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DNA polymerases: Mechanistic insight from biochemical and biophysical studies

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1. ABSTRACT

In vivo the DNA polymerases are responsible for replicative and repair DNA synthesis. These enzymes use the pre-existing 3'-OH group of a primer annealed to a single-stranded DNA template to incorporate monophosphate deoxynucleosides (dNMPs) in a sequential and directional manner. Although all DNA polymerases share a similar catalytic core constituted by a palm, a thumb and a fingers domain and a similar chemical mechanism of dNMP incorporation that requires two metal cations, they intrinsically differ by the nature of the step that controls the incorporation of dNMP and by their capacity to cope with lesions. Several factors, such as the size of the active site, the flexibility of the DNA in the active site or the presence of protein subdomains devoid of known catalytic activity but able to accommodate small DNA loops, control the fidelity of DNA polymerases. Auxiliary replication factors, such as the processivity factor or the single-stranded DNA binding protein, can also modulate the intrinsic properties of DNA polymerases and therefore fine-tune the cellular function of DNA polymerases.

Table 1. Properties of template-dependent DNA polymerases from *E. coli* and Mammals

DNA polymerase	Family	Gene name	Function	Additional activities	Mutation rate
in *Escherichia coli*					
I	A	polA	Maturation of Okazaki fragment Nucleotide excision repair	5'->3' exonuclease 3'->5' exonuclease	10^{-5}-10^{-6}
II	B	polB	Translesion synthesis Replication restart	3'->5' exonuclease	10^{-5}-10^{-6} -2 frameshifts
III	C	dnaE	Replication	3'->5' exonuclease (carried by the epsilon subunit)	10^{-5}-10^{-6}
IV	Y	dinB	Translesion synthesis	None	10^{-3}-10^{-4} -1 frameshifts
V	Y	umuDC	Translesion synthesis	None	10^{-3}-10^{-4} T to C transitions
In Mammals					
alpha-primase	B	POLA	Priming	RNA synthesis	10^{-4}-10^{-5}
beta	X	POLB	Base excision repair Translesion synthesis Double-strand break repair	dRP/AP lyase terminal transferase	10^{-4}-10^{-5}
gamma	A	POLG	Mitochondrial maintenance	3'->5' exonuclease dRP lyase	10^{-5}-10^{-6}
delta	B	POLD1	Replication Nucleotide excision repair Base excision repair	3'->5' exonuclease	10^{-6}-10^{-7}
epsilon	B	POLE1	Replication	3'->5' exonuclease	10^{-6}-10^{-7}
zeta	B	REV3L	Translesion synthesis	None	10^{-4}-10^{-5}
eta	Y	POLH	Translesion synthesis	None	10^{-2}-10^{-3}
theta	A	POLQ	Translesion synthesis Base excision repair	DNA dependent ATPase/dRP lyase	10^{-2}-10^{-3}
iota	Y	POLI	Translesion synthesis Base excision repair	dRP lyase	10^{-1}
kappa	Y	POLK	Translesion synthesis Nucleotide excision repair	None	10^{-2}-10^{-3}
lambda	X	POLL	Non homologous end joining Base excision repair V(D)J recombination	dRP lyase Terminal transferase Polynucleotide synthase	10^{-4}-10^{-5}
mu	X	POLM	Non homologous end joining V(D)J recombination	Terminal transferase	10^{-4}-10^{-5}
nu	A	POLN	Translesion synthesis	None	10^{-3}
REV1	Y	REV1	Translesion synthesis	dCTP transferase	

Adapted with permission from (273, 274)

2. MUTLIPLE DNA POLYMERASES WITH SPECIFIC FUNCTIONS

In 1956, Arthur Kornberg and his colleagues identified and partially purified from crude extracts of *Escherichia coli* an enzyme, subsequently named DNA polymerase I, that could catalyze the incorporation of deoxyribonucleotides into DNA *in vitro* (1). Following this initial discovery and over the past five decades, a plethora of DNA polymerases with specific functions was isolated, and it is now well recognized that more than a dozen different DNA polymerases can be expressed in mammalian cells (Table 1). Unicellular organisms such as *E. coli* possess fewer DNA polymerases than multicellular organisms. Based on primary sequence (2-4) and 3D structure (5, 6) comparisons, DNA polymerases have been classified into 6 families (A, B, C, D, X, and Y; Table 1) excluding reverse transcriptases. Members of the C family are restricted to Eubacteria, whereas all D family members belong to Archea. Even though all isolated DNA polymerases catalyze the same chemical reaction using a similar kinetic pathway, their intrinsic fidelity (Table 1), speed, and processivity have tuned them to a specific cell function. For example, replicative DNA polymerases that belong to the A, B or C families are very accurate due to a high capacity to discriminate between very similar substrates, and to an intrinsic or associated 3'->5' exonuclease proofreading activity. In contrast members of the Y and some of the A (*e.g.* DNA polymerase nu or theta) and B (*e.g.* DNA polymerase II or zeta) families of DNA polymerases, often referred to as "specialized" DNA polymerases, can replicate over DNA lesions with reduced fidelity *via* a process called translesion DNA synthesis (TLS). The low fidelity of DNA polymerases of the Y family leads to the generation of an enormous repertoire of antibodies with different affinities for antigens, *via* a process called somatic hypermutation (SHM) (7). This feature specific of the Y family of DNA polymerases however, seems to be at the root of the acquired resistance of tumors, thus limiting the success of cancer therapy (8).

DNA polymerases of the Y family come into play after a lesion has induced blockage of DNA replication. Interestingly, recent studies suggest that DNA polymerases of the Y family, such as the *E. coli* DNA polymerase IV, can also be recruited at a stalled transcription elongation complex (9, 10). The mechanism by which a specialized DNA polymerase is recruited at a stalled DNA or RNA polymerase is not fully understood. In the case of a stalled DNA replication fork, a model based on competition among DNA polymerases has been proposed for *E. coli* (11). Recent studies have pointed out the essential role of the processivity factor in the DNA polymerase exchange process as this protein is proposed to serve as a molecular tool belt (for reviews see (12, 13)). In addition in Eukaryotes, post-translational modification of the processivity factor, the proliferating cell nuclear antigen (PCNA) - a topic not discussed in this review - might represent a key event in the process of coordinating DNA polymerase activities at the fork. The recruitment of an inappropriate DNA polymerase at the fork may result in mutations and contribute to pathologies such as cancers or the variant form of *Xeroderma pigmentosum* (XP-V) (14-17). Most of the DNA polymerases of the X family have no detectable proofreading activity and their fidelity is intermediate between replicative DNA polymerases and the Y family of DNA polymerases. These DNA polymerases are involved in (i) base excision repair (*e.g.* DNA polymerase beta), (ii) non homologous end joining (*e.g.* DNA polymerases lambda and mu), or (iii) V(D)J recombination (*e.g.* DNA polymerases lambda and mu), a recombination mechanism that allows the rearrangement of the variable (V), diversity (D) and joining (J) segments of the antigen receptor gene and, together with SHM, leads to the generation of a stock of antigen receptors with different specificities. It is interesting that among the DNA polymerases of the Y family, Rev1 DNA polymerase displays a highly specific activity. Although structurally similar to other Y family of DNA polymerases (18, 19), Rev1 is indeed a G template-specific DNA polymerase with a specific dCTP transferase activity (20, 21).

In addition to the 5'->3' DNA polymerization activity that allows incorporation of dNMPs into a growing polynucleotide chain, DNA polymerases can carry additional enzymatic activities (Table 1). As mentioned above, the 3'->5' exonuclease activity associated with replicative DNA polymerases and initially discovered by the *in vitro* characterization of an antimutator mutant of the T4 DNA polymerase (22), removes misincorporated dNMPs, thus increasing DNA polymerase fidelity. The DNA polymerase alpha-primase by combining a DNA and an RNA synthesis activity initiates leading and lagging strand DNA syntheses in Eukaryotes. *E. coli* DNA polymerase I possesses a 5'->3' exonuclease activity that is essential to remove the RNA primer that starts each Okazaki fragment. The DNA deoxyribophosphodiesterase (dRP lyase) activity excises the 2-deoxyribose-5'-phosphate residue generated during the initial steps of base excision repair (BER) and allows the DNA polymerases beta, theta or lambda to perform the post-incision events associated with BER. The TLS DNA polymerase iota also possesses a dRP lyase activity (23). *In vivo* (24) and *in vitro* (23, 25) studies suggest the participation of this DNA polymerase in BER. DNA polymerase mu of the X family has a template-directed DNA synthesis activity and a terminal transferase (template-independent) activity. It has been proposed that the terminal transferase activity of DNA polymerase mu is specifically required during non homologous end joining (NHEJ) to create or increase complementarity of DNA ends. Recent data suggest that the regulation of the DNA polymerase and the terminal transferase activities of DNA polymerase mu by Arg 387 prevents excessive mutagenic events during NHEJ (26).

3. NUCLEOTIDE INCORPORATION REACTION OF REPLICATIVE AND REPAIR DNA POLYMERASE

3.1. Minimal scheme for dNMP incorporation

The basic chemical reaction performed by a DNA polymerase is the incorporation of a dNMP onto the 3' end of a DNA or RNA primer annealed to a template DNA strand. This template-directed 5'->3' DNA synthesis reaction can be divided into several steps (Figure 1). First, the DNA polymerase must bind its substrate, either a primer-template (p-t) junction for replicative and TLS DNA polymerases, or a gapped DNA for repair DNA polymerases involved in BER or nucleotide excision repair (NER). This binary complex (E-DNA) selects the correct dNTP, primarily according to the Watson-Crick base pairing rules. Within the ternary complex (E-DNA-dNTP) catalysis can take place with the nucleophilic attack by the 3'-OH primer terminus on the alpha-phosphate of the selected dNTP. This step that corresponds to the formation of a new phosphodiester bond is designated the chemistry step in what follows, and leads to the incorporation of dNMP into the elongating DNA chain. Inorganic pyrophosphate (PPi) is next released and the DNA polymerase can translocate by one nucleotide along the template strand to position the 3' end of the DNA chain to be extended within its active site. The new binary complex with a DNA chain extended by one residue can either dissociate from the substrate (distributive DNA synthesis) or be incorporated into a new catalytic cycle (processive DNA synthesis).

The nucleotidyl transfer reaction catalyzed by the DNA polymerases requires two divalent metal cations (such as Mg^{2+} that is probably the physiological relevant cation although Mn^{2+} might be used by enzymes with terminal transferase activity) located in the A and B sites of the DNA polymerase, and carboxylate ligands from two or three aspartyl side chains that interact with both divalent cations. The function of the metal ion in the A site is to lower the pKa of the 3'-OH terminal group of the primer, thus generating an oxyanion than can attack the alpha-phosphorus atom of the incoming dNTP. The metal ion located in the B site ligates the oxygen atoms in the triphosphate tail of the dNTP. It stabilizes the developing negative charge in the pentavalent transition state and assists the departure of PPi (27, 28). In the recent crystal structure of Pol3 the catalytic subunit of yeast DNA polymerase delta, interacting with a p-t junction and an incoming dNTP, a third metal ion is observed, coordinated by the gamma-phosphate of the complementary incoming dNTP and three carboxylates from acidic residues (Asp 608, Glu 800 and Glu 802) (29). Mutagenesis studies performed on Glu 800 and 802 suggest that this putative third metal binding site modulates Pol3 catalytic activity. In addition to the conserved aspartic acids that chelate the divalent cations, site directed mutagenesis studies identified additional residues important for catalysis (30-35), such as those that interact with the beta- and gamma- phosphate of the selected dNTP and permit proper orientation of the alpha-phosphate for the reaction (36). As discussed below, kinetic and structural characterizations made it possible to refine this minimal scheme of dNMP incorporation and to

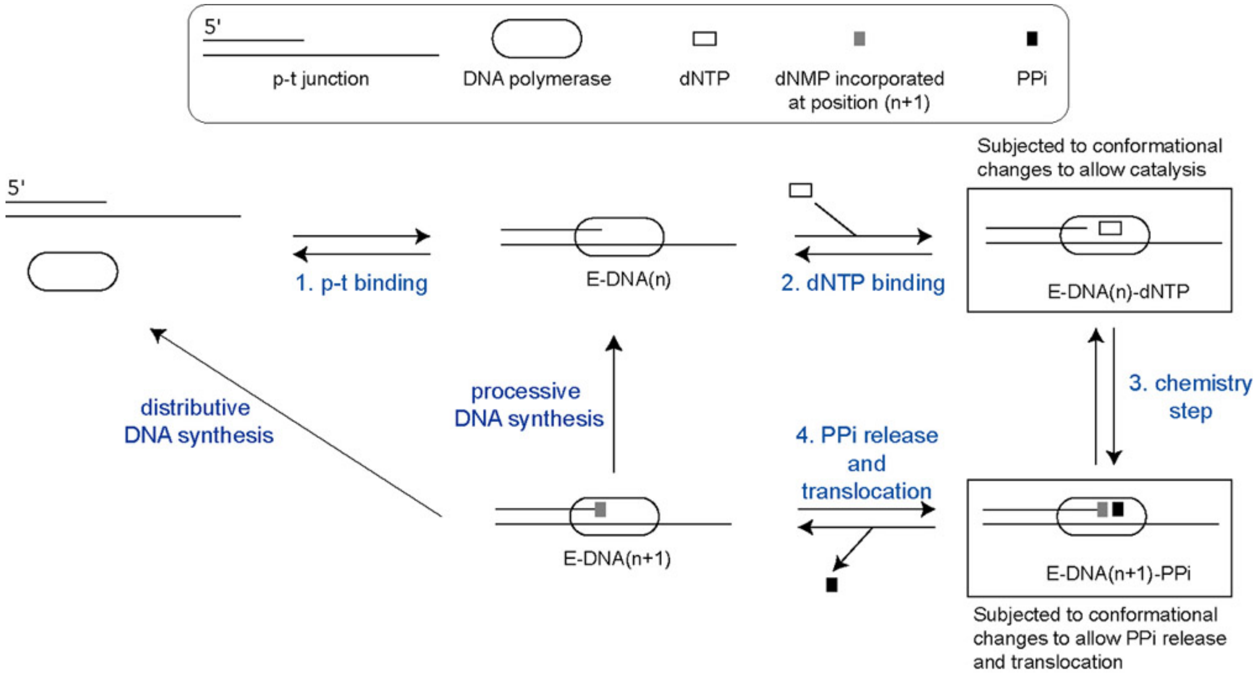


Figure 1. Sequential steps of a nucleotide incorporation cycle. The incorporation of a nucleotide into the 3' end of a DNA chain includes four steps: 1. binding of the DNA polymerase to the DNA substrate (*e.g.* a primer-template (p-t) junction as shown here); 2. binding of the complementary dNTP; 3. chemistry step; 4. inorganic pyrophosphate (PPi) release and translocation. After one cycle of dNMP incorporation, the DNA polymerase can either dissociate from the p-t junction extended by one nucleotide (distributive DNA synthesis) or be incorporated at step 2 of the reaction cycle (processive DNA synthesis). DNA(n) and DNA(n+1) signify that the primer of the p-t junction is (n) or (n+1) nucleotides long. E-DNA(n)-dNTP and E-DNA(n+1)-PPi are subjected to conformational changes (see text for details).

introduce at least two conformational changes taking place before and after the chemistry step whose molecular nature still needs to be defined.

3.2. Overall architecture of unliganded and liganded DNA polymerases

The overall shape of the polymerase domain of DNA polymerases, as revealed by X-ray structures, consists of a right hand with subdomains referred to as fingers, palm and thumb. The active site within the palm subdomain carries catalytically essential amino acids, including the highly conserved aspartic acids that coordinate divalent cations. The palm subdomain is located at the base of a crevice formed between the fingers and the thumb subdomains. The fingers subdomain is important for dNTP recognition and binding, and interacts with the 5' single-stranded template. The thumb subdomain is important for binding of the nascent duplex DNA. The structures of the fingers and thumb subdomains are family specific, whereas the structure of the palm subdomains falls into two folds. The classical palm fold consists of a beta-sheet composed of four antiparallel strands, whereas in the pol beta-like nucleotidyltransferase (NT) palm fold, a fifth beta-strand is inserted in the middle of the beta-sheet (Figure 2A). The classical palm fold is found in family A, B and Y of DNA polymerases. The palm fold of the X and C families of DNA polymerases belongs to the pol beta-like NT superfamily. Interestingly, DNA polymerases known to greatly differ by their speed, fidelity and processivity of DNA synthesis can share the same palm fold (37-41). This is indeed the case of the alpha catalytic subunit of the replicative DNA polymerase III from *Thermus aquaticus* (a C family member) and of the repair DNA polymerase beta (an X family member) whose palm fold belongs to the pol beta-like NT superfamily and whose orientation of their DNA substrates relative to their palm domains are nearly identical (Figure 2B). The similarities between these two DNA polymerases are however limited to the palm domain, as the DNA path outside the active site differs between these two proteins (41). The fact that specific properties (such as fidelity) can be acquired by a DNA polymerase thanks to structural features positioned a long distance from the active site has also been suggested by structural comparisons between two B family members, the replicative DNA polymerase from bacteriophage RB69 and the TLS of the *E. coli* DNA polymerase II (42).

Crystal structures of various DNA polymerases alone or complexed with a p-t junction (binary complex) or with a p-t junction and the complementary dNTP (ternary complex) identified two major DNA polymerase conformations that essentially differ by the position of the fingers (37, 41, 43, 44). These conformations have been named open and closed for the binary and ternary complex, respectively, as fingers close upon dNTP binding. They possibly reflect two states adopted during catalysis. The open state of the DNA polymerase engaged in a binary complex with a p-t junction makes it possible for the dNTP

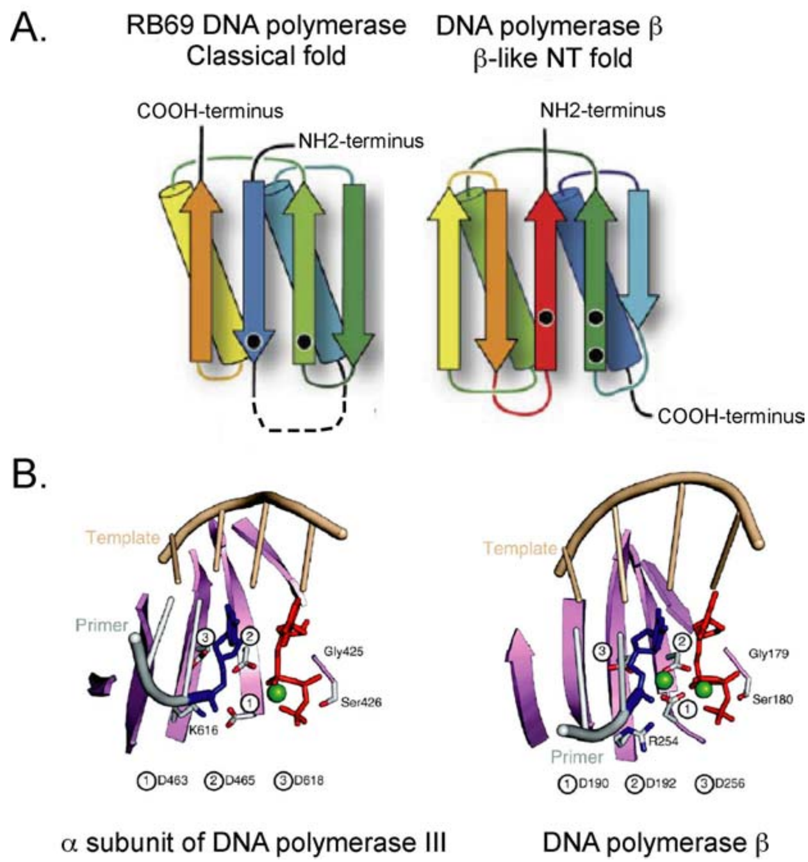


Figure 2. Comparison of the two palm folds and the active sites of the alpha subunit of DNA polymerase III and DNA polymerase beta. A. The topologies of the classical palm fold of the RB69 DNA polymerase (left) and of the beta-like nucleotidyl transferase (beta-like NT) palm fold of DNA polymerase beta (right) are shown. The black circles indicate the positions of the conserved catalytic carboxylate residues. The two palm folds differ by the number of beta-strands that constitute the palm (4 in the case of the classical fold and 5 in the case of the beta-like NT fold). Adapted with permission from (37). B. The active sites of the alpha subunit of DNA polymerase III and DNA polymerase beta are shown. The template and primer strands are colored in brown and grey, respectively. The 3' terminal base of the primer (blue) and the incoming dNTP (red) are shown as sticks. The beta-sheets of the palm are in pink. The conserved aspartate residues are designated by the number 1, 2, and 3. Divalent cations are as green spheres. Although belonging to different families, the active sites of the alpha subunit of DNA polymerase III (a C family member) and DNA polymerase beta (an X family member) are structurally very similar. Adapted with permission from (41).

substrate to rapidly diffuse into the active site. In the closed state observed in the ternary complex, the fingers subdomain is reoriented and closed around the incoming dNTP; the catalytic residues are now optimally aligned for catalysis. In the case of the T7 (45) and RB69 (46) DNA polymerases, reopening of the fingers domain before catalysis is slow when the DNA polymerase has selected the correct dNTP. The slow reopening of the fingers subdomain (relative to fingers domain closure and chemistry steps) possibly limits the release of the correct dNTP and commits the ternary complex to catalysis.

In the case of the T7 DNA polymerase, fingers closure upon dNTP binding involves a rotation of the O helix of the fingers subdomain that allows (i) the O helix to abut the nucleotide binding site, and (ii) functionally important residues to interact with the incoming dNTP (43). The open to closed transition of the large fragment of *T. aquaticus* DNA polymerase I known as Klentaq I also affects the orientation of the O helix and allows the formation of an active site poised for catalysis (44). The space occupied by the side chain of Tyr 671 - this amino acid is located at the base of the O helix - in the binary complex is filled by the next templating base in the ternary complex and can base pair with the incoming complementary dNTP. In the case of the catalytic alpha subunit of *T. aquaticus* DNA polymerase III, a rotation of 15° of the index finger region within the fingers domain inward toward the palm helps to form the dNTP binding pocket (38, 41). In all three ternary structures described above (that of the T7 DNA polymerase, Klentaq I and the alpha subunit of *T. aquaticus* DNA polymerase III), fingers closure introduces a sharp kink in the downstream single-stranded template as it exits the active site (41, 43, 44). The situation is slightly different in the case of the rat repair DNA polymerase beta when complexed with a p-t junction, as transition from an open to a closed complex involves a large motion of the 8-kDa amino-terminal domain of the protein relative to the fingers, thumb and palm carboxy-terminal domains (47). This additional domain does not belong to the DNA polymerase core. It carries the dRP lyase activity of DNA polymerase beta and is tethered to the thumb subdomain by a flexible linker. Its function is to maintain a grip on the 5' phosphate of the downstream strand of the one-nucleotide gapped intermediate that is formed during BER. Indeed, in the ternary complex formed with a one-nucleotide gapped DNA substrate, the dRP lyase domain binds the downstream duplex. Comparing the structures of binary and ternary complexes formed with the physiological one-nucleotide gapped BER intermediate revealed a significant rotation of the N-subdomain (equivalent to the thumb) around the alpha-helix N that makes it possible for the side chains of amino acids from the alpha-helix N to directly stack against the nascent base pair and for the active site to assemble ((47-49); for a review see (50)).

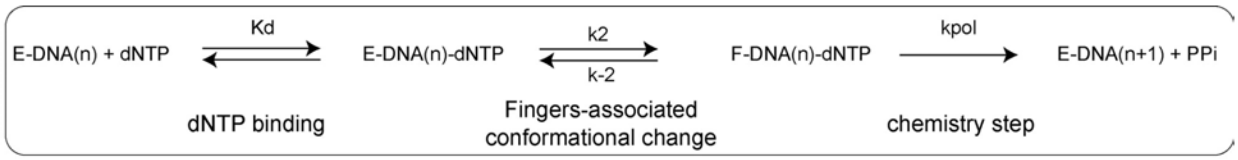
The crystal structures of the repair DNA polymerase lambda with a one-nucleotide gapped DNA indicate that DNA polymerase lambda already adopts a closed conformation even prior to dNTP binding (51, 52). dNTP binding triggers the motion of some specific structural elements of the palm and thumb subdomains and of a few side chains of amino acids (including Arg 517) that are part of the dNTP binding pocket. In addition, the template strand of the DNA substrate moves relative to the primer strand upon dNTP binding and adopts a catalytic conformation. Altered motion of the template strand relative to the primer strand might be responsible for strand slippage. Mutagenesis studies performed on the Arg 517 revealed that this amino acid possibly regulates strand slippage by limiting the flexibility of the template strand (53). Surprisingly, the crystal structure of DNA polymerase lambda interacting with a two-nucleotide gapped DNA substrate, a potential NHEJ intermediate, is almost identical to that observed when the enzyme interacts with a one-nucleotide gapped DNA (54). The enzyme achieves this conformation by scrunching the template strand. One of the template nucleotides from the gap is in an extrahelical position. The binding pocket that interacts with the extrahelical nucleotide is large enough to accommodate pyrimidine and purine residues, although amino acid residues that interact with the extrahelical base might adopt conformations that are pyrimidine and purine specific. Simulation studies suggest that DNA polymerase lambda could accommodate more than one uncopied template residue in the extra binding pocket. Equipped with a domain that binds specifically the 5' phosphate of the downstream DNA of a gap, and a binding pocket capable of accommodating a few uncopied template nucleotides from the gap, DNA polymerase lambda on its own can fulfill the function of a DNA polymerase specialized in filling short gaps processively.

3.3. Dynamics of unliganded and liganded DNA polymerases

Contrary to X-ray crystallography that requires a uniform and homogeneous population and has permitted to elucidate the structure of a variety of unliganded and liganded DNA polymerases, single molecule Förster resonance energy transfer (FRET) can examine the dynamic behavior of a population and detect subpopulations with specific properties. This technique has recently been applied to DNA polymerase I and revealed a millisecond timescale dynamics in the apo-enzyme with conformational transitions between the open and closed states characterized by X-ray structures (55). Similar conformational dynamics exist in the binary and ternary complexes, and each of these complexes can populate the less favored conformations to a significant extent (55). An equilibrium between two binary complexes that possibly differ by at least the position of the templating base and the Tyr 671 residue from the fingers subdomain has also been reported in the case of the Klentaq I enzyme (56). It is possible that the DNA sequence context shifts the equilibrium of the binary and ternary complexes toward a specific conformation, thus regulating the rate of the DNA polymerases. As discussed in the next paragraph, the ternary complex is also subject to conformational transitions involving the movement of the fingers subdomain and possibly other subtle transitions.

3.4. Rate limiting step during correct dNMP incorporation reaction

Kinetic experiments initially performed with replicative or repair DNA polymerases such as the T7 DNA polymerase (57-59) or the Klenow fragment (60) identified a conformational change in the ternary complex that was rate limiting and permitted the formation of a catalytically competent complex. This conformational change has been proposed to be part of an induced-fit mechanism that controls the DNA polymerase fidelity. The conclusion that the conformational change in the ternary complex was rate limiting was based on comparing the kinetics of incorporation of dNMP using either a dNTP or a dNTP-alpha-S (a chemical dNTP analog in which a nonbridging oxygen on the alpha-phosphate of the incoming dNTP has been replaced by a sulfur atom) as a substrate of the reaction. A significant decrease in the rate of dNMP incorporation upon sulfur substitution suggested that the formation of the phosphodiester bond was rate limiting. However, it has been realized that the magnitude of the "thio" effect might not be the correct criteria to determine whether chemistry is rate limiting or not and must be interpreted with caution (61). For instance, in the case of the repair DNA polymerase beta, the use of a fluorescent p-t junction or protein made it possible to follow the structural transitions in the template strand and in the protein during dNMP incorporation and clearly revealed that during correct dNMP incorporation the rate limiting step is not the conformational change preceding chemistry but the formation of the phosphodiester bond (Figure 3A) (62-65). Similar recent experiments conducted with a fluorescently labeled T7 DNA polymerase revealed that the conformational change preceding chemistry is faster than chemistry in the case of incorporation of a correct dNMP (Figure 3B) (45). The exact step that controls the dNMP incorporation reaction catalyzed by the Klenow fragment is still under debate (60, 64, 66).



A. Repair DNA polymerase β interacting with a two-nucleotide gapped substrate

	correct (T:A)	incorrect (T:G)
Kd (μM)	30	433

	correct (T:A)	incorrect (T:G)
k2 (sec)$^{-1}$	116	255

	correct (T:A)	incorrect (T:G)
kpol (sec)$^{-1}$	42.9	5.7

B. T7 DNA polymerase interacting with a p-t junction

	correct (G:C)	incorrect (G:G)
Kd (μM)	28	200

	correct (G:C)	incorrect (G:G)
k2 (sec)$^{-1}$	660	220
k-2 (sec)$^{-1}$	1.6	420

	correct (G:C)	incorrect (G:G)
kpol (sec)$^{-1}$	360	0.3

C. Repair DNA polymerase β interacting with a p-t junction

	correct (T:A)	incorrect (T:G)
k-2 (sec)$^{-1}$	84.3	565

Figure 3. Scheme and kinetic constants for correct and incorrect dNTP incorporation in the case of the repair DNA polymerase beta and T7 DNA polymerase. Top: E designates the open conformation of the DNA polymerase and F its closed conformation. DNA(n) and DNA(n+1) signify that the primer of the DNA is (n) or (n+1) nucleotides long, respectively. PPi means pyrophosphate. The open binary complex (E-DNA(n)) binds dNTP with an equilibrium dissociation constant Kd. The open ternary complex (E-DNA(n)-dNTP) undergoes a conformational change associated with fingers closure leading to the closed ternary complex (F-DNA(n)-dNTP). k2 and k-2 are the rates of fingers closure and reopening, respectively. The chemistry step corresponds to the formation of the phosphodiester bond and occurs at a rate of kpol. After the incorporation of a dNMP the primer has been extended by one nucleotide. When indicated, the base pairs are designated by two letters separated by ":". The first letter corresponds to the templating base and the second letter following ":" corresponds to the incoming dNTP that will be incorporated. A. The kinetics of incorporation of a correct or an incorrect dNMP by the repair DNA polymerase beta is shown. The substrate used in this study is a two-nucleotide gapped substrate. The rate limiting step of the correct and incorrect dNMP incorporation is the chemistry step. Adapted with permission from (65). B. The kinetics of incorporation of a correct or an incorrect dNMP by the T7 DNA polymerase is shown. The substrate used in this study is a p-t junction. The rate limiting step of the correct and incorrect dNMP incorporation is the chemistry step. Adapted with permission from (45). C. The reverse rates of the conformational change during a correct or an incorrect dNMP incorporation by the repair DNA polymerase beta are reported. The substrate used in this study is a p-t junction. Adapted with permission from (89).

What could be the molecular nature of the conformational change that controls dNMP incorporation of some DNA polymerases? It was initially hypothesized that the conformational change that takes place upon dNTP binding before catalysis consists of the large open-to-closed structural transition affecting the fingers subdomain of the DNA polymerase pinpointed by crystallographic studies (see 3.2.). Mutational and computational studies together with stopped flow fluorescence assays involving fluorescently labeled protein and/or DNA (reviewed in (67); see also (68-73)) confirmed the existence of a fingers motion upon dNTP binding. They however revealed that the open-to-closed transition affecting the fingers subdomain of the DNA polymerase is fast and not rate limiting when the correct dNTP has been selected by the DNA polymerase. Studies of DNA polymerase beta (63, 74) and of the RB69 DNA polymerase (75) showed that the formation of the closed ternary complex does not require that the A metal binding site be filled with a metal cation, and that filling the A site stabilizes this closed ternary structure. It is therefore possible that the conformational change limiting the rate of dNMP incorporation of some DNA polymerases corresponds to entry of the metal ion into the A site (73).

3.5. dNTP selectivity

As DNA polymerases are central to the overall fidelity of DNA synthesis and to genome stability, understanding the mechanisms of dNTP selection is essential. *In vivo*, with the assistance of replication auxiliary factors and repair proteins (*e.g.* mismatch repair proteins) replicative DNA polymerases replicate DNA with an error rate approaching one in a billion (76). This high fidelity of accurate DNA polymerases stems in part from (i) the high capacity of replicative DNA polymerases to discriminate between correct and incorrect dNTPs during 5’->3’ DNA synthesis, and (ii) the 3’->5’ proofreading exonuclease activity that is activated upon incorporation of a mispaired dNMP. The critical role of proofreading in maintaining eukaryotic genome stability is illustrated by genetic studies of yeast strains harboring 3’->5’ exonuclease–deficient pol delta, epsilon or gamma, all of which have a mutator phenotype (77-81). Similarly, mice harboring exonuclease–deficient polymerase delta have a shortened life span and increased susceptibility to several types of cancer (82, 83). Inactivation of the 3’->5’ exonuclease activity of DNA polymerase gamma elevates the levels of mitochondrial DNA mutations and leads to loss of mitochondria and premature ageing (84).

A full understanding of DNA polymerase fidelity requires a comparison of both matched and mismatched dNTP incorporation pathways, including dNTP binding, conformational changes and the chemistry step. In principle, each step in the dNTP incorporation pathway can participate in overall nucleotide selectivity, and the relative contribution of each step in enzyme fidelity may be DNA polymerase dependent and dictated by the DNA polymerase function. As a consequence, a unified strategy shared by all DNA polymerases to gain fidelity might not be expected, and each DNA polymerase might possess a specific mechanism to regulate its fidelity during DNA synthesis that is fine-tuned to its role in the cell. Part of the high dNTP selectivity of DNA polymerases can take place at the initial dNTP binding step. The open conformation of the DNA polymerases indeed binds incorrect dNTPs with a weaker affinity than the correct dNTP (Figure 3A-B) (45, 65). However the affinity difference of the DNA polymerases in their open conformation between a correct and an incorrect dNTP does not suggest an active contribution of the DNA polymerase during the dNTP selection (45, 61, 65). Instead, the discrimination level achieved at this initial step reflects the difference of stability between a Watson-Crick a non Watson-Crick base pair (36). The conformational change that follows dNTP binding and that takes place before the chemistry step can be considered as a checkpoint or a switch and can improve dNTP discrimination by providing opportunities to reject the mispaired dNTP. This conformational change is preferentially induced when the correct dNTP is bound in the active site of the DNA polymerase. This step can be rate limiting in the case of correct dNMP incorporation (see 3.4.) but in general does not control the misincorporation reaction. In contrast, this is the chemistry step that is in most cases rate limiting when a mispaired dNMP is incorporated. Whether fidelity is gained when the conformational change is rate limiting during the incorporation of a correct dNMP has been previously discussed and depends in part on which step controls the incorporation of an incorrect dNMP (61). Finally fidelity can be gained during the chemical reaction if the transition state of the ternary complex containing a mismatch in the active site is not as stabilized as that containing a perfect base pair. The repair DNA polymerase beta acquires most of its fidelity past the binding of the dNTP by the binary complex, by lack of stabilization of the mispaired ternary complex and transition state (65). It is possible that a mispair in the active site of the DNA polymerase prevents binding of Mg^{2+} in the A site and further stabilization of the reaction intermediates. In addition, biochemical characterization of mutator mutants of DNA polymerase beta suggests that fidelity requires a minimum degree of flexibility of the fingers subdomain. Restricting the motion of this subdomain indeed lowers fidelity (85). How the motion of the fingers subdomain contributes to the stabilization of the transition state is still unknown. In the case of the human DNA polymerase lambda, structural studies combined with biochemical characterizations suggest that the repositioning of the palm subdomain upon correct dNTP binding controls the motion of both the template strand and the 3’-OH primer terminus and thus can regulate the fidelity of the enzyme (86). When these structural changes occur precisely during the dNMP incorporation reaction and how they potentially contribute to the stabilization of reaction intermediates need to be investigated.

The group of Johnson recently developed a new paradigm for DNA polymerase selectivity, based on experiments that measured forward (k2) and reverse (k-2) rates of the conformational change of the DNA polymerase when incorporating a correct or an incorrect dNMP (36, 45, 87). This new paradigm has been recently extended to the HIV reverse transcriptase when discriminating against nucleotide analogs (88). Reaction rates measured in the case of correct and incorrect dNMP incorporation (Figure 3B) suggest that dNTP discrimination depends on the *relative* ratio of the nucleotidyl transfer rate (kpol) and the rate of fingers reopening (k-2) in the ternary complex that can contain either the correct or the incorrect dNTP (36, 45, 87). For instance, the closed ternary complex filled with the correct dNTP is more prone to commit into the chemistry step since kpol >>> k-2 (Figure 3B). In contrast the closed ternary complex filled with an incorrect dNTP is more prone to reject the mispaired dNTP since k-2 >>> kpol. Recent experiments performed with replicative RB69 DNA polymerase and aiming at measuring the rate of fingers subdomain reopening before the chemistry step seem to support this new model (46). The situation might be different for the repair DNA polymerase beta. Measurement of the reverse rate of the conformational change step (89) and its comparison with the nucleotidyl transfer rate (65) in the case of a correct or an incorrect dNMP incorporation suggest that the reopening step of the fingers subdomain is not as critical for fidelity as reported for a replicative DNA polymerase (Figure 3A, C). It is possible that discriminating between correct and incorrect dNTPs by regulating the reverse rate of the conformational change is specific to high-fidelity DNA polymerases that synthesize DNA faster than repair DNA polymerases.

3.6. Choice of the right sugar

In addition to discriminating against incorrect dNTPs, DNA polymerases also select the correct sugar choosing dNTPs over rNTPs (90). This selection represents a big challenge given that the intracellular concentration of rNTPs is at least 10-fold greater than that of dNTPs (91, 92). For instance, a recent study indicates that in yeast, the intracellular molar rNTP:dNTP ratios range from 36:1 for cytosine to 190:1 for adenine (93). In the case of the RB69 DNA polymerase, sugar discrimination is provided mainly by the Tyr 416 side chain that can sterically block the 2'-OH group of an incoming rNTP (94). Similarly, Klenow fragment discrimination against rNTPs occurs within the ternary complex during the open to closed transition *via* steric interference by a tyrosine side chain when the active site reorganizes to be poised for phosphoryl transfer (73). Such a mechanism allows the Klenow fragment to select dNTPs over rNTPs by several thousand fold (31). In contrast, DNA polymerases of the X family do not rely on a protein side chain to exclude rNTP binding, but instead, employ the protein backbone itself to reject rNTPs (95, 96). The rNTP exclusion mechanism based on protein backbone may not be as efficient as the one relying on an amino acid side chain since DNA polymerase beta may insert 1 rNMP every 81 dNMP incorporations (97). A recent study compared the ability of yeast DNA polymerase alpha, delta and epsilon to discriminate against rNMP incorporation (93). Among the three DNA polymerases tested, DNA polymerase alpha has the lowest discrimination ability incorporating 1 rNMP for 625 dNMPs. DNA polymerase delta and epsilon incorporate 1 rNMP every 5000 and 1250 dNMPs, respectively. All these data suggest that rNMPs might well be the most common non canonical nucleotides incorporated into DNA. In striking contrast to replicative DNA polymerases, repair DNA polymerase mu is very poor at discriminating against rNTPs (98-100). Indeed, DNA polymerase mu incorporates rGTP almost as efficiently as dGTP. Considering the role of this DNA polymerase in double-stranded break repair and the higher thermodynamic stability of RNA:DNA over DNA:DNA hybrids, incorporation of rNMPs might stabilize the reaction intermediate that is formed during the NHEJ reaction and that has limited base pairing.

3.7. Conformation of a ternary complex with a paired or mispaired dNTP/rNTP

The formation of a closed ternary complex with a mispaired dNTP similar to that formed with correct dNTP has been recently questioned. Indeed, in the case of the T7 DNA polymerase (36, 45), the fluorescence properties of the ternary complexes formed with correct and incorrect dNTP are different, suggesting the existence of at least three states: open, closed and mismatched recognition. Single molecule FRET experiments performed with the Klenow fragment showed that the ternary complex formed in the presence of incorrect dNTP is distinct from the open and closed complexes with a correct incoming dNTP (55). Ensemble FRET-based kinetics indicate that the Klenow fragment detects mispaired dNTPs very early in the reaction pathway before the DNA rearrangement that precedes fingers subdomain closure; in addition, the selection of the wrong dNTP by the Klenow fragment may drive the complex into a different pathway leading to dissociation of the dNTP and of the entire binary complex (73). The same lack of stabilization of a mismatched ternary complex applies to repair DNA polymerase beta, although mismatched and matched dNTP incorporations seem to proceed *via* an analogous pathway (65). The pre-equilibrium of the binary complex formed with the Klentaq I enzyme may represent a key step in initial dNTP selection as this step has been proposed to make the templating base available for a “preview” by the incoming dNTP (56).

Single molecule FRET experiments performed with the Klenow fragment showed that the ternary complex formed in the presence of complementary rNTP is distinct from the open and closed complexes formed with a complementary dNTP, and it is likely that the ternary complex formed with a complementary rNTP corresponds to a partially closed conformation limited by steric constraints (55). The inability of this complex to proceed further along the reaction pathway results in rNTP rejection.

3.8. Inorganic pyrophosphate release and translocation

Translocation is the processive movement of the DNA polymerase along the DNA substrate between two rounds of the phosphoryl transfer reaction. To prevent frameshift mutations and base substitutions, it is critical to restrict the directional motion of the DNA polymerase to one nucleotide per cycle and to inhibit translocation after a misincorporation event. In the case of correct incorporation, PPi release and translocation are fast and not the rate limiting steps of dNMP incorporation (58). In contrast, PPi release is slow in the case of incorporation of modified dNMPs allowing reversal of the chemistry step and dissociation of the modified dNTP (101, 102). Even though directional translocation has been difficult to study using standard techniques, structural studies and computer simulation analyses have been very helpful in elucidating its mechanism.

In the case of the A family of DNA polymerases, structural studies (103-105) proposed the existence of two distinct pre-insertion sites, one for the incoming dNTP and one for the next templating base. The pre-insertion site of the incoming dNTP is located near the fingers subdomain, and once bound to its pre-insertion site the incoming dNTP has been proposed to be escorted into its insertion site. The pre-insertion site for the next templating base is located at the interface between the O and O1 helices of the fingers subdomains and its conformation changes during the dNMP incorporation cycle. A recent study used computational methods and X-ray structural information from the *Bacillus stearothermophilus* DNA polymerase I large fragment (104) and the T7 RNA polymerase (106) to identify the sequential events leading to opening of the fingers subdomain and translocation of this A family of enzymes along the DNA (107). In this model, as for the T7 RNA polymerase (106), PPi release triggers fingers subdomain opening and DNA translocation. Opening of the fingers is associated with bending of the O helix that first involves the upper end of the helix (see transition from “Closed 1” to “Closed 2” state in Figure 4A). The lower end of the O helix then starts rotating on itself and the O1 helix undergoes a “gating motion” (see transition from “Closed 2” to “Translocated” state in Figure 4A). These concerted motions allow (i) the terminal base pair to be displaced into the post-insertion site, (ii) the side chain of the conserved Tyr 714 to stack against the last templating base and to position itself into the insertion site of the

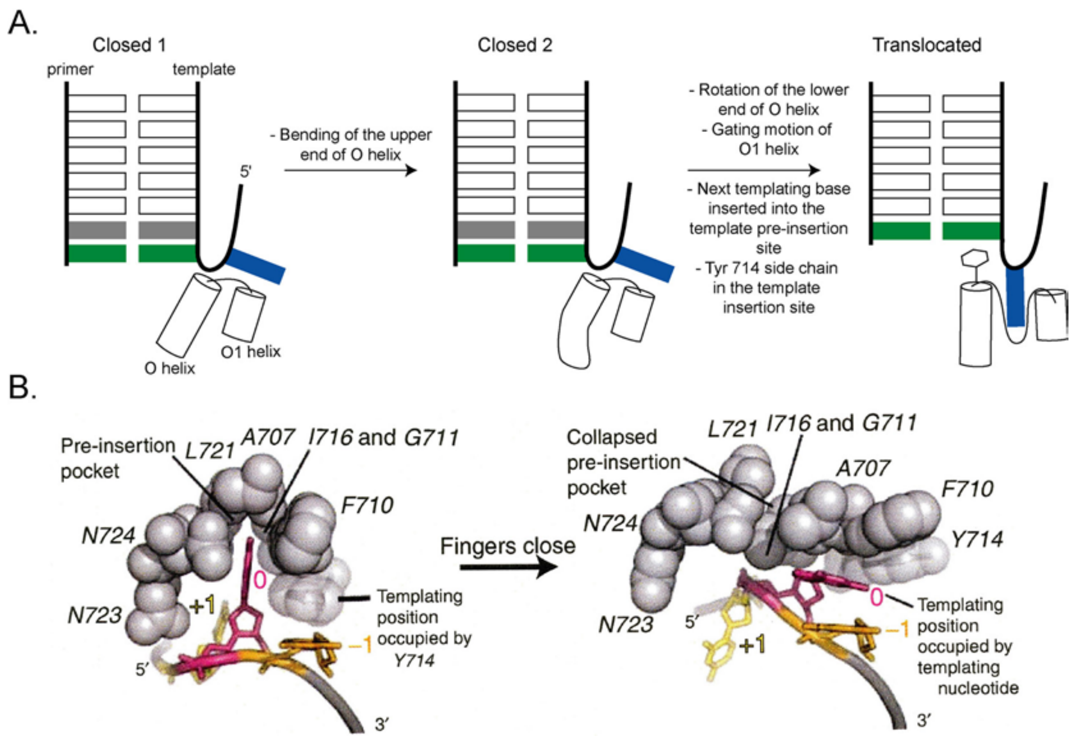


Figure 4. Translocation of *B. stearothermophilus* DNA polymerase I large fragment. A. The active site of the DNA polymerase during fingers subdomain opening and translocation is schematically represented. In all states, the next templating base is in blue. The O and O1 helices are part of the fingers subdomain. In the "Closed 1" and "Closed 2" states, the gray base pair is in the post-insertion site, the terminal base pair (in green) is in the insertion site, and the pre-insertion site of the next templating base has not yet assembled. In the "Translocated" state, the post-insertion site is filled by the green base pair, the insertion site for the next templating base is filled by the side chain of Tyr 714 ⬠ newly assembled pre-insertion site of the next templating base. See text for details. Adapted with permission from (107). B. The motion of the templating bases during the closure of the fingers subdomain of *B. stearothermophilus* DNA polymerase I is represented. As the fingers subdomain closes, the templating nucleotide (in red and numbered 0) is transferred from the pre-insertion site to the insertion site. The transfer of the templating nucleotide is associated with the collapse of the pre-insertion binding pocket. Adapted with permission from (108).

templating base, (iii) the pre-insertion site of the next templating base to assemble in a pocket, and (iv) the next templating base to be incorporated into the newly constituted pre-insertion site ("Translocated" state in Figure 4A). How and when the templating base located in the pre-insertion site is incorporated into the insertion site is still unclear. However, structural (104) and fluorescence (66) studies suggest that when fingers close, residues from the templating base pre-insertion site collapse, Tyr 714 moves out and the templating base moves into the insertion site (Figure 4B).

A similar mechanism has been proposed for the B family phi29 DNA polymerase based on static X-ray structures of binary and ternary complexes (108). Structure comparisons suggest that translocation takes place after phosphodiester bond formation, as for the A family of DNA polymerases (Figure 5A-B). During the opening of the fingers subdomain, the interactions between the PPi and the basic residues of the fingers subdomain are broken; PPi is released from the enzyme. In addition, when fingers open, two conserved tyrosine residues, Tyr 390 and Tyr 254, are proposed to move in a concerted manner into the dNTP insertion site, thus facilitating translocation of the newly formed base pair into the post-insertion site; DNA polymerases of the B family do not have a residue homologous to Tyr 714 of the *B. stearothermophilus* DNA polymerase I large fragment. Therefore, contrary to what has been proposed for the A family of DNA polymerases, the next templating base is not accommodated into a pre-insertion site but is directly transferred to the templating base insertion site as fingers open, from where it is subjected to only subtle changes during fingers closure (Figure 5C).

Recently, single molecule FRET has been used to measure the movement of the Klenow fragment on the DNA template with single base pair resolution (109). This technique led to the identification of a new transient intermediate after incorporation of a correct dNMP. The FRET signal of this intermediate indeed suggests that the DNA polymerase may have translocated by more than one nucleotide along the template. This previously unobserved step in the mechanism of DNA synthesis may position the primer terminus in a new site and may be part of the proofreading process.

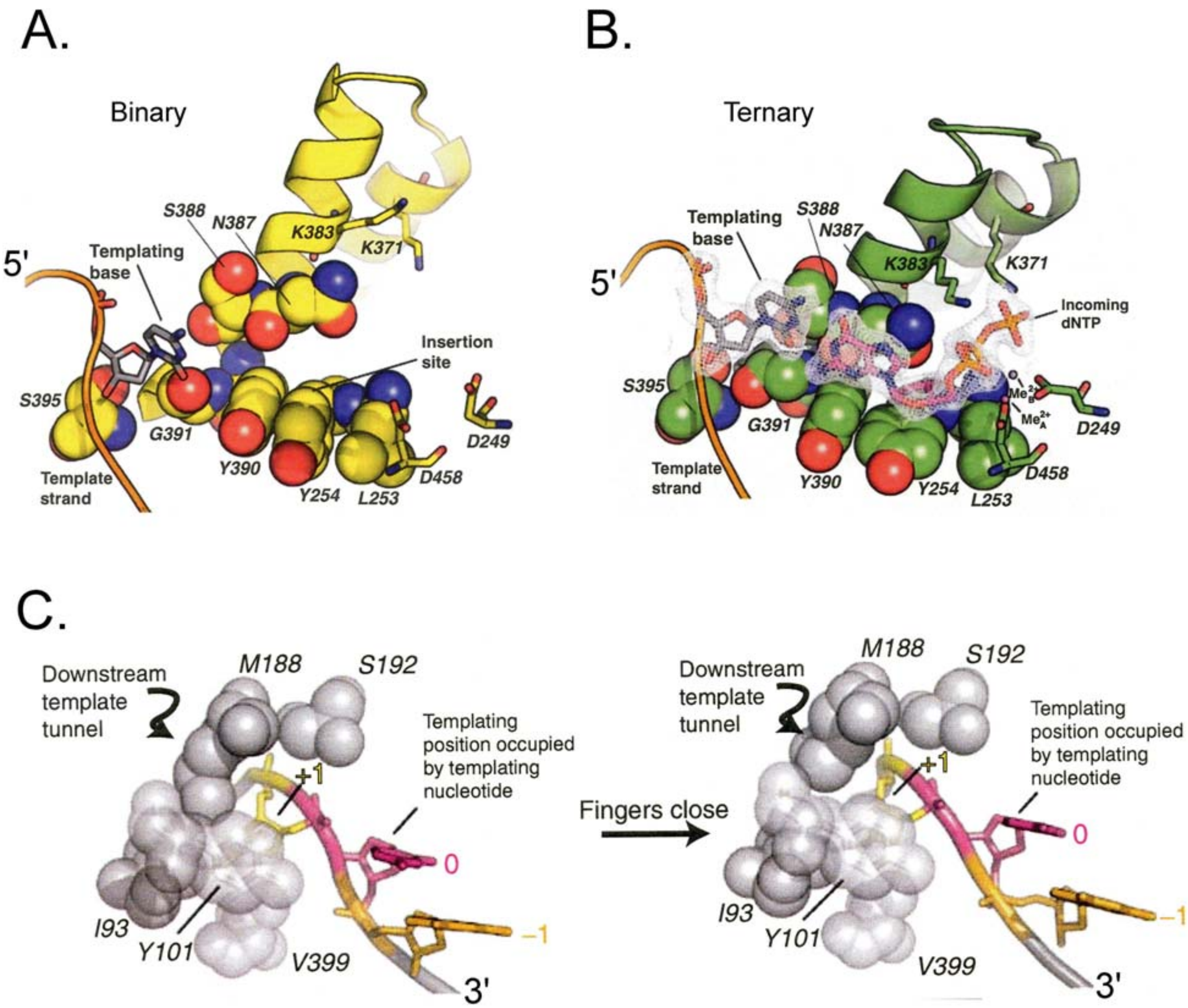


Figure 5. Translocation of phi29 DNA polymerase deduced from the comparison of the binary and ternary complex structures. Binary (A.) and ternary (B.) complexes are shown in yellow and green, respectively. The residues that form the base pair binding pocket are as spheres, the aspartic acids that binds the Mg^{2+}, the templating base and the incoming dNTP as sticks. The side chains of conserved lysines that interact with the phosphate tail of the incoming dNTP in the ternary complex are also shown as sticks. Their conformation changes upon dNTP binding to establish electrostatic bonds with the triphosphate tail of the dNTP. The conformation of the side chain of Tyr 390 and 254 also changes upon dNTP binding to create room for the incoming dNTP and to possibly facilitate translocation of the newly formed base pair into the post-insertion site. Adapted with permission from (108). C. The motion of the templating bases during the closure of the fingers subdomain of the phi29 DNA polymerase is represented. Limited motion of the template nucleotides occurs during fingers subdomain closure of the phi29 DNA polymerase. Adapted with permission from (108).

3.9. Replicative and repair DNA polymerases and 8-oxoguanine lesion

DNA lesions that are created either spontaneously or by external agents (*e.g.* reactive oxygen species, UV light or gamma-rays) are removed from the cell *via* efficient repair pathways (*e.g.* BER or NER). Nevertheless, some of them can persist and be encountered by the replication machinery. Although replicative DNA polymerases are very sensitive to the quality of the template DNA, they can bypass certain lesions such as the non-distorting DNA adduct, 7,8-dihydro-8-oxoguanine (8-oxoguanine) or O6-alkylguanines. The bypass of damaged DNA by replicative DNA polymerases can be highly mutagenic, as recently exemplified in the case of various O6-alkylguanine adducts (110). In this section, the DNA synthesis activity of replicative and repair DNA polymerases across 8-oxoguanine is described from a kinetic and structural point of view.

8-oxoguanine is the most prevalent mutagenic lesion derived from the interaction of reactive oxygen species (ROS) with DNA. Nuclear and mitochondrial

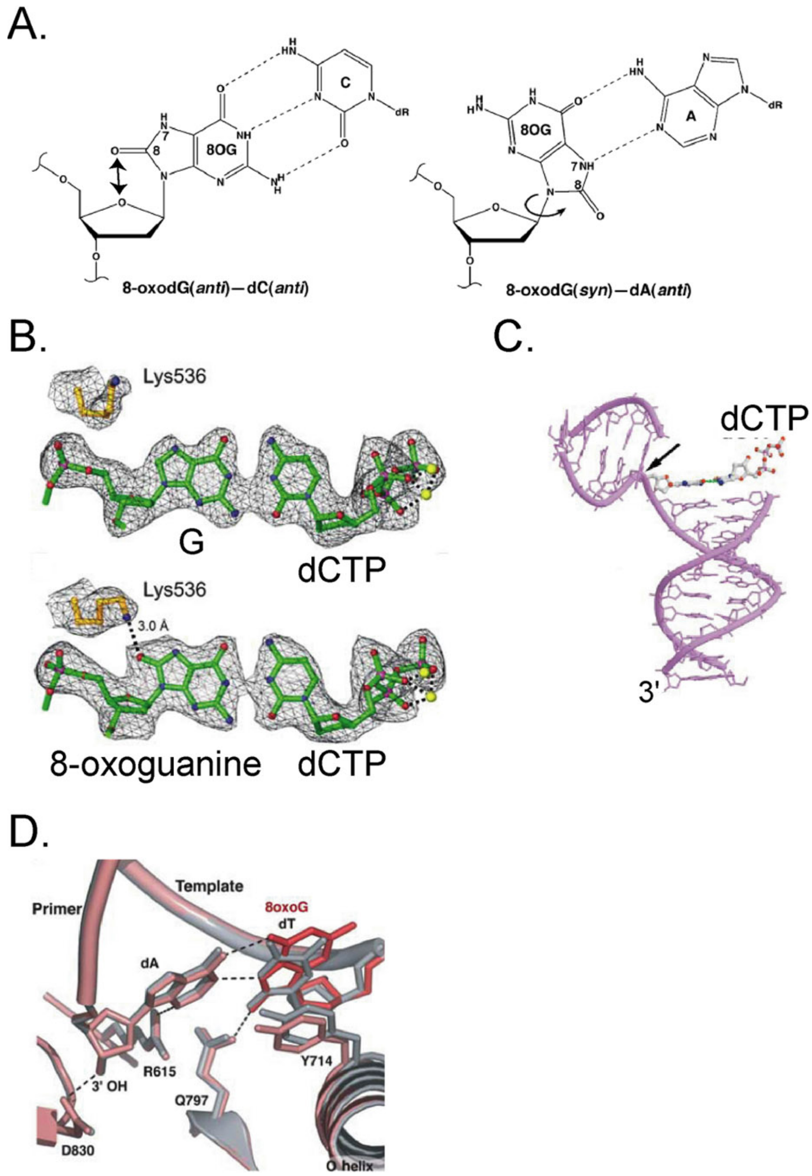


Figure 6. Conformation of the 8-oxoguanine lesion in the active site of various DNA polymerases. A. Base pairing between an 8-oxoguanine in an *anti* (left) or *syn* (right) conformation with a C or an A, respectively. The electronic repulsion between the oxygen of the deoxyribose and the O8 of the 8-oxoguanine kept in an *anti* conformation is indicated by a double arrow. B. Base pairing between a G (top) or an 8-oxoguanine (bottom) in an *anti* conformation with a dCTP in the active site of T7 DNA polymerase. The yellow spheres that interact with the phosphate tail of the incoming dCTP are Mg^{2+}. Note the hydrogen bond between O8 of the lesion kept in an *anti* conformation and the Lys 536 side chain. Adapted with permission from (116). C. When bound by DNA polymerase beta, a gapped DNA containing an 8-oxoguanine as the nucleotide to be copied is sharply kinked. The 8-oxoguanine in an *anti* conformation paired with a dCTP are as sticks. The black arrow points to phosphodiester bond 5' to the lesion that is severely bended. Adapted with permission from (121). D. In the active site of the *B. stearothermofilus* DNA polymerase I large fragment, a *syn*-8-oxoguanine:*anti*-A base pair mimics the geometry of a normal Watson-Crick *anti*-T:*anti*-A base pair. The ternary complex with unmodified DNA is shown in grey. The ternary complex with modified DNA is shown in pink with the 8-oxoguanine (8-oxoG) colored in red. Adapted with permission from (117).

DNAs are both targets of ROS and in all tissues and species studied, the level of oxidative damage is significantly higher in mitochondrial DNA than in nuclear DNA due to the proximity of the mitochondrial DNA to the electron transport chain (111). Approximately 10^4 8-oxoguanine adducts arise per cell and per day, but most of them are repaired by very efficient repair enzymes (112). The equilibrium between the *anti* and *syn* conformations of the base is shifted towards the *syn* conformation when a guanine is oxydized on position C8 (Figure 6A). An electronic repulsion indeed exists between the oxygen of the deoxyribose and the O8 of the 8-oxoguanine kept in an *anti* conformation. Due to the ability of 8-oxoguanine in a *syn* conformation to base pair with dATP (Figure 6A), 8-oxoguanine can cause G to T transversions *in vivo*.

In vitro studies showed that DNA polymerases bypass 8-oxoguanine with different efficiencies and fidelity (101, 113-120). The faithful bypass of an 8-oxoguanine lesion requires the DNA polymerase to incorporate dCMP across the lesion and extend from the correct but non canonical C:8-oxoguanine base pair. Specific structural features can stabilize the lesion in an *anti* conformation to favor base pairing with dCTP. For example, T7 DNA polymerase deficient in its 3->5' exonuclease (T7 DNA polymerase exo-) tolerates the lesion by introducing a strong kink in the DNA template (116) and stabilizes the *anti* conformation of 8-oxoguanine by establishing an interaction between the side chain of Lys 536 in the O1 helix of the fingers subdomain and the O8 group of the lesion (Figure 6B). The essential role of Lys 536 in modulating the miscoding potential of the 8-oxoguanine lesion by interfering with base pairing between 8-oxoguanine in a *syn* conformation and an incoming dATP has been confirmed by the kinetic and structural characterization of the Lys536Ala T7 DNA polymerase exo- mutant (118). Similarly, in the case of the repair DNA polymerase beta, the 5' phosphate backbone of the templating 8-oxoguanine is severely kinked, thus permitting the lesion in an *anti* conformation to be accommodated in the enzyme active site without steric clash and to establish Watson-Crick interactions with the incoming dCTP (Figure 6C) (121).

In contrast, the preference of the high-fidelity *B. stearothermophilus* DNA polymerase I large fragment to incorporate dATP opposite an 8-oxoguanine lesion stems from the formation of a *syn*-8-oxoguanine:*anti*-A base pair that mimics the geometry of a normal Watson-Crick *anti*-T:*anti*-A base pair and thus evades the DNA polymerase mismatch detection mechanism (Figure 6D) (117). The inefficient removal of dAMP opposite the 8-oxoguanine by the 3'->5' exonuclease activity is a property also shared by other DNA polymerases such as T7 DNA polymerase (116) or DNA polymerase gamma (101).

The efficiency of the extension reaction from a C:8-oxoguanine or A:8-oxoguanine terminal base pair is dictated by the efficiency of translocation after dCMP or dAMP incorporation across the lesion, the affinity of the DNA polymerase complexed with a non canonical terminal base pair for the next correct incoming dNTP and the dNMP incorporation reaction itself. The RB69 DNA polymerase extends very poorly from a C:8-oxoguanine or A:8-oxoguanine terminal base pair possibly because its translocation to vacate the active site requires the phosphate backbone of the templating strand to reorient itself (119). Similar to the Klenow fragment exo- (113), the T7 DNA polymerase exo- prefers to extend from an A:8-oxoguanine base pair, due to a higher affinity of the binary complex for correct dNTP and a higher maximal rate of incorporation compared to the same values measured for the extension from a C:8-oxoguanine base pair (114). In addition the structure of the post-insertional ternary complex with a C:8-oxoguanine base pair as the terminal base pair revealed a significant change in the conformation of the sugar-phosphate backbone of the templating nucleotide, which might destabilize base pairing at the active site (116). Such a conformational change in the sugar-phosphate backbone is not observed with the post-insertional ternary complex formed with an A:8-oxoguanine base pair as the terminal base pair, providing a possible explanation for the preferential extension of the A:8-oxoguanine base pair (114).

All together these *in vitro* studies show that 8-oxoguanine can be bypassed by DNA polymerases *via* incorporation of dCMP or dAMP opposite the lesion. The efficiency of the complete bypass reaction (incorporation opposite the lesion and extension from the unusual terminal base pair) depends on several parameters: capacity of the lesion to be accommodated and stabilized in the active site of the DNA polymerase and to base pair with the incoming dNTP, efficiency of the dNMP incorporation and proofreading reactions, ability to translocate and extend from a non canonical base pair. The efficiency of lesion bypass and the fidelity of the DNA polymerases can be modulated by auxiliary factors such as the processivity factor and single-stranded DNA binding proteins (see 5.).

4. NUCLEOTIDE INCORPORATION REACTION OF TLS DNA POLYMERASES

TLS DNA polymerases, including the Y family of DNA polymerases, the B family DNA polymerase zeta, the B family DNA polymerase II from *E. coli*, the A family DNA polymerase nu and the X family DNA polymerase lambda, can introduce mutations at a high rate due to their low fidelity (for reviews on the TLS and Y family DNA polymerases see (122-125)). The low fidelity of TLS DNA polymerases is in part due to lack of proofreading activity. Yet, *E. coli* DNA polymerase II is an exception among the TLS DNA polymerases as it possesses a 3'->5' exonuclease domain. However, a recent biochemical and structural study revealed that the *E. coli* DNA polymerase II proofreading activity is weak compared to that of the RB69 DNA polymerase from the same family of DNA polymerase and that structural features might alter DNA partitioning between the active and the exonuclease sites, thus favoring primer extension over degradation (42). The next sections describe the structural specificities that make the Y family of DNA polymerases, and the TLS DNA polymerases in general, (i) less accurate than replicative DNA polymerases on undamaged DNA, (ii) capable of synthesizing across a lesion, and (iii) capable of extending from a "non typical Watson-Crick" base pair.

4.1. Domain organization of the Y family of DNA polymerases

As also the DNA polymerases from other families, the catalytic core of the Y family of DNA polymerases is composed of three subdomains, fingers, palm and thumb (Figure 7). The palm is the only domain that has a topology similar to that of most other DNA polymerases (the classical palm fold; Figure 2A). It is

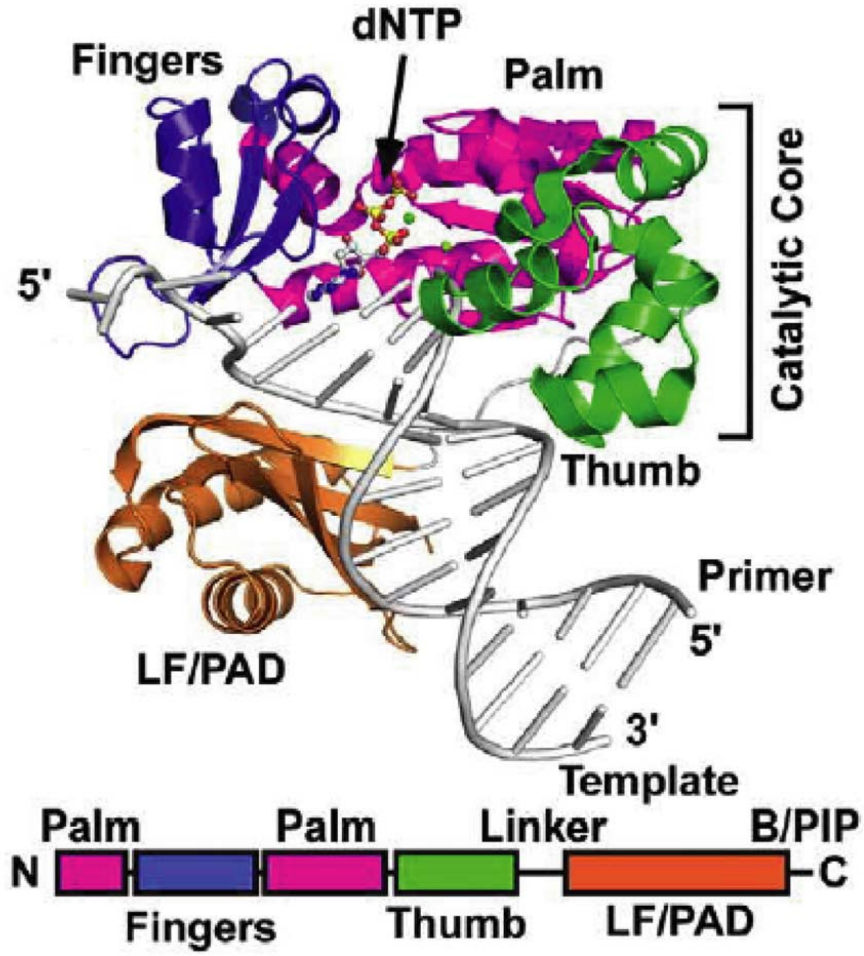


Figure 7. Structure of the *S. solfataricus* Dpo4 enzyme. The ribbon diagram of a ternary complex of *S. solfataricus* Dbo4 (top) and the diagram showing polymerase domains (bottom) are presented. The palm, fingers, thumb and little finger (LF/PAD) are colored in pink, blue, green and orange, respectively. The primer and the template are in grey, and the dNTP is highlighted. Adapted with permission from (125).

therefore not surprising that, just as the DNA polymerases from other families, the DNA polymerases of the Y family use a two metal ion catalytic mechanism to incorporate a new dNMP into the 3’ end of a primer. In addition, the equivalent of the O helix of the fingers subdomain, important for incoming dNTP binding in the case of replicative DNA polymerases and during fingers subdomain closure (see 3.2.), is absent from the DNA polymerases of the Y family. The carboxy-terminal domain is specific of the DNA polymerases of the Y family and is commonly referred to as the little finger (LF) in bacterial and archeal enzymes or as the polymerase associated domain (PAD) in eukaryotic proteins (Figure 7). A flexible linker connects the catalytic core to the LF/PAD domains. Interestingly, although not predictable from primary structure comparisons, the tertiary structure of the FL/PAD subdomain is quite well conserved and is composed of a four-stranded beta-sheet flanked on one side by two alpha-helices. In all the Y family of DNA polymerase structures examined (for a review see (125)), the thumb subdomain contacts the sugar phosphate backbone of the double-stranded DNA across the minor groove, whereas the LF/PAD subdomain contacts the sugar phosphate backbone of the double-stranded DNA across the major groove. The 5’ single-stranded template DNA of a p-t junction interacts with the fingers and/or LF/PAD subdomains. Due to its small size relative to that of other DNA polymerases, the fingers subdomain establishes very few contacts with the major groove of the nascent base pair. Interactions between the palm subdomain and the minor groove of the nascent base pair are also very limited.

4.2. Base pair active site

Crystal structures from various apo-enzymes of the Y family initially suggested that the nascent base pair would not be as constrained in the active site as observed in high-fidelity DNA polymerases (126-128). In the first ternary complex structure obtained with the *Sulfolobus solfataricus* P2 DNA polymerase IV, Dpo4, the enzyme establishes limited and non specific contacts with the replicating base pair, thus relaxing base selection, and possibly explaining the low fidelity on undamaged templates of DNA polymerases of the Y family (129). The base pair binding pocket of the Y family of DNA polymerases is more open and accessible to solvents than that of high-fidelity DNA polymerases, and can accept not only Watson-Crick but also non Watson-Crick base pairing such as Hoogsteen base pairing as in the case of DNA polymerase iota (130, 131), or reverse wobble base pairing as in the case of *S. solfataricus* Dpo4 (132)). The X-ray crystal structure of *S. solfataricus* Dpo4 complexed with a UV-induced lesion, a *cis-syn* cyclobutane thymine (TT) dimer, indicates that the spacious and solvent accessible active site can accommodate two templating bases and two incoming dNTPs. In this structure, the 3' T of the lesion pairs with a ddATP in a Watson-Crick manner and the 5' T of the lesion makes Hoogsteen hydrogen bonds with ddATP in a *syn* conformation (133). The spacious and open active site of yeast (134) and human (135) DNA polymerase eta permits the enzyme to tolerate *cis-syn* cyclobutane pyrimidine dimers without any hindrance. Interactions between specific amino acids of yeast DNA polymerase eta and the two Ts of the *cis-syn* TT dimer maintain the lesion in a stable conformation. In human DNA polymerase eta a continuous and positively charged groove, similar to a molecular splint and unique among other DNA polymerases of the Y family, maintains the DNA rigid and straight in a B-conformation (135). In addition dATP pairs in a similar manner with the 3' T of an undamaged or damaged *cis-syn* TT dimer, explaining the equal capacity of yeast DNA polymerase eta to copy undamaged and UV-damaged DNA.

The size of the active site of DNA polymerase kappa is intermediate between that found in high-fidelity DNA polymerases and DNA polymerase eta and can only accommodate a single templating base. Due to its unique N-clasp domain, this DNA polymerase can tolerate distorted primer termini, and its major cellular role might be to catalyze extension of mispaired primer termini (136). The active site of Rev1 is unique since this DNA polymerase uses a protein-template nucleotide incorporation mechanism to specify incorporation of dCMP (18, 19). More specifically, in the crystal structure reported by Nair *et al.* (18) the templating G is evicted from the double helix and the space vacated is filled by the side chains of a leucine and an arginine. The arginine makes a set of hydrogen bonds with the incoming dCTP, thus acting as the templating base. Similar bonds could not easily be formed with any other dNTPs, explaining the specificity of Rev1 for dCTP incorporation. dNTP binding studies confirmed this structural prediction as dCTP binds at least 10 times more tightly than other dNTPs opposite a templating guanine (137). Similarly, the space occupied by the templating guanine cannot easily accept any other base, or O6-adducted guanines. In contrast N2-adducted guanines could be evicted from the DNA explaining the ability of Rev1 to promote faithful replication across those lesions (138). Again pre-steady state kinetic experiments confirmed this prediction and revealed also that Rev1 discriminates between the four dNTPs not only at the dNTP binding step but also at the incorporation step (137).

The fact that some DNA polymerases of the Y, B and X families can accommodate two templating bases, two dNTPs or misaligned DNA intermediates (42, 139-144) may explain the elevated frameshift mutation rate that such DNA polymerases can generate *via* template slippage or a dNTP-stabilized misalignment mechanism (145-147). For instance, the small cavities that mark the path of the template strand in *E. coli* DNA polymerase II may help stabilize the putative -2 frameshift intermediate during the error-prone bypass of a single N-2-acetylaminofluorene (AAF) adduct within the *NarI* mutation hot spot (11, 42).

4.3. dNMP incorporation reaction on undamaged DNA

X-ray structure comparisons initially suggested that the fingers subdomain of the DNA polymerases of the Y family lacks flexibility and that ternary complex formation only leads to subtle repositioning of residues from the active site (134, 135, 148, 149). However, experiments performed in solution (*e.g.* reactions of hydrogen-deuterium exchange in tandem with mass spectrometry) made it possible to pinpoint significant structural changes within the *S. solfataricus* Dpo4 thumb and fingers subdomains upon correct dNTP binding (150). In addition, in the case of the *S. acidocaldarius* DinB homolog, Dbh, three non covalent steps between the binding of a correctly paired dNTP and the rate limiting step for dNMP incorporation have been proposed; one of them may be related to unstacking of the 5' neighboring templating base, a feature revealed by crystal structures that may prepare the active site for phosphoryl transfer (144).

As for other DNA polymerases, for yeast DNA polymerase eta, a conformational change occurring before phosphodiester bond formation is rate limiting in the case of correct incorporation on an undamaged DNA template and its function might be to permit proper alignment of chemical groups involved in catalysis (151). In constrast, pre-steady-state kinetics performed on the *S. solfataricus* Dbh suggest that the rate limiting step of correct nucleotide incorporation is the chemical step itself, and not the conformational change preceding the chemistry step, making *S. solfataricus* Dbh similar to repair DNA polymerase beta (152). The rate limiting step of dNMP incorporation in the case of *S. solfataricus* Dpo4 is still under debate (153-156).

Very recently, using two FRET systems that monitor the motion of specific residues on each domain of the enzyme relative to the DNA substrate and the motion of the fingers subdomain relative to the LF domain, Xu *et al.* characterized the global conformational dynamics of the *S. solfataricus* Dpo4 enzyme during a single correct nucleotide incorporation event (157). Surprisingly, in contrast to high-fidelity DNA polymerases for which PPi release triggers DNA polymerase translocation (see 3.8.) FRET signal changes suggest that dNTP binding induces fast DNA translocation that frees the dNTP binding site previously occupied by the 3'-OH primer terminus. After dNTP binding and translocation, fingers and palm domains close to grip the DNA while the LF domain moves away from the fingers and the DNA. The active site is re-organized to properly align all substrates. This step is thought to be rate limiting. The phosphodiester bond forms and after catalysis, fingers and palm subdomains reopen permitting PPi release. The inward movement of the LF domain that follows may inhibit translocation. Once returned to a relaxed conformation, the enzyme may either dissociate or commit itself to a next round of nucleotide incorporation.

4.4. dNTP incorporation reaction on damaged DNA

In this section, the bypass by TLS DNA polymerases of three lesions that represent a strong block for replicative DNA polymerases are discussed: the two UV photoproducts and the abasic (AP) site. The bypass of other DNA lesions by TLS DNA polymerases has been investigated and will not be discussed here due to space constraints. (See (158-162) for N2-alkylguanine and O6-alkylguanine lesions, (143, 163-166) for 8-oxoguanine, (167, 168) for thymine glycol).

4.4.1. UV photoproducts

UV light induces the formation of two major photoproducts, *cis-syn* cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone adducts [(6-4) PPs]. The yeast DNA polymerase eta incorporates two As opposite the two Ts of a *cis-syn* TT dimer without any steric hindrance and with kinetics similar to those measured using undamaged DNA (169). The use of nucleotide analogs that specifically disrupt the Hoogsteen or Watson-Crick base pairing revealed that yeast DNA polymerase eta replicates through a *cis-syn* TT dimer by retaining both bases of the dimer in the active site and directly incorporating two As opposite the 3' T and the 5' T of the dimer by using the intrinsic Watson-Crick base pairing capacity of the lesion (169, 170). *S. solfataricus* Dpo4 is severely blocked by a *cis-syn* TT dimer and uses a different mechanism to synthesize across this lesion. Incorporation of an A across the 3' T occurs *via* an AP site-like intermediate (the enzyme "sees" the 3' T of the dimer as a non instructional nucleotide), whereas the more efficient incorporation of an A across the 5' T occurs *via* Watson-Crick base pairing (170). This mechanism was not predicted by structural studies (133). Recent *in vivo* studies performed with mammalian cells confirmed the involvement of DNA polymerase eta in the error-free bypass of UV-induced pyrimidine dimers that biochemical and structural studies had anticipated (171). In the absence of DNA polymerase eta, such as in the cells from XP-V patients, *in vivo* studies suggest cooperation between DNA polymerase iota, kappa and zeta that leads to an error-prone bypass of CPDs (172).

In contrast to *cis-syn* CPDs that have a modest effect on DNA structure, (6-4) PPs introduce a large structural distortion in the DNA. As a consequence, this lesion represents a severe block for yeast and human DNA polymerases of the Y family and biochemical analyses performed *in vitro* with DNA polymerases eta, iota and zeta had predicted an error-prone mechanism of TLS (reviewed in (122)). However, a recent study revealed a predominant error-free bypass of (6-4) PPs in human and mouse cells involving DNA polymerase zeta among other protein partners (173). In this model, an as yet unidentified polymerase carries out the accurate insertion opposite the 3' T or 3' C of a (6-4) PP, from which the DNA polymerase zeta then extends. As *in vitro* studies have not yet been able to recapitulate the *in vivo* situation, one may hypothesize that auxiliary replication factors modulate the intrinsic activity of TLS DNA polymerases.

4.4.2. AP sites

AP sites arise in DNA as a result of spontaneous depurination or during BER since the removal of a damaged base by a DNA glycosylase produces an AP site. Because no base is available to instruct incorporation of an incoming dNMP, bypass of an AP site is expected to be highly mutagenic. dAMP is preferentially incorporated opposite an AP site, a phenomenon known as the "A rule", which may rely on the greatest pi-stacking energy of this base (174). Yeast and human DNA polymerases eta (169, 175, 176), and yeast DNA polymerase zeta (177) very inefficiently incorporate nucleotides opposite an AP site. The poor efficiency of yeast DNA polymerase eta to synthesize across an AP site is consistent with the fact that DNA polymerase eta in yeast modestly contributes to translesion DNA synthesis opposite an AP site (178). This recent genetic study showed that dAMP is preferentially incorporated opposite an AP site on both leading and lagging strand templates and that DNA polymerase zeta is indispensable for the bypass of an AP site (178). Since DNA polymerase zeta can efficiently extend from nucleotides inserted opposite an AP site (177) and considering that only the catalytic activity of Rev1 is dispensable (178), it has been suggested that dAMP is incorporated opposite the AP site by the replicative polymerase. DNA polymerase zeta, structurally assisted by Rev1, then extends from the primer terminus (178). This model is supported by *in vitro* data showing that under single-hit conditions yeast replicative DNA polymerase epsilon bypasses a natural AP site with preferred incorporation of dAMP across the AP site (179). Efficient bypass of an AP site can also be reconstituted *in vitro* with the combined action of the replicative DNA polymerase delta that preferentially incorporates dAMP across the AP site, and the DNA polymerase zeta that extends from the AP site:A mispair (177).

S. solfataricus Dpo4 DNA polymerase is slightly more efficient than human DNA polymerase zeta at incorporating a dNMP across an AP site and the preferred nucleotide incorporated opposite the AP site is dAMP (176). Human DNA polymerase iota is one of the few DNA polymerases that does not follow the A-rule as it slightly preferentially incorporates dGMP opposite an AP site. Structural studies (180) indicate that the AP lesion and the incoming dNTP are confined in a constricted active site cleft. Each of the three tested dNTPs (dGTP, dATP, dTTP) is engaged in a specific network of hydrogen bonds to fill the "void" opposite the lesion. The difference in patterns of hydrogen bonds and stacking interactions between the three dNTPs studied may underlie the small preference of DNA polymerase iota for the insertion of dGTP over the other dNTPs opposite an AP site.

AP site bypass also results in frameshift errors, and single base deletions may constitute up to 10-25% of bypass events (176). It has been proposed that *E. coli* DNA polymerase IV generates a -1 frameshift deletion when bypassing an AP site by a dNTP-stabilized misalignment mechanism (181). This model is supported by the X-ray crystal structure of *S. solfataricus* Dpo4 complexed with a p-t junction containing an AP site in which the incoming dNTP does not pair with the AP site but instead pairs with the base 5' of the lesion (139).

All the structural and biochemical features described in this section are relevant to the ability of the Y family of DNA polymerases to bypass lesions more efficiently than high-fidelity DNA polymerases. The recruitment of a given TLS polymerase at a lesion site may be dictated by the chemical nature of the DNA damage itself. For instance, *in vivo* and *in vitro* studies have clearly established the implication of *E. coli* DNA polymerase V in UV mutagenesis (182). In humans, DNA polymerase eta is the main cellular DNA polymerase coping faithfully with CPDs adducts (183). Nevertheless as *in vitro* systems do not always reproduce *in vivo* situations, it is possible that auxiliary cellular factors modulate the intrinsic DNA synthesis activity of TLS DNA polymerases. The next section focuses on the functional interaction between DNA polymerases and auxiliary proteins and their functional consequences.

5. INFLUENCE OF DNA POLYMERASE AUXILIARY PROTEINS ON DNA POLYMERASE ACTIVITY

In vivo, DNA polymerases work in concert with an ensemble of cellular proteins that regulate their DNA synthesis activity. For instance, it has been recently proposed that the selection of an error-free TLS DNA polymerase over an error-prone DNA polymerase at a specific damaged site may rely on protein-protein interactions (184). Similarly, *E. coli* $UmuD_2$ is a protein encoded by the *UmuDC* operon that, upon proteolytic cleavage and association with UmuC, constitutes DNA polymerase V. Recent studies showed that in *E. coli*, $UmuD_2$ interacts with DNA polymerase IV and increases DNA polymerase fidelity on homopolymeric nucleotide runs by inhibiting template slippage and slipped intermediate formation (185, 186). Among the replication factors that interact with the DNA polymerase at the fork are the processivity factor (also called sliding clamp) and the single-stranded DNA binding (SSB) protein. The replicative helicase is also a central component of the replisome as its directional double-stranded DNA unwinding activity provides the naked single-stranded DNA that will be ultimately copied by the DNA polymerases. A functional interaction between the replicative DNA polymerase and the replicative helicase has been demonstrated in the case of *E. coli* (187, 188), the bacteriophages T4 (189) and T7 (190). Its main function is to stimulate the DNA synthesis activity of the DNA polymerase and the unwinding activity of the helicase. A model of mutagenesis involving uncoupling between the replicative helicase and the leading DNA polymerase has been proposed to explain that trinucleotide repeats tend to delete in dividing cells and that their instability depends on the orientation of the replication fork (191). The Werner helicase, a helicase deficient in patients suffering from the Werner syndrome, has been reported to specifically stimulate DNA polymerase delta (192, 193) and some TLS DNA polymerases including DNA polymerase eta, kappa and iota (194). The stalling of a replication fork induced either by a lesion or an unusual secondary structure on the templating DNA strand may be rescued by the cellular coupling between the Werner helicase and the DNA polymerases. The functional interaction between helicase and DNA polymerase will not be discussed here due to space constraints, and the following section describes the recent progress made on the physical and functional interactions between the DNA polymerase, its processivity factor and the SSB protein on undamaged and damaged DNA.

5.1. Processivity factor

The processivity of a DNA polymerase can be defined as the number of dNMPs incorporated by the enzyme between two dissociation events. At physiological salt concentrations, processivity of most DNA polymerases is low, limited to a few nucleotides, and replicative DNA polymerases acquire the processivity needed for genome duplication by physically interacting with a processivity factor.

5.1.1. Advantages of a ring-shaped oligomeric structure: stability and multiple identical binding sites

In *E. coli*, bacteriophage T4 and in Eukaryotes, the processivity factor has the shape of a ring that once loaded onto DNA by a clamp loader - the protein complex that loads the processivity factor at a p-t junction - physically encircles the DNA. Most of the processivity factors have an oligomeric structure; therefore their stability on circular double-stranded DNA depends on the strength of the interaction between subunits. The *E. coli* processivity factor, known as the beta clamp, and the eukaryotic processivity factor PCNA are highly stable once loaded onto DNA with a half-life time of one hour and 24 minutes, respectively (195). Their clamp loader must be used to unload them after completion of an Okazaki fragment to permit their recycling (195). Once loaded around DNA, the processivity factor can move freely along double-stranded DNA, as revealed by single molecule approaches (196, 197). Interaction between the processivity factor and the DNA may also contribute to the stability of the protein on the DNA. Indeed, in the crystal structure of the *E. coli* beta clamp bound to a p-t junction, interactions between the protein and the double-stranded and single-stranded part of the primed DNA are observed (198). Surprisingly, the p-t junction binds the *E. coli* beta clamp in a reverse orientation. Interaction with the double-stranded part of the p-t junction is allowed by a sharp tilt (22°) in the DNA. Furthermore, although the interaction observed between the single-stranded region of the p-t junction and the *E. coli* beta clamp involves an adjacent clamp, the authors propose that this interaction (i) provides specificity for a single-stranded/double-stranded junction with a 5' single-stranded tail, (ii) facilitates ring closure around the DNA during the clamp-loading process, and (iii) holds the protein at the primed site where it will ultimately interact with the DNA polymerase (198). Keeping the processivity factor at the primed site is also facilitated by the single-stranded DNA binding protein, as diffusion of the *E. coli* beta clamp along double-stranded DNA is strongly inhibited on a primed DNA coated by the *E. coli* SSB protein (196). Finally, due to their oligomeric structure, processivity factors can recruit several proteins and serve as a molecular tool belt (199, 200). As discussed below (see 5.1.4.), this characteristic is at the basis of current models for lesion bypass *in vivo*.

5.1.2. Processivity factor and stability of the DNA polymerase on DNA

Determination of thermodynamic parameters, such as the dissociation constant Kd of a DNA polymerase from a p-t junction or the half-life time of a DNA polymerase bound to a p-t junction, showed that the processivity factor increases the affinity of the DNA polymerase for a p-t junction. For example, the affinity of the *E. coli* DNA polymerase III for a p-t junction is increased at least 5 fold by the beta clamp (201). The same is true of the T4 processivity factor that increases the affinity of the T4 DNA polymerase nearly 170 fold (202, 203). The *E coli* DNA polymerase IV cannot form a stable complex with a p-t junction in the absence of the beta clamp whereas its half-life time on a p-t junction in the presence of the beta clamp is around 2 minutes (204), surprisingly less than the half-life time of the beta clamp alone (one hour; (195)). Measurements of the half-life times of the calf thymus DNA polymerase delta on a p-t junction in the absence or presence of PCNA indicate that PCNA stabilizes the DNA polymerase delta on the p-t junction more than 1900 fold, leading to a half-life time for the PCNA-polymerase delta complex on a p-t junction of more than two hours (205). Stabilization of a replicative DNA polymerase by its processivity factor on a p-t junction leads to highly processive DNA synthesis. In addition the processivity factor may stimulate the rate of the dNMP incorporation reaction of some DNA polymerases (*e.g.* yeast DNA polymerase delta (206, 207)), thus permitting fast and processive DNA synthesis *in vivo*.

During lagging strand DNA synthesis, the lagging strand DNA polymerase must rapidly dissociate after completion of each Okazaki fragment and be recycled to a new upstream RNA primer to initiate synthesis of the next Okazaki fragment. Recycling of the lagging DNA polymerase can be achieved either by a collision release or by a premature release mechanism. In the collision release mechanism, the DNA polymerase disengages from the processivity factor and from the DNA upon completion of an Okazaki fragment. This recycling mode is used in most circumstances by *E. coli* DNA polymerase III (208) and yeast lagging DNA polymerase delta (209). In *E. coli* it has recently be shown that the loss of single-stranded DNA at the junction with the beginning of an Okazaki fragment is perceived by two single-stranded DNA binding elements of the DNA polymerase III holoenzyme, the OB fold of the alpha subunit of DNA polymerase III and the tau subunit of the clamp loader. As a consequence, the affinities of DNA polymerase III for DNA and for the beta clamp are lowered, triggering release of the DNA polymerase from the DNA and its processivity factor (201, 210). In the second type of recycling, designated premature release mechanism or premature signaling mechanism, which is dominant in the case of the T4 replication system (211), the lagging DNA polymerase disengages from the processivity factor before finishing an Okazaki fragment. Priming and clamp loading onto the primed DNA are proposed to be part of this mechanism of lagging strand DNA polymerase recycling (211).

It is interesting that even though human DNA polymerases eta, iota, kappa and lambda physically interact with human PCNA, only DNA polymerase lambda becomes significantly more processive in the presence of the processivity factor, suggesting that the major function of the interaction between PCNA and TLS DNA polymerases might be to target these TLS polymerases to a stalled replication machinery rather than increasing their processivity (212-215). Considering the low fidelity of TLS DNA polymerases, low processivity for these enzymes is nevertheless desirable.

5.1.3. Thioredoxin: an example of a non ring-shaped monomeric processivity factor

Bacteriophage T7 does not rely on a ring-shaped protein to provide processivity to its DNA polymerase. To insure its propagation, the phage borrows from its host, *E. coli*, a small protein of 12 kDa, thioredoxin, that forms a tight 1:1 complex with T7 DNA polymerase (for a review on the T7 DNA polymerase see (216)). The affinity of the processive DNA polymerase for a p-t junction is increased at least 20 fold compared to that of the unprocessive T7 DNA polymerase (217). The mechanism by which thioredoxin confers processivity to the T7 DNA polymerase has recently been unraveled using the single molecule imaging approach (218). Direct visualization of the non specific interaction between fluorescently labeled T7 DNA polymerase and double-stranded DNA led to the characterization of the diffusion of the enzyme along double-stranded DNA under various conditions. Analysis of the salt dependence of diffusion helped define the translocation mode used by processive and non processive T7 DNA polymerase and made it possible to distinguish between a hopping and a sliding mechanism of diffusion. The results indicate that contrary to the non processive T7 DNA polymerase that microscopically hops as it diffuses along double-stranded DNA, the processive T7 DNA polymerase slides along double-stranded DNA without dissociating during the transfer from one binding site to another. Therefore thioredoxin increases T7 DNA polymerase processivity by suppressing microscopic hopping of the enzyme on and off the DNA. Increased affinity of the processive DNA polymerase for double-stranded DNA is possibly due to the additional electrostatic interaction found in the complex between processive polymerase and double-stranded DNA.

5.1.4. Introducing the notion of dynamic processivity

Recent measurements of DNA polymerase processivity using wild type and specific mutant DNA polymerases in the context of a full replisome led us to revisit our vision of processivity and think of processivity as a dynamic process. Indeed, studies performed with the

T4 replication machinery showed that DNA polymerases could exchange at the replication fork without affecting continued DNA synthesis (219). It was then suggested that the processivity factor mediates DNA polymerase exchange by binding the replicating DNA polymerase and a spare DNA polymerase available to replace the replicating DNA polymerase disengaged from the terminus of the primer. The capacity of the processivity factor to bind multiple DNA polymerases is certainly made possible by the oligomeric structure of most processivity factors, although a monomeric processivity factor may in principle carry multiple binding sites for DNA polymerases. Considering that the crystal structure suggests a loose and flexible connection between the DNA polymerase and its processivity factor (220), the processivity factor may simply tether the DNA polymerase. It is therefore likely that the enhanced processivity conferred by the processivity factor stems from the increased local concentration of DNA polymerase and p-t junction since both factors interact with the processivity factor. Consequently, a DNA polymerase that has transiently dissociated from its substrate has a higher probability to rebind to it (221). Applied to a replicative DNA polymerase stalled by damage on the template, this exchange mechanism facilitates the transfer of a p-t junction to a specialized DNA polymerase capable of lesion bypass, provided the TLS polymerases interact with the processivity factor. Interestingly, it has been reported that all five *E. coli* DNA polymerases interact with the beta clamp, further validating the idea of the sliding clamp as a tool belt carrying additional replication proteins whose activities may be required under specific circumstances (199). A precise description of the interaction between each DNA polymerase and the processivity factor during protein exchange is still lacking. Whereas the tool belt model states that the stalled DNA polymerase interacts with a specific domain (named the hydrophobic cleft) of one monomer of the processivity factor and the spare DNA polymerase with the hydrophobic cleft of the second monomer (Figure 8A), a recent study combining *in vivo* and *in vitro* approaches proposes a variation of this model (222, 223). In this model (Figure 8B), the stalled DNA polymerase interacts with the hydrophobic cleft of one subunit of the processivity factor and the spare DNA polymerase with the so-called rim domain of the adjacent subunit. During DNA polymerase exchange, the DNA polymerases simply trade positions on the processivity factor and the DNA does not need to be "shuttled" from one protomer of the processivity factor to the other, as required by the tool belt model (Figure 8A). To facilitate DNA polymerase exchange, the spare DNA polymerase bound to the processivity factor may weaken the interaction between the stalled DNA polymerase and the processivity factor as suggested in the case of the switch between the *E. coli* DNA polymerase III holoenzyme and DNA polymerase IV (224).

A similar mechanism of DNA polymerase exchange has been proposed in the case of the T7 DNA replication machinery except that the replication protein that stores spare DNA polymerases is not the processivity factor but the helicase (225). A recent study refined this model by defining two modes of binding of the processive DNA polymerase to the helicase: a tight polymerizing mode that is used by the DNA polymerase when it synthesizes DNA, and an electrostatic mode that retains the spare DNA polymerase close to the DNA (226). In *E. coli*, it is possible that the DNA polymerase interacting with the hydrophobic cleft or that interacting with the rim domain of the beta clamp represents two binding modes of the processive DNA polymerase.

5.1.5. Effect of the processivity factor on DNA polymerase fidelity on undamaged DNA

Since several genetic studies performed either in yeast (227, 228) or in mammalian cells infected by the herpes simplex virus type 1 (229, 230) identified mutations in the processivity factor that increased the mutation rate, it was legitimate to investigate the effect of the processivity factor on the fidelity of DNA polymerases on undamaged DNA (Table 2). In a DNA synthesis-fidelity assay in which a single dNTP is provided to extend a primer annealed to a template, PCNA promotes misincorporation catalyzed by the calf thymus DNA polymerase delta (231). In a similar assay, the processivity factor of herpes simplex virus type 1 has been shown to increase the fidelity of its DNA polymerase (232). This enhanced fidelity possibly relies on the increased residence time of the DNA polymerase on DNA by the processivity factor giving more opportunities to the DNA polymerase to excise a misincorporated dNMP. Another DNA synthesis-fidelity assay that scores errors made during the filling of a short gap showed that thioredoxin can enhance or reduce the fidelity of the T7 DNA polymerase depending on the error considered (233). Using the same assay, similar results were found for the T4 DNA replication system (234). In the case of the RB69 DNA polymerase, the processivity factor decreased polymerase fidelity by a small factor (1.6) (235). An increase in the formation of mispairs and misaligned intermediates, a decrease in the proofreading efficiency or an increase in the extension efficiency of a mispair or slipped intermediate may account for this effect (235). More recently, the yeast PCNA was reported to strongly suppress (by $\approx$ 10 fold) the formation of large deletions occurring between direct repeats during a gap-filling reaction performed *in vitro* by the yeast DNA polymerase delta, by possibly preventing the primer terminus from fraying and reannealing to the downstream repeat (236). On the other hand, it has been reported that the yeast PCNA had little effect on the fidelity of TLS DNA polymerase zeta on undamaged DNA, increasing or decreasing fidelity by at most a factor of 2 depending on the error considered (237).

5.1.6. Effect of the processivity factor on the capacity of DNA polymerases to bypass lesions

Possibly related to its function of tethering DNA polymerases, the processivity factor has been shown to increase the lesion bypass capability of some DNA polymerases (Table 3). For example, PCNA has been reported to be an important accessory factor for incorporation across the 8-oxoguanine and extension beyond this lesion by calf thymus DNA polymerase delta (238, 239). The same is true of CPDs (240), AP sites and C8-(aminofluorenyl)guanine modifications (238).

Table 2. Effect of the processivity factor on the fidelity of various DNA polymerases on undamaged DNA

DNA polymerases	Modification of the fidelity of the DNA polymerase by the processivity factor on undamaged DNA	References
Calf thymus DNA polymerase delta	Decrease of fidelity	(231)
Herpes simplex virus type 1 DNA polymerase	Increase of fidelity	(232)
T7 DNA polymerase	Increase or decrease of fidelity	(233)
T4 DNA polymerase	Increase or decrease of fidelity	(234)
RB69 DNA polymerase	Decrease of fidelity	(235)
Human DNA polymerase lambda	No effect	(255)
Yeast DNA polymerase zeta	Increase or decrease of fidelity	(237)

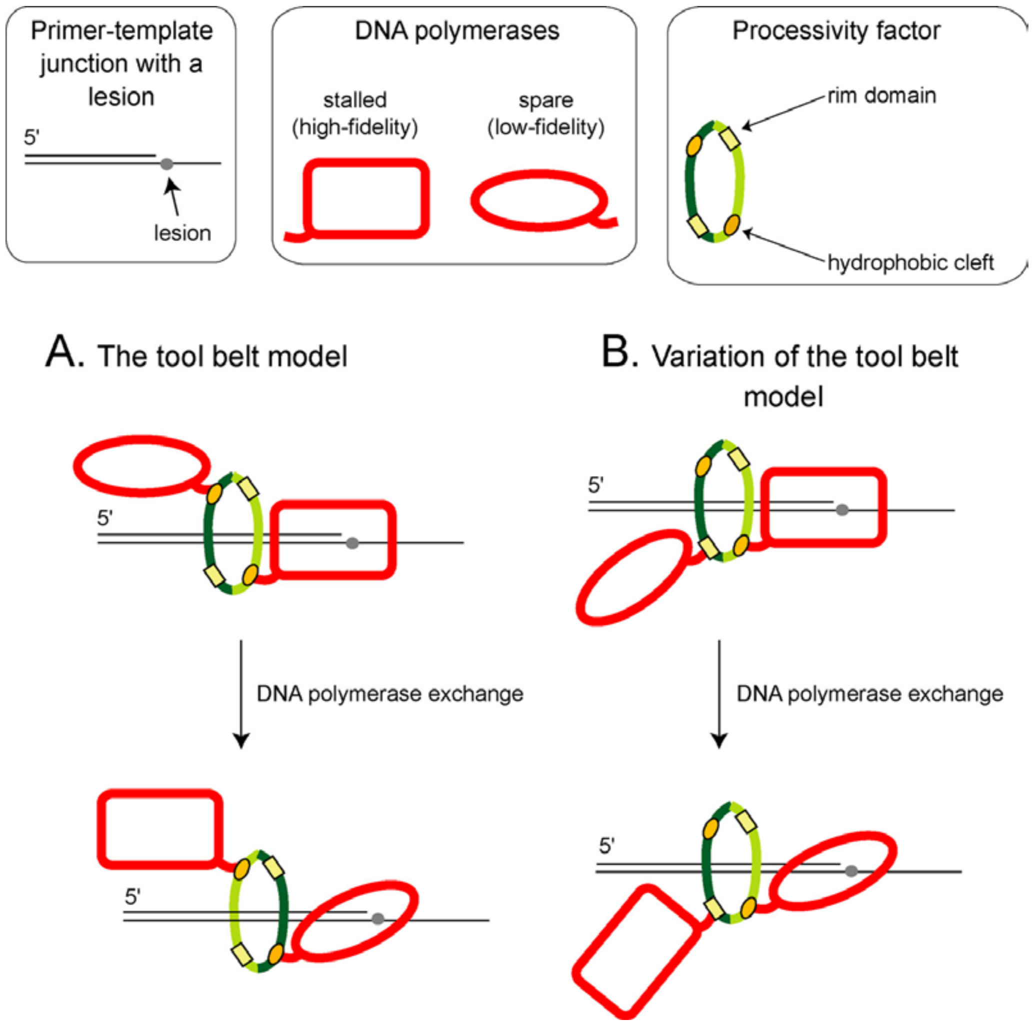


Figure 8. Models of the DNA polymerase exchange at a stalled replication fork. Top: The lesion that blocks the replication fork is shown as a grey ball on the template strand. The stalled (high-fidelity) DNA polymerase is shown as a red rounded rectangle and the spare (low-fidelity) DNA polymerase as a red oval. The processivity factor is a presented as a homo-dimer composed of a light and a dark green half-oval. Each subunit of the processivity factor carries a rim domain (black rectangle filled in yellow) and a hydrophobic cleft (black oval filled in orange). To circumvent the blockage of DNA synthesis, the spare DNA polymerase trades position with the stalled DNA polymerase at the site of the lesion. A. In the tool belt model the stalled and the spare DNA polymerases are both bound to the dimeric processivity factor through the hydrophobic cleft of each subunit. During DNA polymerase exchange, the DNA polymerases maintain their interaction with the processivity factor *via* the hydrophobic cleft. The processivity factor undergoes a rotation around the axis of the DNA to position the previously spare DNA polymerase at the site of the lesion. B. The variation of the tool belt model proposes that the stalled DNA polymerase binds one subunit of the processivity factor through the hydrophobic cleft and the spare DNA polymerase binds the second subunit of the processivity factor through the rim domain. During DNA polymerase exchange, the two DNA polymerases trade position on the sliding clamp. The previously spare DNA polymerase ends up interacting with the processivity factor through the hydrophobic cleft and is ready to cope with the lesion whereas, the previously stalled DNA polymerase interacts with the rim domain of the other subunit of the processivity factor.

Table 3. Effect of the processivity factor on the capacity of various DNA polymerases to bypass lesions

DNA polymerases	Lesion	Modification of the capacity of the DNA polymerase to bypass lesion by the processivity factor	References
Calf thymus DNA polymerase delta	8-oxoguanine CPD Abasic site C8-aminofluorenylguanine	Increase of lesion bypass capacity (Incorporation + Extension steps)	(239) (240) (238) (238)
Human DNA polymerase delta	Abasic site	No effect	(215)
Human DNA polymerase lambda	Abasic site	Increase of lesion bypass capacity (Extension step)	(215)
T4 DNA polymerase exo+ T4 DNA polymerase exo-	Abasic site Abasic site	No effect Increase of lesion bypass capacity (Incorporation + Extension steps)	(242)
Yeast DNA polymerase delta	Abasic site	Increase of lesion bypass capacity (Incorporation + Extension steps)	(241)
Yeast DNA polymerase zeta	Abasic site	Increase of lesion bypass capacity (Incorporation + Extension steps)	(241)
Yeast DNA polymerase eta	Abasic site	Increase of lesion bypass capacity (Incorporation + Extension steps)	(241)

Table 4. Effect of the processivity factor on the fidelity of various DNA polymerases on damaged DNA

DNA polymerases	Lesion	Modification of the fidelity of the DNA polymerase by the processivity factor	References
Human DNA polymerase beta	8-oxoguanine	No effect	(243)
Human DNA polymerase delta	8-oxoguanine	Increase of fidelity	(243)
Human DNA polymerase lambda	8-oxoguanine	Increase of fidelity	(243)
Human DNA polymerase eta	8-oxoguanine	Increase of fidelity	(243)

Mechanistically, PCNA can alter the dissociation rate constant (k_{off}) of the DNA polymerase from the p-t junction, the affinity of the binary complex for dNTPs or the rate of the conformational change of the DNA polymerase (239). Yeast PCNA has also been shown to stimulate DNA polymerases delta, eta and zeta in the bypass of an AP site (241). Interestingly, the human PCNA did not stimulate elongation of the recombinant human DNA polymerase delta past an AP site but had a stimulatory effect on human DNA polymerase lambda (215). The T4 processivity factor allows the T4 DNA polymerase deficient in 3’->5’ exonuclease activity to synthesize across an AP site lesion located on a circular DNA substrate, but has no effect on the wild type enzyme (242). This new property of bypassing lesions gained by the loss of proofreading activity has to be taken into account considering that mice bearing a mutation in the proofreading activity of the replicative DNA polymerases delta (nucleus) (82, 83) or gamma (mitochondria) (84) develop epithelial cancers and show signs of premature ageing.

5.1.7. Effect of the processivity factor on the fidelity of DNA polymerases to bypass lesions

Considering the stimulatory effect of the processivity factor on the capacity of various DNA polymerases to bypass lesions, it is reasonable to characterize the effect of this factor on the fidelity of DNA polymerases on damaged DNA (Table 4). When incorporation of dNMP by human DNA polymerases delta, eta and lambda across an 8-oxoguanine was investigated in the absence or presence of PCNA, it was found that PCNA can suppress dAMP incorporation and favor incorporation of dCMP opposite the lesion by a factor of 6, 10 and 2, respectively (243). The mechanism underlying this phenomenon still needs to be elucidated. No effect of the human PCNA on human DNA polymerase beta was observed (243).

In conclusion, the effect of the processivity factor on the fidelity of the DNA polymerases on undamaged DNA seems to depend on the DNA polymerases and the type of errors (Table 2). In contrast, a unifying rule can be drawn from the study of the processivity factor on the lesion bypass capacity of DNA polymerases. This factor indeed stimulates the capacity of most DNA polymerases to bypass DNA lesions (Table 3). Although studied with only a single lesion, the 8-oxoguanine, PCNA increases the fidelity of most of the DNA polymerases (Table 4) by a still unknown mechanism.

5.2. Single-stranded DNA binding protein

SSB proteins are ubiquitous and essential proteins involved in multiple aspects of DNA metabolism (for reviews see (244-249)). The primary function of SSB proteins is to cover single-stranded naked DNA exposed by helicases or nucleases to prevent secondary structure formation or nuclease digestion. In addition, SSB proteins bind multiple protein partners, and facilitate the assembly of genome maintenance complexes and the coordination of their activities.

It is well established that the SSB protein stimulates DNA polymerases, primarily by removing secondary structures that can impede DNA polymerase progression. For example, the DNA synthesis activity of human DNA polymerase lambda is specifically stimulated by the human SSB protein, the human replication protein A (RPA), in a dose-dependent manner, while its nucleotidyl transferase activity is inhibited (250). We recently reported that the T4 SSB protein relieved the T4 DNA polymerase impediment created by trinucleotide repeat sequences *in vitro* (191). Interestingly, a recent study using the FRET-based approach to study single DNA polymerases undergoing DNA synthesis through a DNA hairpin indicates that the rate of strand displacement DNA synthesis is not slower than the rate of primer extension, and that DNA polymerase pauses depend on the primary structure of the DNA and not on the secondary structure of the DNA template (251). This result is in agreement with a bulk DNA synthesis assay showing that DNA polymerase progression is not always correlated with the thermodynamic stability of the DNA template (191). In this

Table 5. Effect of SSB protein on the fidelity of various DNA polymerases

DNA polymerases	DNA	Modification of the fidelity of the DNA polymerase by SSB proteins	References
Human DNA polymerase alpha	undamaged DNA 8-oxoguanine	Increase of fidelity Increase of fidelity	(254) (243)
Human DNA polymerase beta	8-oxoguanine	No effect	(243)
Human DNA polymerase lambda	undamaged DNA	Increase of fidelity	(255)
Yeast DNA polymerase delta	Direct repeats	Increase of fidelity	(236)
Yeast DNA polymerase zeta	Undamaged DNA	Increase of fidelity	(237)

Table 6. Combined effect of the processivity factor and SSB protein on the fidelity of various DNA polymerases

DNA polymerases	DNA	Modification of the fidelity of the DNA polymerase by the processivity factor and SSB protein	References
Human DNA polymerase beta	8-oxoguanine	Increase of fidelity	(243)
Human DNA polymerase delta	8-oxoguanine	Increase of fidelity (as with PCNA alone)	(243)
Human DNA polymerase lambda	Undamaged DNA 8-oxoguanine 2-hydroxy-adenine	Increase of fidelity (as with RPA alone) Increase of fidelity Increase of fidelity	(255) (243) (257)
Human DNA polymerase eta	Undamaged DNA 8-oxoguanine	Increase of fidelity Increase of fidelity	(212) (243)
Human DNA polymerase kappa	Undamaged DNA	Increase of fidelity	(214)
Human DNA polymerase iota	Undamaged DNA 8-oxoguanine	Increase of fidelity Increase of fidelity	(213) (243)
Yeast DNA polymerase delta	Direct repeats 8-oxoguanine	Increase of fidelity Very little effect	(236) (166)
Yeast DNA polymerase eta	Undamaged DNA cis-syn TT dimer 8-oxoguanine	Very little effect Very little effect Very little effect	(166) (256)
Yeast DNA polymerase zeta	Undamaged DNA	Increase of fidelity	(237)

context, SSB proteins might remodel single-stranded DNA such that the primary sequence would no longer influence the DNA polymerase activity. Pauses in DNA synthesis would then be reduced.

During unperturbed DNA replication, SSB proteins bind to the lagging strand template exposed by the helicase, and the lagging DNA polymerase copies the lagging strand template covered by SSB proteins. In Eukaryotes, DNA polymerase delta has been suggested to be responsible for copying the lagging strand template (252) and, contrary to *E. coli* in which DNA polymerase I is involved in Okazaki fragment maturation, DNA polymerase delta is also required for Okazaki fragment processing. During the processing of Okazaki fragments, human RPA limits the strand displacement activity of human DNA polymerase delta such that the size of the displaced strand does not exceed 30 nucleotides (253). This critical size corresponds to the stretch of nucleic acid synthesized by the DNA polymerase alpha-primase and to the size of the RPA binding site. This suggests that once the displaced strand reaches 30 nucleotides in length, RPA binds to it, and the strand displacement activity of DNA polymerase delta is inhibited. The stabilized flap structure is then an ideal substrate for the DNA2 helicase and the flap-specific endonuclease FEN1. The nicked DNA that is then produced after removal of the flap is sealed by the DNA ligase.

Table 5 summarizes the results of studies investigating the potential influence of the SSB protein on DNA polymerase fidelity. In 2001, it was proposed that RPA serves as a "fidelity clamp" for the DNA polymerase alpha-primase since it significantly (up to 6 fold) reduces misincorporation efficiency of this enzyme (254). Detailed kinetic studies of correct *versus* incorrect single nucleotide addition indicate that RPA decreases both the Michaelis constant Km and the catalytic constant kcat for misincorporations, whereas it only slightly stimulates correct nucleotide incorporation. The same suppression of misincorporation by human RPA has been reported for human DNA polymerase lambda (255). Precise stoichiometry between DNA polymerase lambda and RPA is required, and this effect is specific of RPA since neither PCNA, BSA nor *E. coli* SSB could reproduce this effect (250, 255). The increased fidelity of DNA polymerase lambda by RPA is mainly due to decreased affinity (by a factor of 10 to 50) of the binary complex for the mismatched nucleotides. In the case of yeast DNA polymerase zeta, its average base substitution fidelity on undamaged DNA is increased ≈ 2 fold by yeast RPA (237). Like yeast PCNA and possibly using a similar mechanism, yeast RPA can strongly suppress (≈ 10 fold) the formation of large deletions occurring between direct repeats during a gap-filling reaction performed *in vitro* with yeast DNA polymerase delta (236).

5.3. Combined effects of the processivity factor and the SSB protein

To mimic a more physiological reaction, the combined effect of the processivity factor and the SSB protein on the fidelity of DNA synthesis has been investigated for a variety of DNA polymerases, especially TLS DNA polymerases, on undamaged and damaged DNA (Table 6).

5.3.1. Fidelity on undamaged DNA

On undamaged DNA, PCNA and RPA increase the efficiency of human DNA polymerase eta to insert the correct dNMP ≈ 15 fold (212). The same is true of human DNA polymerase kappa (stimulation of 50 to 200 fold, (214)), and human DNA polymerase iota (stimulation of 80 to 150 fold, (213)). For all three DNA polymerases this stimulation is due to a decrease in the Km value for the

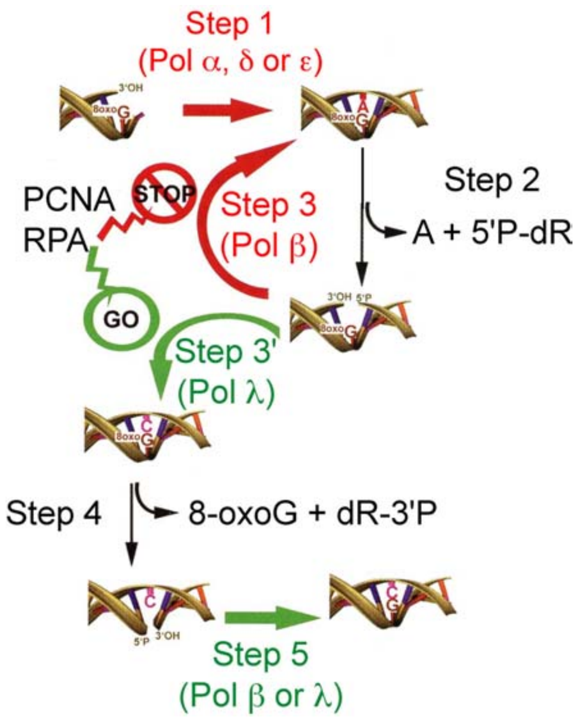


Figure 9. Model of coordinated action of RPA, PCNA and DNA polymerase lambda for the error-free repair of an 8-oxoguanine lesion. The error-free and error-prone steps are in green and red, respectively. Step 1: Error-prone DNA synthesis across an 8-oxoguanine by the replicative DNA polymerase (alpha, delta or epsilon) leads to the formation of an 8-oxoguanine:A mispair. Step 2: In humans, the repair of this mismatch involves proteins from the MYH-dependent BER pathway (including the MutY Homolog glycosylase and AP endonuclease 1 (APE1)) that remove the adenine (A) across the lesion and a 5' phosphate deoxyribose (5'P-dR), leaving a one-nucleotide gapped intermediate. Step 3: dAMP can be incorporated across the 8-oxoguanine (error-prone repair by DNA polymerase beta) and the pathway returns to step 2. Step 3': Alternatively, PCNA and RPA can facilitate the recruitment of DNA polymerase lambda on the gapped substrate that preferentially incorporates a dCMP across the lesion, allowing an error-free bypass of the lesion. The 8-oxoguanine lesion paired with a dCMP can be faithfully repaired by the 8-oxoguanine glycosylase-dependent BER pathway. Step 4: The damaged base (8-oxoG) and the deoxyribose 3' phosphate (dR-3'P) are excised. Step 5: This leaves a one-nucleotide gapped intermediate that can be filled in an error-free manner by DNA polymerase beta or lambda. Adapted with permission from (272).

correct incoming dNTP. The effect of PCNA and RPA on the fidelity of DNA polymerase eta and zeta has also been investigated using a DNA synthesis-fidelity assay that scores errors made during the filling of a short gap. Using this assay, it was found that the fidelity of the yeast DNA polymerase eta on undamaged DNA was not significantly (≤ 2) affected by PCNA and RPA (256) and that the average fidelity of base substitution of yeast DNA polymerase zeta on undamaged DNA was increased ≈ 2 fold by yeast RPA and PCNA (237). On the other hand, the combined effect of yeast PCNA and RPA makes it possible to diminish deletions between direct repeats generated by DNA polymerase delta by a factor of ≈ 90 (236).

5.3.2. Capacity of bypassing lesions

The capacity of the human DNA polymerase kappa to insert dAMP across an AP site is stimulated by the presence of PCNA and RPA. However, the efficiency of this reaction is very low, and an AP site still remains a strong block for this DNA polymerase (214). Among the UV photoproducts, the *cis-syn* TT dimer constitutes a strong block for the human DNA polymerases iota (213) and kappa (214) even in the presence of PCNA and RPA. Addition of these two factors stimulates the incorporation of human DNA polymerase iota opposite the 3' T of a (6-4) TT photoproduct or an AP site, by 30 or 60 fold, respectively. Extension of these intermediates is however blocked, even in the presence of PCNA and RPA (213).

5.3.3. Fidelity on damaged DNA

By performing primer extension assays with a primer annealed to a template containing a specific lesion and analyzing the bypass products both *in vivo* and *in vitro*, it was found that the yeast PCNA and RPA had very little effect on the fidelity of yeast DNA polymerase eta across *cis-syn* TT dimers or 8-oxoguanine lesions (166, 256). The same was true of yeast DNA polymerase delta across an 8-oxoguanine lesion (166). The bypass of 2-hydroxy-adenine lesions by human DNA polymerase lambda has recently been investigated in the presence of RPA and PCNA (257). Whereas the ratio to incorporate a dTMP *versus* a dGMP opposite a 2-hydroxy-adenine equals 23:1 in the absence of PCNA and RPA, it rises to 166:1 in the presence of these two factors. Steady state kinetics indicates that PCNA and RPA impede dGMP incorporation by increasing the Km and lowering the kcat for this specific dNTP. A recent study investigated the DNA synthesis activity of a variety of human DNA polymerases across an 8-oxoguanine lesion in the presence of PCNA and RPA (243). A major and specific effect of these two factors was found for the DNA polymerases eta and lambda. RPA and PCNA inhibit incorporation of dAMP *versus* dCMP opposite the lesion, raising the preference for error-free *versus* error-prone bypass to 68 and 1200 fold for DNA polymerases eta and lambda, respectively. In contrast, DNA polymerases delta, beta and iota showed a much lower efficiency of faithful bypass.

5.3.4. Coordinated activity of DNA polymerases, RPA and PCNA to faithfully repair A:8-oxoguanine mismatches

Despite the existence of accurate mechanisms that reduce the deleterious consequences of 8-oxoguanine lesions, dAMP can still be incorporated across an 8-oxoguanine adduct (step 1, Figure 9). In humans, the first step in the repair of this mispair is removal of the adenine by the combined action of the MutY homolog DNA glycosylase, the AP endonuclease 1 (APE1) and a dRP lyase, leading to the formation a one-nucleotide gapped intermediate with the 8-oxoguanine as the templating base to be copied (step 2, Figure 9). A recent biochemical study

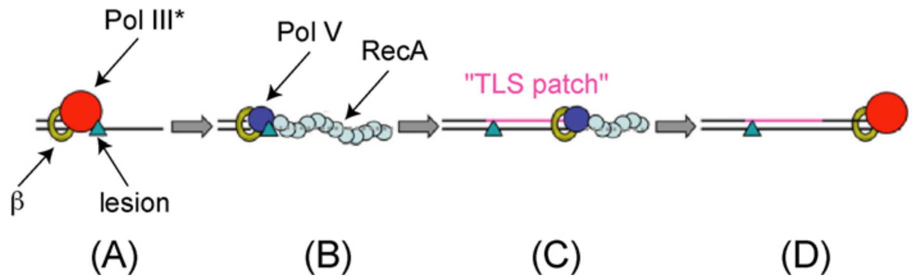


Figure 10. Simplified model for the error-free TLS mediated by the *E. coli* DNA polymerase V. Pol III* (red ball) refers to DNA polymerase III holoenzyme, beta (yellow ring) to the beta sliding clamp or processivity factor, and Pol V (blue ball) to DNA polymerase V. In state (A), PolIII* is blocked by a lesion (turquoise filled triangle). The efficient bypass of the lesion in an error-free manner requires DNA polymerase V (i) to be stabilized at the lesion site by the beta clamp, and (ii) to be activated by a RecA nucleofilament (light blue balls) provided either in *cis* (as shown in state (B)) or in *trans*. Once activated, DNA polymerase V bypasses the lesion and synthesizes a short patch of DNA (as shown in state (C)). The patch of DNA must however be long enough to allow elongation (and not exonucleolytic digestion) by the DNA polymerase III holoenzyme after a second DNA polymerase exchange event (as shown in state (D)). Adapted with permission from (263).

mimicked the repair of such a one-nucleotide gapped substrate and showed that PCNA and RPA activate the highly efficient and faithful repair activity of DNA polymerase lambda (step 3', Figure 9) while repressing DNA polymerase beta activity (step 3, Figure 9) (258). Indeed, binding studies showed that in the presence of RPA, DNA polymerase lambda is more efficiently recruited at the lesion site than DNA polymerase beta. In addition, at physiological salt concentrations, RPA and PCNA favor dCMP incorporation across the 8-oxoguanine by DNA polymerase lambda (step 3', Figure 9; see 5.3.3.). The correct C:8-oxoguanine base pair can be faithfully repaired by the enzymes from the BER pathway including the 8-oxoguanine glycosylase, APE1, a dRP lyase (step 4, Figure 9) and the DNA polymerase beta or lambda (step 5, Figure 9). This study demonstrates how PCNA and RPA coordinate the selection of the appropriate DNA polymerase during the repair of an 8-oxoguanine located in a one-nucleotide gapped structure. The precise role of PCNA and RPA still needs to be investigated since on such a one-nucleotide gapped intermediate processivity is not required and no RPA binding site exists.

5.3.5. Coordination of protein activity during the error-free bypass of a strongly blocking lesion

Another example of protein orchestration that has been beautifully reconstituted *in vitro* is the faithful bypass of the strong blocking AAF lesion using purified proteins from *E. coli* (Figure 10) (259-263). In *E. coli*, error-free bypass of an AAF adduct is mediated by DNA polymerase V. Also required for this process are the processivity factor (beta clamp) and the recombination protein RecA (for a review see (264)). It has been proposed that when the replicative DNA polymerase III holoenzyme encounters an AAF lesion, it stalls, and a gap of single-stranded DNA between the stalled DNA polymerase III and the moving helicase is created (state (A), Figure 10) (265). Initially covered by SSB proteins, the single-stranded DNA is then converted into a RecA nucleofilament by the recombination proteins, RecFOR. As a consequence of its inability to bypass the blocking lesion, DNA polymerase III can dissociate locally from the primer terminus. As discussed above (see 5.1.4.), a TLS DNA polymerase, such as DNA polymerase V, can be recruited at the site of the lesion *via* its interaction with the beta clamp, bind to the p-t junction (state (B), Figure 10) and bypass the lesion (state (C), Figure 10). Successful lesion bypass by DNA polymerase V requires not only the beta clamp to stabilize the DNA polymerase V at the site of the lesion but also the RecA nucleofilament. The RecA nucleofilament can be provided either in *cis* by the filament assembled closed to the lesion or in *trans* by a separate single-stranded DNA covered by RecA. It allows DNA polymerase V to acquire its active form by associating with an ATP bound form of RecA (266, 267). The active form of DNA polymerase V is therefore DNA polymerase V-RecA-ATP. It is possible that the stretched conformation of the DNA in the RecA nucleofilament near the site of the lesion (268) facilitates smooth elongation by DNA polymerase V. The DNA patch synthesized by DNA polymerase V must reach a certain length to resist the 3'->5' exonuclease activity of the DNA polymerase III holoenzyme. Like mismatches than can be detected either directly or indirectly by DNA polymerases when they are up to four or five base pairs from the primer terminus (29, 39, 269), DNA polymerase III can sense the lesion located up to five base pairs away. Therefore the second DNA polymerase switch to reposition the replicative DNA polymerase III holoenzyme at the p-t junction (state (D), Figure 10) does not take place before DNA polymerase V has synthesized a DNA fragment of at least five nucleotides. The integrated view of TLS across a model blocking lesion may represent a paradigm of lesion bypass in other organisms.

6. CONCLUSION

All DNA polymerases catalyze the incorporation of dNMPs into DNA in a 5'->3' direction using a single-stranded DNA as template. To accomplish this directional template-dependent DNA synthesis, they use a common catalytic mechanism involving two metal cations. The kinetic pathway shared by all DNA polymerases to incorporate a dNMP into the 3'-OH end of a primer includes several steps. The step that controls the reaction of DNA synthesis depends on the DNA polymerase and on the quality of the DNA template (*e. g.* presence of lesions). DNA polymerases differ by their intrinsic speed, processivity, fidelity and ability to bypass lesions. Several factors can affect the fidelity of DNA polymerases. For example, reduced fidelity can stem from the architecture of the active site itself as exemplified by the DNA polymerases of the Y family whose preformed active site has room to accommodate DNA lesions or non Watson-Crick base pairs. The flexibility of the DNA template in the active site of the DNA polymerase is also an important parameter for fidelity, and its limitation by keeping the base pair binding pocket rigid for example, may increase fidelity (42, 53, 119, 270). Recent structural studies have also revealed that protein domains devoid of known catalytic activity and located at some distance from the active site can modulate the fidelity of DNA polymerases. For instance, *E. coli* DNA polymerase II possesses small cavities remote from the active site that make it possible for the template strand of the newly synthesized duplex to loop out and therefore for the enzyme to promote deletions and perform error-prone TLS (42). On the other hand, the repair DNA polymerase lambda is equipped with specific structural devices that allow the enzyme to fill processively and faithfully DNA gaps of a few nucleotides without the assistance of additional proteins. Indeed, DNA polymerase lambda can bind the upstream and downstream strands of the gap and store the additional template nucleotides in an extrahelical position within a specific binding pocket before copying them in an error-free manner (54). These two examples illustrate the structural adaptation of DNA polymerases to their cellular function. In the cell, auxiliary proteins assist DNA polymerases and modulate their intrinsic properties. For instance, the SSB protein not only removes secondary structures in the DNA to facilitate the progression of DNA polymerases but also increases their fidelity (254, 255). Similarly, the ring-shaped processivity factor not only increases the processivity of the DNA polymerases but also ensures the interplay between DNA polymerases and possibly other proteins (271). As a consequence, the DNA polymerases are only one piece of sophisticated macromolecular machines, and their structural and functional diversity allow them to adjust to an ever changing cell environment.

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