mg^{2+}-mediated chemical selection in the DNA synthesis reaction. The requirement for three Mg^{2+} ions is universal, and conformational selection (boxed) occurs in replicative and a few repair polymerases. $Mg^{2+}{}_A$ binding and conformational change can occur without one another in replicative polymerases. (c) In the DNA synthesis (finger-closed) mode, a replicative polymerase (B-family RB69 as an example, PDB: 3NCI) interacts with the DNA and nascent base pair seamlessly. In the proofreading mode (PDB: 2P5O), DNA is detached from the open finger and disordered thumb, and primer strand is partially separated from the template and migrates to the Exo active site for cleavage.

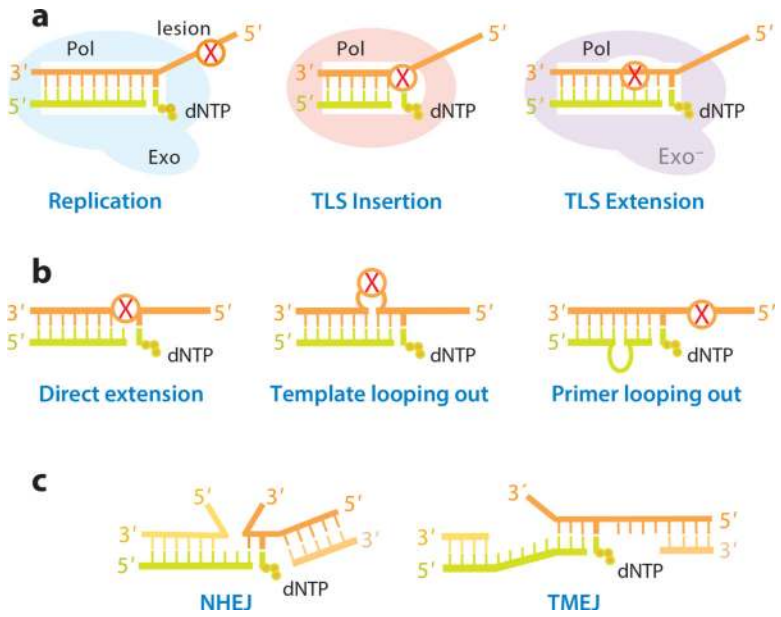

Figure 3.

Mechanisms for DNA translesion and repair synthesis. (a) Diagram of insertion and extension during TLS. (b) Three possible ways to achieve TLS extension. (c) Discontinuous strands of broken DNA can be accommodated similarly to looping out.

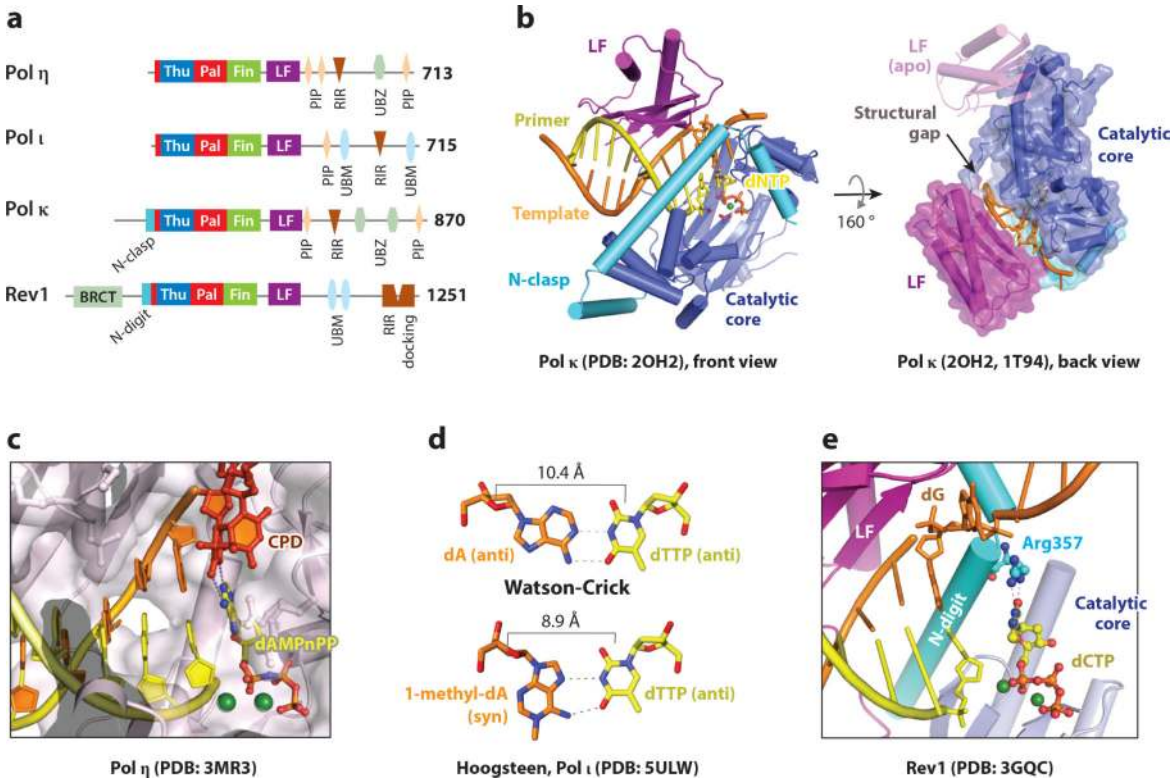

Figure 4.

Mechanism of TLS insertion by Y-family polymerases. (a) Primary structures of human Y-family polymerases. (b) Crystal structures of Pol κ. In the ternary complex (PDB: 2OH2), the N-clasp stabilizes the LF and the structural gap between the catalytic core and LF to accommodate a bulky adduct. Without DNA the LF is flexible (PDB: 1T94). (c) Crystal structures of Pol η accommodating a thymine dimer in the active site (PDB: 3MR3). (d) The Pol ι active site favors a narrow Hoogsteen basepair (PDB: 5ULW) and not WC pair. (e) Crystal structure of Rev1, which utilizes an Arg sidechain to select dCTP for incorporation (PDB: 3GQC). The N-digit is inserted between the LF and catalytic core.

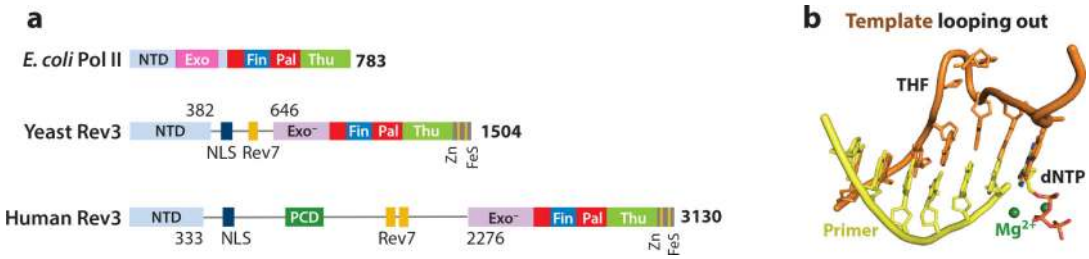

Figure 5.

TLS extension by Pol ζ and *E. coli* Pol II. (a) Primary structures of *E. coli* Pol II, yeast and human Rev3. (b) Two nucleotides including an AP site (THF) on the template strand are looped out in complex with *E. coli* Pol II (PDB: 3K5M).

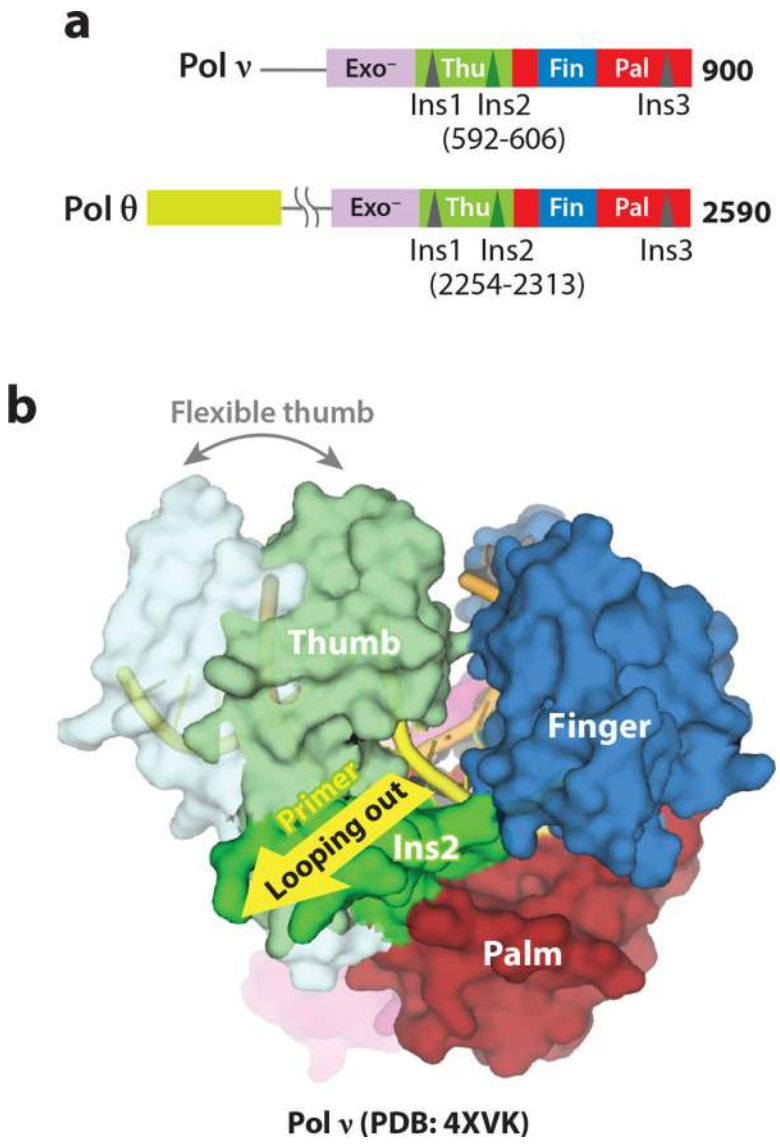

Figure 6.

TLS and end joining by Pol ν and θ. (a) Primary structures of human Pol ν and θ. (b) Ins 2 in Pol ν results in the cavity and a flexible thumb for primer looping out (PDB:4XVK, 4XVM).

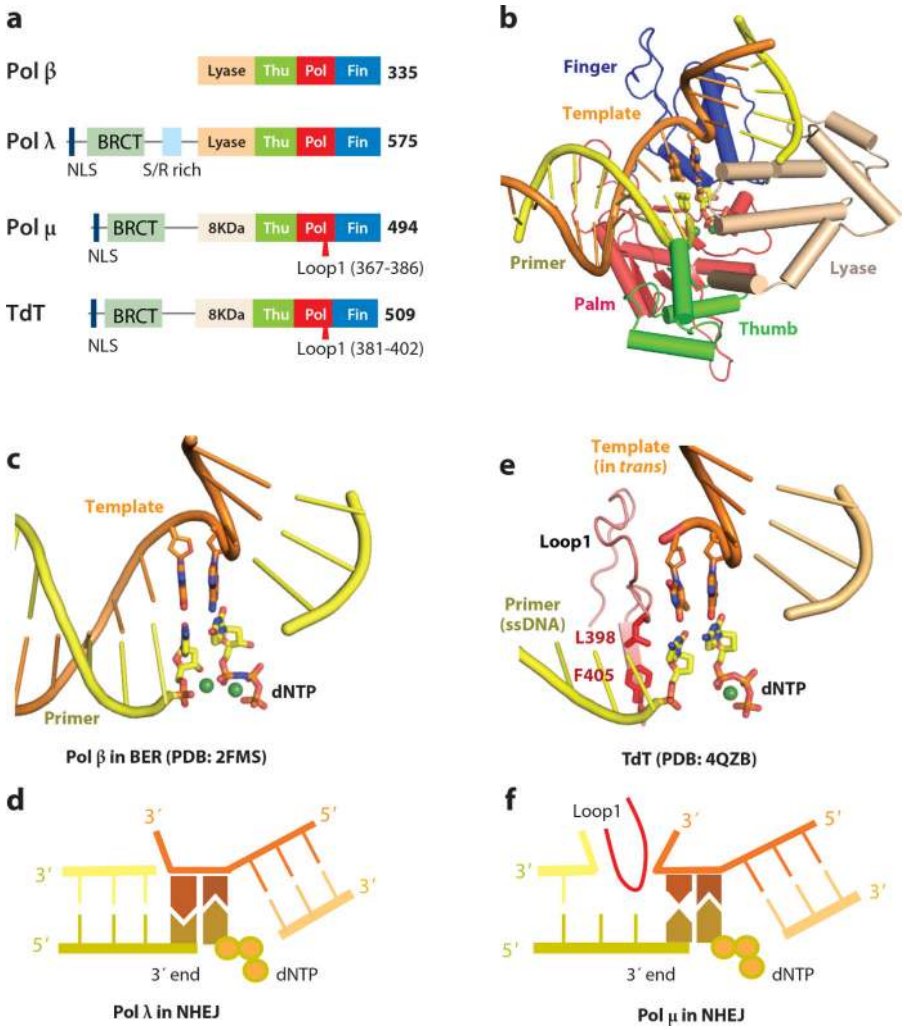

Figure 7.

sGRS by the X-family polymerases. (a) Primary structures of Pol β, λ, μ and TdT. (b) Crystal structure of the Pol β-DNA-dNTP complex (PDB: 2FMS). Domains are labeled according to the right-hand convention. In the left-hand definition, finger and thumb would be swapped. (c) Gap filling synthesis and dRP removal by Pol β. The two DNA segments are at ~90° angle. (d) Pol λ can bridge two DNA ends by one base pair and incorporate a dNTP to match the trans template. (e) Structure of TdT bridging two DNA ends without base pairing (PDB: 4QZB). (f) Pol μ can bridge two DNA ends by a mismatched base pair and incorporate a dNTP that matches the trans template. Loop1 insertion in TdT and Pol μ segregates the two base pairs in the active site from upstream DNA.

Table 1.

DNA polymerase families

Family	Function	3′–5′ Exo	Conf. selection	Error rate[1]	Examples			
					Bacterial	Archaeal	Humans (function)	PDB[2]
A	Replication	Yes	Yes	$10^{-5} – 10^{-7}$	Pol I, T7 Pol		Pol γ (mitochondrial)	5C53
	Repair	No	Yes	$10^{-3} – 10^{-4}$			Pol θ (TMEJ) Pol ν (end processing)	4X0Q 4XVK, 4XVM
B	Replication	Yes	Yes	$10^{-4} – 10^{-7}$	RB69 Pol ϕ29 Pol	PolB	Pol α (primer extension) Pol δ (lagging strand & large gap filling, lGRS) Pol ε (leading strand)	4QCL, 5EXR 3IAY 4M8O, 4PTF
	TLS extension	Yes/No	Yes	$10^{-3} – 10^{-6}$	Pol II		Pol ζ (TLS extension)	3K5O, 3K5M
C	Replication	Yes	Yes	$10^{-5} – 10^{-7}$	Pol III, PolC			3F2C, 5M1S
	Repair			$10^{-2} – 10^{-3}$	S. pyo DnaE			
D	Replication	Yes		$10^{-4} – 10^{-5}$		Pol D		
X	BER BER/sGRS sGRS sGRS	No	Yes No No No	$10^{-2} – 10^{-4}$	D. rad Pol X	Pol X	Pol β (BER, sGRS) Pol λ (BER, NHEJ) Pol μ (NHEJ) TdT (NHEJ)	2FMS, 4KLH 2PFO 4M04 4QZ9(B)
Y	TLS insertion	No	No No No No	$10^{-2} – 10^{-4}$ $3 – 10^{-4}$ $10^{-3} – 10^{-4}$ —	Pol IV (DinB) Pol V (UmuC)	Dpo4, Dbh	Pol η (CPD, SHM) Pol ι Pol κ (steroid, BPDE) Rev1 (AP, SHM)	3MR3, 4ECV 5ULW 1T94, 2OH2 3GQC
RT	Replication	No	Yes	$10^{-3} – 10^{-4}$			HIV-1 RT TERT (telomerase)	3KK2 3KYL
PrimPol	Repair	No	Yes	$10^{-2} – 10^{-4}$	PolDom	PolDom	PrimPol	5L2X

[1] Error rates are measured without accessary components of replisome.

[2] Representative structures for each DNA polymerase family are listed.