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Structure and Function of 2:1 DNA Polymerase·DNA Complexes

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DNA polymerases are required for DNA replication and DNA repair in all of the living organisms. Different DNA polymerases are responsible different stages of DNA metabolism, and many of them are multifunctional enzymes. It was generally assumed that the different reactions are catalyzed by the same enzyme molecule. In addition to 1:1 DNA polymerase·DNA complex reported by crystallization studies, 2:1 and higher order DNA polymerase·DNA complexes have been identified in solution studies by various biochemical and biophysical approaches. Further, abundant evidences for the DNA polymerase–DNA interactions in several DNA polymerases suggested that the 2:1 complex represents the more active form. This review describes the current status of this emerging subject and explores their potential in vitro and in vivo functional significance, particularly for the 2:1 complexes of mammalian DNA polymerase β (Pol β), the Klenow fragment of *E. coli* DNA polymerase I (KF), and T4 DNA polymerase.

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Since the studies of the *Escherichia coli* DNA polymerase I (or the Kornberg enzyme) were first reported (Lehman et al., 1958), many DNA polymerases have been discovered and identified (Friedberg, 2006). All of the characterized DNA polymerases can be divided into seven families: families A, B, C, D, X, Y, and RT, based on sequence homology (Goodman and Tippin, 2000), and each of the DNA polymerases possesses different properties and plays various roles in DNA replication and/or DNA repair (Friedberg et al., 2006; Garcia-Diaz and Benenek, 2007). Most of the function–structure relationship studies for DNA polymerases have focused on the fidelity of nucleotide (dNTP) incorporation into the DNA (Steitz, 1998; Beard and Wilson, 2006; Showalter et al., 2006). Relatively less is known about the DNA polymerase–DNA interactions in solution. Considering that DNA polymerases have apparently evolved to distinct patterns of substrate specificities (Prakash et al., 2005), more knowledge on the interactions of DNA polymerase and different types of DNA substrates are necessary to understand the biological functions and mechanisms of DNA polymerases (Bebenek and Kunkel, 2004).

Our knowledge in the mechanism of DNA polymerases has been enormously enhanced by the crystallographic studies (Sawaya et al., 1997; Wang et al., 1997; Brautigam and Steitz, 1998; Doublie et al., 1998; Ling et al., 2001; Garcia-Diaz et al., 2005; Nair et al., 2005), and much useful information about the active site has been acquired from high-resolution crystal structures. Nevertheless, it is also known that many important mechanistic questions cannot (solely) rely on the crystal structures, and that other approaches are required in order to complement the crystallographic studies and provide the necessary information. Several biophysical and biochemical methods have been developed in studying DNA polymerase–DNA interactions; for example, analytical ultracentrifugation (Delagoutte and Von Hippel, 2003), size exclusive chromatography, surface plasmon resonance (Tsoi and Yang, 2002, 2004), electrophoretic mobility shift assays, UV/visible spectrum, circular dichroism (Reisbig et al., 1979), NMR chemical shift perturbation (Showalter et al., 2001; Bose-Basu et al., 2004), fluorescence intensity (Tleugabulova and Reha-Krantz, 2007), fluorescence titration (Bujalowski, 2006), single molecular fluorescence resonance energy transfer (Kapanidis et al., 2006; Luo et al., 2007), and small-angle X-ray/neutron scattering (Ho et al., 2004; Tang et al., 2008). Several studies have employed more than one of the approaches described above.

Except for the *E. coli* DNA polymerase III holoenzyme, the only characterized dimeric DNA polymerase with 14 subunits per monomer that plays different roles of replicating leading and lagging strands (Onrust et al., 1995), all of the crystal structures of DNA polymerases are in the monomeric form in the presence or absence of DNA (Sawaya et al., 1994; Garcia-Diaz and Benenek, 2007). In contrast, DNA polymerase·DNA complexes with the ratio of 2:1 have been detected in solution by various biochemical and biophysical approaches (Ahn et al., 1998; Rajendran et al., 2001; Tsoi and Yang, 2002; Bailey et al., 2007; Jezewska et al., 2007; Tang et al., 2008). Various fluorescence approaches together with sedimentation equilibrium have been frequently used to acquire the average molecular mass of polymerase·DNA complexes (Rajendran et al., 2001; Bujalowski, 2006; Bailey et al., 2007). Alternatively, recent advancements in sedimentation velocity (Brown and Schuck, 2006) have overcome the difficulty in indirectly determining the molecular mass of sedimentation species, and the stoichiometries of DNA polymerase·DNA complexes can be reasonably estimated by monitoring sedimentation velocity at both wavelength 260 nm (for DNA) and 280 nm (for protein) (Delagoutte and Von Hippel, 2003; Tang et al., 2008).

In addition to the 2:1 complex, higher order (ratio >2:1) DNA polymerase·DNA complexes were also reported for

Abbreviations: ASFV Pol X, African swine fever virus DNA polymerase X; BER, base excision repair; HhH motif, helix–hairpin–helix motif; KF, the Klenow fragment of *Escherichia coli* DNA polymerase I; Pol β, mammalian DNA polymerase β.

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various DNA polymerases with longer DNA substrate (Rajendran et al., 2001; Jezewska et al., 2007). One of the most important questions for the 2:1 and higher order DNA polymerase·DNA complexes is whether it is a specific functional form or a non-specific aggregate. In this mini-review we mainly focus on the studies of reported 2:1 DNA polymerase·DNA complexes on mammalian DNA polymerase β (Pol β), the Klenow fragment of *E. coli* polymerase I (KF), bacteriophage T4 DNA polymerase, and African swine fever virus DNA polymerase X (ASFV Pol X).

The Reported 2:1 DNA Polymerase·DNA Complexes

Pol β·DNA complex

Pol β is one of the most extensively investigated DNA polymerases. It is a 39 kDa monomeric DNA polymerase responsible for the DNA base excision repair (BER) pathway in mammalian cells (Beard and Wilson, 2006), and in contrast to many characterized high-fidelity DNA polymerases, Pol β does not have the proofreading exonuclease activity. It is known to be a multi-functional enzyme, which possesses a 5′-deoxyribose phosphodiesterase (5′-dRP lyase) activity with the N-terminal 8 kDa lyase domain and a nucleotidyl transferase activity with the C-terminal 31 kDa nucleotidyl transferase domain (Sawaya et al., 1994); the latter contains the DNA-binding, nucleotidyl transferase, and dNTP-binding subdomains. Pol β uses the DNA-binding motifs (the helix–haipin–helix (HhH) motifs) in the 8 kDa domain and the DNA-binding subdomain to interact with the DNA backbone as shown in the structure of the 1:1 Pol β·DNA complex, and closing of the 8 kDa domain upon DNA binding has been well established (Sawaya et al., 1997). Both gapped (preferred) and template-primer DNAs can serve as DNA substrates of Pol β (Beard and Wilson, 2006; Showalter et al., 2006).

The 2:1 Pol β·DNA complexes have been reported by various methods in some laboratories. Bujalowski and coworkers reported two distinct single-stranded DNA binding modes, $(\text{Pol }\beta)_5$ and $(\text{Pol }\beta)_{16}$, through steady-state fluorescence titration and sedimentation equilibrium (Jezewska et al., 1998). By surface plasmon resonance measurements, Tsoi and Yang reported that a 2:1 complex is formed with the biologically relevant gapped DNA and template-primer DNA, whereas only 1:1 complex is formed with double-helix DNA and single-stranded DNA (non-natural DNA substrates of Pol β) (Tsoi and Yang, 2002). With the biologically relevant gapped DNA substrate used previously in functional and structural studies (Sawaya et al., 1997; Kraynov et al., 2000), Tsai and coworkers used small-angle X-ray scattering (SAXS) and sedimentation velocity (SV) to show that the 2:1 complex is the predominant form in the presence of substoichiometric amounts of DNA, whereas the 1:1 complex is the major form with stoichiometric or excess amount of DNA (Tang et al., 2008).

KF·DNA complex

KF, the Klenow (large) fragment of *E. coli* DNA polymerase I, was the first characterized DNA polymerase (Lehman et al., 1958), and the structures and functions of KF have been investigated for several decades. KF works as a replicative DNA polymerase by filling the necessary nucleotides of the Okazaki fragments on the lagging strand, 3′–5′ exonuclease, and solely interacts with the template-primer DNA. Similar to Pol β, both 1:1 and 2:1 KF·DNA complexes in solution have been reported. The low-resolution solution structure of the 1:1 complex of *Thermus aquaticus* (*Taq*) DNA polymerase (homologous to *E. coli* DNA polymerase I) at the stoichiometric amount of DNA ([enzyme]/[DNA] = 1/1) was reported by small-angle neutron scattering (Ho et al., 2004), and a 2:1 KF·DNA complex has been indirectly suggested by the stopped-flow fluorescence assays, in which more than twofold excess amount of enzyme over DNA was used (Purohit et al., 2003), and directly characterized by fluorescence anisotropy titration and analytical ultracentrifugation (Bailey et al., 2007). It needs to be mentioned that in contrast to the free form of Pol β, where non-specific association was negligible (Tang et al., 2008), concentration-dependent aggregation has been detected in free forms of KF, *E. coli* DNA polymerase I, and *Taq* DNA polymerase (Joubert et al., 2003; Bailey et al., 2007).

T4 DNA polymerase·DNA complex

T4 bacteriophage DNA polymerase (a Family B replicative DNA polymerase), like KF, also exhibits polymerase and exonuclease activities. A dimeric form of free T4 DNA polymerase was reported earlier by cross-linking (Salinas and Benkovic, 2000), but later studies indicated that the dimer formation was likely due to non-specific association (Delagoutte and von Hippel, 2001). A 2:1 T4 DNA polymerase·DNA complex was shown by cross-linking with or without the presence of sliding clamp protein (Ishmael et al., 2003), whereas a 1:1 complex was reported in the absence of sliding clamp by sedimentation velocity (Delagoutte and Von Hippel, 2003). Recent studies suggested that the sliding clamp (gp45 protein in bacteriophage T4) and maybe also the sliding clamp loader (a gp44/62 complex in bacteriophage T4) are required for the formation a 2:1 T4 DNA polymerase·DNA complex, which may exist as a short-lived transition complex proposed by biochemical assays (Yang et al., 2004), thus the formation of 2:1 complex of T4 DNA polymerase is not exclusively depending on polymerase–DNA interactions.

ASFV Pol X·DNA complex

ASFV Pol X (a 20 kDa monomer), the smallest DNA polymerase reported to date, is only half the size of Pol β and is missing the N-terminal 8 kDa lyase domain and the DNA-binding subdomain. Two forms of the ASFV Pol X have been identified: the oxidized form with a disulfide bond in the nucleotidyl transferase subdomain (Showalter et al., 2001), and the reduced form with no disulfide linkage (Maciejewski et al., 2001). The global structures of these two forms are similar (Maciejewski et al., 2001; Showalter et al., 2001). ASFV Pol X can bind DNA as tightly as Pol β, despite lacking of the DNA-binding domains/motifs found in Pol β (Showalter et al., 2001). A few structural and biochemical studies for the ASFV Pol X·DNA complex have been reported recently (Showalter et al., 2001; Jezewska et al., 2006, 2007; Tang et al., 2008), indicating that a 1:1 ASFV Pol X·DNA complex is formed at high ionic strength ($\geq$0.4 M KCl) at various ratios of ASFV Pol X/DNA (Showalter et al., 2001; Jezewska et al., 2007; Tang et al., 2008), and that a 2:1 ASFV Pol X·DNA complex was identified in low salt buffer by fluorescence studies (Jezewska et al., 2006, 2007). In contrast to Pol β (Rajendran et al., 2001), no sedimentation equilibrium data were reported for the 2:1 complex of ASFV Pol X.

Structural and Functional Studies of the Reported 2:1 Polymerase·DNA Complexes

Pol β·DNA complex

Two binding models of 2:1 Pol β·DNA complexes have been reported. One is the studies by Bujalowski and coworkers (Rajendran et al., 2001; Bujalowski, 2006) that a 2:1 complex is formed with two different binding modes, $(\text{Pol }\beta)_{16}$ and $(\text{Pol }\beta)_5$, where one Pol β molecule has larger DNA-binding interface in the $(\text{Pol }\beta)_{16}$ mode and the other Pol β has smaller contact surface in the $(\text{Pol }\beta)_5$ mode (Fig. 1A). Their studies suggested that two DNA polymerase molecules bind to different positions on the DNA substrate with the same conformation,

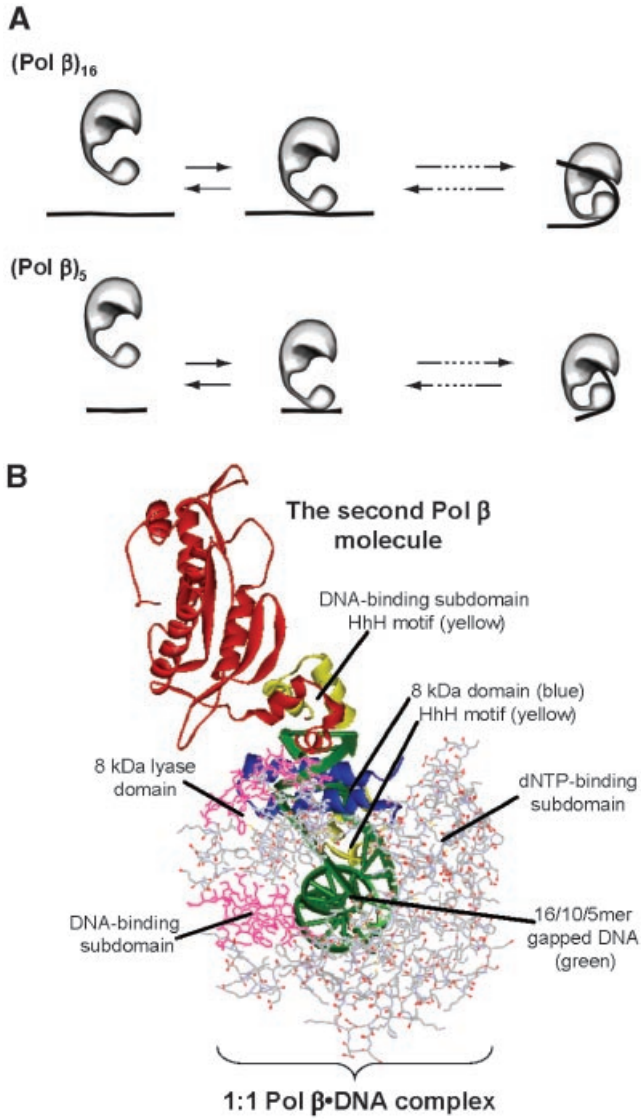

Fig. 1. The studies of 2:1 Pol β·DNA complexes. (A) The schematic model of Pol β binding to the ssDNA in the $(Pol\ \beta)_{16}$ and $(Pol\ \beta)_5$ binding modes (Bujalowski, 2006), and (B) the structural model of the 2:1 complex (Tang et al., 2008). Reprinted with permission for (A) from the reference (Bujalowski, 2006). Copyright 2006 American Chemical Society.

and the two polymerase molecules may or may not interact with each other. The DNA-binding affinity is dependent on the binding position on the DNA substrate (Tsoi and Yang, 2002).

The structural model of the 2:1 complex generated by SAXS and SV (Fig. 1B) (Tang et al., 2008) indicates that the Pol β-DNA interactions in the 1:1 complex employ the HhH motifs in both the 8 kDa lyase domain and the DNA-binding subdomain, while the second Pol β molecule contacts the nascent base pair binding pocket of the 1:1 Pol β·DNA complex with the HhH motif in the 8 kDa domain. The two Pol β molecules bind to the same site of DNA and display different conformations in the 2:1 complex. This is the only reported model of a 2:1 complex that contains structural insight, and it is not clear how it is related to the models mentioned in Figure 1A. Regardless, it is important to note that Pol β should exist in the 2:1 form predominantly under the conditions used for the pre-steady state kinetic studies. It has been reported by Tsai laboratory (Werneburg et al., 1996) and others (Vande Berg et al., 2001) that the Pol β·DNA complex seems to have optimal activity when the enzyme/DNA ratio is approximately 2:1. Thus a [Pol β]/[DNA] ratio of 2:1 to 3:1 was chosen to form the Pol β·DNA complex in the stopped-flow fluorescence assays (Dunlap and Tsai, 2002; Bakhtina et al., 2005). Additionally, previous 5′-dRP lyase studies reported ~5-fold increase in the 5′-dRP lyase activity when Pol β is in excess relative to DNA (Prasad et al., 1998a,b, 2005). Together, both the nucleotidyl transferase and 5′-dRP lyase activities of Pol β are enhanced when the enzyme is in excess of DNA.

The structural model shown in Figure 1B is consistent with the activity enhancement for the 2:1 Pol β·DNA complex. The reactions of nucleotidyl transferase and 5′-dRP lyase are proposed to be sequential in BER (Wilson and Kunkel, 2000; Beard and Wilson, 2006), in which the nucleotidyl transferase activity of Pol β catalyzes single-nucleotide gap-filling synthesis and the 5′-dRP lyase activity of Pol β removes the 5′-dRP moiety of the downstream strand. The structural model implies that the two reactions could be catalyzed by the two Pol β molecules in the 2:1 complex.

The proposed 5′-dRP lyase role is consistent with the following reports: (a) The isolated N-terminal 8 kDa lyase domain alone can function as a 5′-dRP lyase (Matsumoto et al., 1998; Prasad et al., 1998a,b), supporting the second Pol β molecule in Figure 1B processing the 5′-dRP lyase reaction. (b) As we have pointed out in a recent paper (Tang et al., 2008), the crystal structure of the Pol β·DNA·dNTP ternary complex (the active form of DNA polymerase) (Sawaya et al., 1997) (PDB ID code 1BPY) showed that the 8 kDa lyase domain is not at the optimal position and 5′-dRP group is distant from the proposed Schiff base nucleophile (Lys^{72} in the 8 kDa lyase domain), suggesting that the second Pol β molecule may be required to catalyze the 5′-dRP lyase reaction.

KF·DNA complex

By fluorescence anisotropy titration, DNA gel shift assays, and sedimentation equilibrium experiments, Bailey et al. reported that the two KF molecules bind to different template-primer DNA sites; the first KF molecule binds to the dNTP insertion site (3′-end of the primer strand of the template-primer DNA, and the primary binding site) as suggested by the crystal structure of the Klenow fragment of *Taq* DNA polymerase (Li et al., 1998), and the second KF molecule possibly binds to the upstream double-stranded DNA (Fig. 2), as the second KF molecule binding was unaffected when the template overhang was shortened (Bailey et al., 2007). Further, the authors showed that the 2:1 complex is the dominant form for the matched template-primer DNA and only the 1:1 complex can be detected for the mismatched template-primer DNA. Their working hypothesis can be illustrated as follows. Upon matched dNTP incorporation, the KF molecule uses the polymerase domain to interact with DNA (in the "base insertion" mode) and the exonuclease domain to interact with the other KF molecule, so the 2:1 complex is the major form. When the mismatched dNTP is incorporated (in the "editing" mode), the exonulcease is occupied by DNA binding for proofreading thus the second KF molecule cannot bind to the dimerization site and the 1:1 complex becomes the favorable form in the mismatched complex. Thus, the authors postulated that the dimerization interface in the 2:1 complex is located in the exonuclease domain of KF. It remains to be shown whether the putative dimerization domain of KF is correlated with non-specific association of the free form of KF (Bailey et al., 2007) and (full-length) *E. coli* DNA polymerase I (Joubert et al., 2003). It is also highly desirable to use structural tools to develop a more detailed structural model for the 2:1

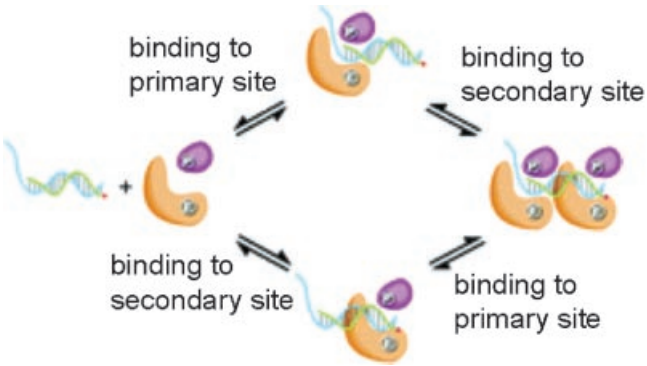

Fig. 2. The schematic model for the formation of 2:1 KF·DNA complex (Bailey et al., 2007). The figure is adopted from the report. The nucleotidyl transferase and exonuclease domains are shown in orange and purple, respectively. The authors proposed that KF uses the nucleotidyl transferase domain to interact with either the 3′-end of the primer strand (the "primary" binding site) or the upstream double-stranded DNA (the "secondary" binding site) of the template-primer DNA, and that the putative dimerization interface in the 2:1 complex may be located in the exonuclease domain of KF. Reprinted with permission from the reference (Bailey et al., 2007). Copyright 2007 American Chemical Society.

complex of KF, for comparison with that of Pol β shown in Figure 1B.

T4 DNA polymerase·DNA complex

Benkovic and coworkers proposed the schematic model of the 2:1 complex of T4 DNA polymerase (Yang et al., 2004), which may be formed either as a semistable complex, where the incoming polymerase molecule binds at the interdomain binding site on the sliding clamp protein (Fig. 3A), or as a short-lived transition complex occurring in the course of the incoming polymerase replacing the initial polymerase (Fig. 3B). Reha-Krantz and coworkers recently suggested (Fidalgo da Silva and Reha-Krantz, 2007) that the proposed 2:1 complex described above may have the mechanistic implication on nucleotidyl transferase-to-exonuclease active site switching for T4 DNA polymerase. They proposed that if the 2:1 complex does occur,

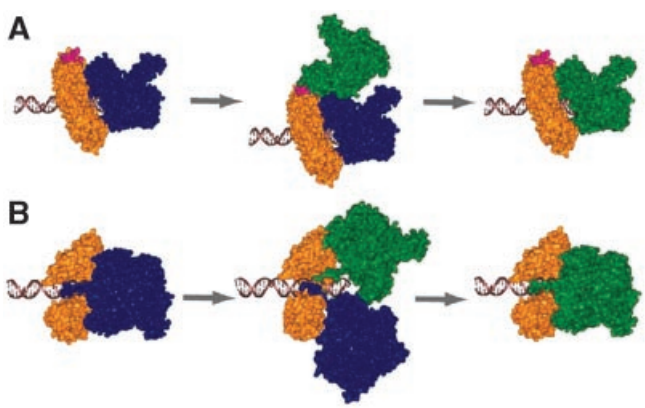

Fig. 3. The schematic models for the formation of 2:1 T4 DNA polymerase·DNA complex proposed on the basis of biochemical assays (Yang et al., 2004). In both parts, the sliding clamp protein is shown in orange, the initial polymerase in blue, and the incoming polymerase in green. The details are described in the text.

two T4 DNA polymerase molecules can potentially bind concurrently to the DNA substrate, where one polymerase molecule functions as a replicative enzyme and the other polymerase molecule as a "spare" enzyme during DNA replication. Conversely, the mismatched dNTP insertion may lead to dissociation of the replicating polymerase and the interaction of "spare" polymerase with the mismatched DNA forms an exonuclease complex as a 1:1 complex. It remains to be established whether the proposed model of 2:1 complex of T4 DNA polymerase described here and that of KF reported above resemble each other.

ASFV Pol X·DNA complex

Structural studies of ASFV Pol X·DNA complexes indicated a distinct strategy employed by ASFV Pol X to interact with DNA. While the interactions between Pol β (and several characterized DNA polymerases) and DNA involve mainly hydrogen bonds between the HhH motifs and the sugar phosphate backbone of DNA (Doherty et al., 1996; Sawaya et al., 1997; Bebenek et al., 2001), the NMR and SAXS studies of ASFV Pol X (Showalter et al., 2001; Tang et al., 2008) indicated that the enzyme binds DNA by use of electrostatic interactions through two positively charged regions: the αC helix in the nucleotidyl transferase subdomain, and the αE helix in the dNTP-binding subdomain (Fig. 4A). These positively charged regions could also be the two single-stranded DNA binding sites detected in the ASFV Pol X–DNA interactions (Jezewska et al., 2006), but no structural evidence is available for the latter. The 2:1 ASFV Pol X·DNA complex reported by Jezewska et al. (Fig. 4B) suggested that each ASFV Pol X molecule binds DNA with the αE helix in the dNTP-binding subdomain in the 2:1 complex, and that the DNA molecule is away from the proposed active site of ASFV Pol X (Showalter et al., 2001). The

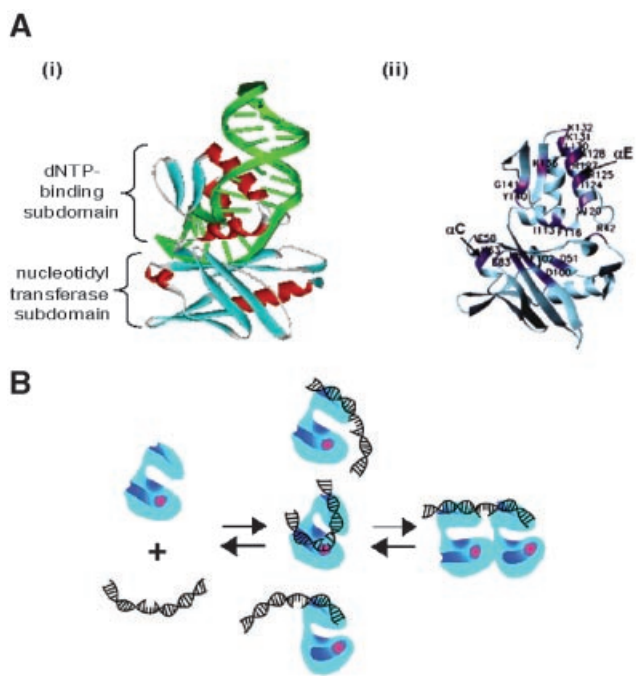

Fig. 4. The formation of ASFV Pol X·DNA complexes. (A) The structural model of the 1:1 complex reported by SAXS (Tang et al., 2008) and NMR chemical shift perturbation (Showalter et al., 2001), and (B) the schematic model for the 2:1 complex (Jezewska et al., 2007). Reprinted with permission for (B) from the reference (Jezewska et al., 2007). Copyright 2007 American Chemical Society.

functional relevance of their proposed 2:1 complex remains to be investigated.

Functional relevance of 2:1 complex in other DNA polymerases

In addition to the studies of Pol β, KF, T4 DNA polymerase, and ASFV Pol X, a survey of the literature suggests that 2:1 DNA polymerase·DNA complexes could also be functionally relevant in several other DNA polymerases. For example, only approximately 50% of DNA substrate was converted into product in the active site titration studies of HIV-1 reverse transcriptase (Suo et al., 1999), mitochondrial replicative DNA polymerase γ (Pol γ) (Hanes and Johnson, 2007), and T7 DNA polymerase (Tsai and Johnson, 2006). Like T4 DNA polymerase and KF, Pol γ and T7 DNA polymerase are replicative polymerases, and also exhibit 3′–5′ exonuclease activities. Also, two-fold excess amount of enzyme relative to DNA was optimized in the pre-steady state studies of the exonuclease activity assays for human DNA polymerase λ (Pol λ) (Johnson and Johnson, 2001). As DNA polymerase proofreading is an extremely important mechanism for enhancing its base-insertion fidelity (Kunkel, 1988) and preventing mutations leading to cancers (Peterson and Cote, 2004), it is useful to probe the proposed nucleotidyl transferase-to-exonuclease roles in the 2:1 complex of KF and T4 DNA polymerase for other high-fidelity DNA polymerases. Moreover, higher 5′-dRP/AP lyase activities in the presence of excess enzyme relative to DNA were also reported in the studies of 5′-dRP and AP lyases of Pol λ (Longley et al., 1998; Garcia-Diaz et al., 2001) and human DNA polymerase ι (Pol ι) (Bebenek et al., 2001), which are thought to play roles in DNA repair (Bebenek and Kunkel, 2004).

The functional relevance presented above is all based on in vitro studies. In vivo evidence for the structure or function of the 2:1 DNA polymerase·DNA complex is highly desirable but not yet available. Nonetheless, one may speculate that some hints exist in the literature—previous studies showed that a reduced level of DNA polymerase δ (Pol δ) leads to *S. cerevisiae* mutants (Kokoska et al., 2000), and that proofreading failures of Pol λ (the only DNA polymerase in human mitochondria) cause mitochondrial toxicity (Kaguni, 2004). One can propose that if two molecules of Pol δ (or Pol λ) are required for dNTP insertion and 3′–5′ exonuclease proofreading activities, insufficient amount of Pol δ (or Pol λ) may affect proofreading and lead to genomic instability.

Conclusion

DNA polymerases are required for DNA metabolism (DNA replication and DNA repair), which is arguably the most important biological activities in the living world. In this review we show that there are compelling evidences in the literature suggesting that the 2:1 complex of DNA polymerase·DNA complex is the more active form of the enzyme. The advantage for the 2:1 complex of Pol β in DNA repair is postulated to be for the first Pol β molecule to carry out dNTP incorporation and the second Pol β molecule to perform the 5′-dRP lyase reaction. Analogously, in the 2:1 complex of KF and T4 DNA polymerase, one molecule could catalyze the nucleotidyl transfer reaction and the other could catalyze the 3′–5′ exonuclease reaction. Considering many DNA polymerases functioning as multifunctional enzymes, these models, if further verified, could represent a paradigm shift in the molecular mechanism of the functions of DNA polymerases.

Acknowledgments

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