# DNA polymerases lose their grip

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**Structural characterization of a variety of DNA polymerases has likened the polymerase domain to a hand that grasps DNA with functional subdomains referred to as fingers, palm and thumb. The solution structure of the African swine fever virus DNA polymerase X indicates that it does not have a hand-like architecture and suggests a mechanism by which the polymerase may compensate for the lack of a dedicated DNA binding subdomain.**



The African swine fever virus (ASFV) is a DNA virus that codes for two DNA polymerases: a B-family replicative DNA polymerase and a DNA polymerase (pol X) of unknown function that has sequence similarity to DNA polymerase β (pol β), a eukaryotic base excision repair enzyme in the X-family of polymerases[1]. Pol X is the smallest naturally occurring polymerase (174 residues, 20 kDa) and lacks accessory activities, such as the lyase activity associated with pol β. The small size of pol X makes this enzyme an ideal system for characterization by NMR. As reported on pages 936 and 942 of this issue of *Nature Structural Biology*, Maciejewski *et al.*[2] and Showalter *et al.*[3] have independently determined the solution structure of ASFV pol X. These structures represent the first solution structures of a full-length DNA polymerase. More importantly, the pol X structures provide valuable insights into how pol X can tightly bind DNA in the absence of a dedicated DNA binding subdomain and the functional significance of relative subdomain positioning upon substrate binding. These insights provide a framework in which to analyze the native function of pol X.

## Pol X structure

The architecture of the prototypical DNA polymerase domain is likened to a right hand that can grasp DNA (for review see ref. 4), with the subdomains referred to as the fingers, palm, and thumb. The palm subdomain has three carboxylates that bind two catalytically essential metals involved in the nucleotidyl transferase reaction. Crystal structures of DNA polymerases from several polymerase families, excluding pol β, indicate that the palm subdomains are structurally homologous, but the fingers and thumb are not. The thumb and fingers subdomains have primary roles in duplex DNA binding and deoxynucleoside 5′-triphosphate (dNTP) selection, respectively. DNA polymerase β has functionally equivalent subdomains, but the topology of the palm is unique[5].

The ASFV pol X shares sequence similarity with the C-terminal region of pol β (Fig. 1*a*). Thus, it is predicted to lack the N-terminal lyase domain and duplex DNA binding subdomain of pol β but retain the catalytic subdomain and the C-terminal subdomain involved in dNTP selection. The solution structures confirm that pol X is indeed composed of only two subdomains and that the N-terminal subdomain includes the metal binding carboxylates: Asp 49, Asp 51, and Asp 100 (Fig. 1*b*)[2,3]. Most importantly, the subdomain organization and overall shape of ASFV pol X is not hand-like (Fig. 1*b*).

Although secondary structural elements of pol β and pol X are similar (Fig. 2), as previously predicted[1], important differences exist. The most notable difference is in the N-terminal catalytic subdomain: αL of the catalytic subdomain of pol β is replaced with a short α-helix (αC) and a β-sheet (strands β2, β3 and β7) in pol X. Nonetheless, the catalytic subdomain resembles that of pol β and is distinct from other DNA polymerases[4,5]. Only minor differences are observed in the C-terminal subdomains: the β-sheet of this subdomain includes an additional strand (β13) in pol X. Although structurally similar, there are functionally important primary sequence differences between pol β and pol X that occur within these structural elements (see below).

Not surprisingly, the two independently determined pol X structures are very similar, and the backbone atoms in the

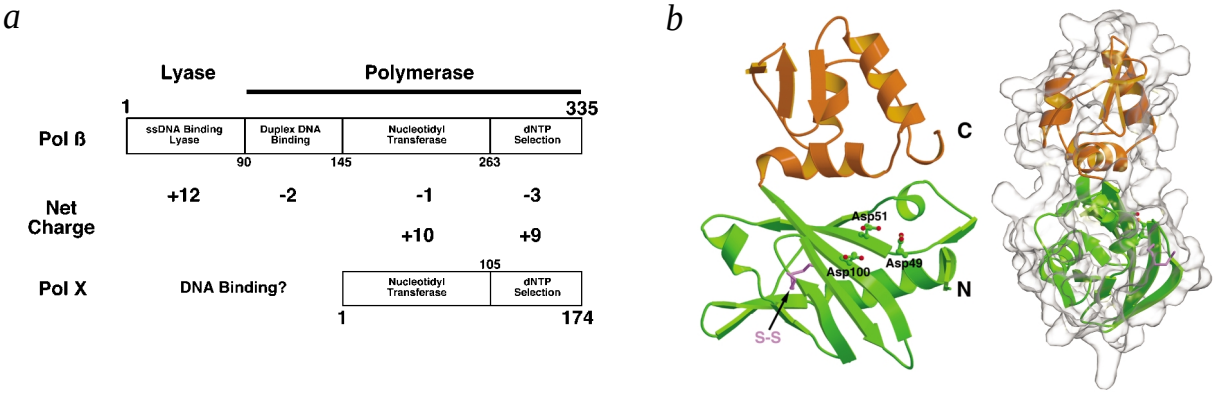


**Fig. 1** Subdomain organization of pol β and pol X. ***a***, Diagram illustrating the subdomain boundaries for pol β and pol X. Pol β is composed of two domains with distinct catalytic activities: an N-terminal lyase domain and a C-terminal polymerase domain. The pol β polymerase domain is comprised of three subdomains with distinct functional roles[5]. ASFV pol X exhibits primary sequence similarity with the polymerase catalytic and C-terminal subdomains[1]. The net charge of each subdomain is determined from the number of acidic and basic residues as well as the contribution of the N- and C-terminus. ***b***, Two views of a ribbon representation of the solution structure of ASFV pol X determined by Showalter *et al.*[3]. The N-terminal catalytic and C-terminal subdomains are colored green and orange, respectively. The disulfide bond observed between Cys 81 and Cys 86 is indicated, as well as the three carboxylates that bind catalytically essential metals. The view on the right is rotated 90° about the y-axis and includes a semi-transparent molecular surface. Figures were made with Molscript[16] and/or GRASP[17] and rendered with Raster3D[18].

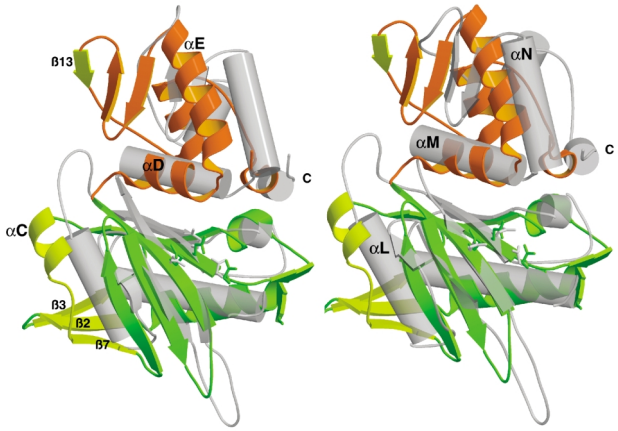


**Fig. 2** Comparison of the structure of human pol β with that of ASFV pol X. The structure of the catalytic and C-terminal subdomains of human pol β is illustrated superimposed with the structure of pol X determined by Maciejewski *et al.*[2]. The left panel shows pol β in an open form and the panel on the right pol β in a closed form. The open and closed forms of pol β represent the binary DNA complex and the ternary complex that includes an incoming dNTP[9]. These ligands are not shown. The α-helices of pol β (gray) are represented as cylinders. Upon binding a correct dNTP, the C-terminal subdomain (αN) rotates about αM to close around the nascent base pair (right panel). Pol X was superimposed with pol β using backbone atoms around the active site carboxylates (residues 190–192 and 255–257 of pol β with residues 49–51 and 99–101 of pol X; r.m.s. deviation of 0.6 Å). The N-terminal catalytic and C-terminal subdomains are colored green and orange, respectively. Structural elements of pol X discussed in the text are indicated in the left panel and in the right panel for pol β. Significant differences in secondary structural elements between pol X and pol β are highlighted in yellow in the pol X structure (see text). The superimposed structures suggest that the structure reported by Maciejewski *et al.*[2] is in a closed conformation.

N-terminal catalytic subdomains superimpose well (Fig. 3). Interestingly, the C-terminal subdomain of the structure determined by Maciejewski *et al.*[2] appears to be rotated toward the N-terminal subdomain about an axis (αD) relative to the position of the C-terminal subdomain in the structure determined by Showalter *et al.*[3]. These structures are reminiscent of the two positions of the C-terminal subdomain of pol β before and after binding a complementary dNTP[6] (see below). The positioning of this subdomain plays a critical role in the fidelity of DNA synthesis. Another notable difference is the presence of a disulfide bond observed in the catalytic subdomain of the structure determined by Showalter *et al.*[3], which is absent in that described by Maciejewski *et al.*[2] (Fig. 1*b*). These structures were determined in the presence of different apparent DTT concentrations, perhaps explaining the different experimental observations; however, the physiological significance of this disulfide bond remains to be determined.

## Substrate binding

The structures of pol X indicate that the enzyme does not possess an obvious DNA binding groove (Fig. 1*b*). Furthermore, the lack of a dedicated DNA binding subdomain suggests that DNA may not bind tightly to pol X. However, a variety of approaches to measure DNA binding affinity indicate that binding is as tight as that observed for pol β[2,3]. A hint on how this might be accomplished can be discerned from the net charge on the subdomains of pol β and pol X (Fig. 1*a*). Whereas the catalytic and C-terminal subdomains of pol β have a net charge of -1 and -2, respectively, pol X is expected to have net charges of +9 and +10 for the equivalent subdomains. The structure of pol X reveals that αC and the C-terminal end of αE (Fig. 2) are more positively charged relative to those in pol β; residues in these structural elements undergo chemical shift changes upon addition of DNA, implicating these regions in DNA binding[2,3]. Compared to pol β, the C-terminal subdomain of pol X reveals a highly positive electrostatic surface potential[3], and this could readily explain the capacity of pol X to tightly bind DNA.

Binding of substrates to DNA polymerases is an ordered process, with DNA binding occurring prior to dNTP binding. A comparison of structures of binary DNA polymerase complexes with ternary complexes that include an incoming dNTP reveals that the fingers subdomain — that is, the C-terminal subdomain of pol β — closes around the nascent base pair[6,7]. Thus, dNTP binding is expected to be much weaker in the absence of a templating nucleotide ($K_d$ >> 100 μM). Maciejewski *et al.*[2] measured the binding affinity for purine and pyrimidine triphosphates using a fluorescent NTP analog in a competition assay. Surprisingly, the $K_d$ values for dNTPs are ~10 μM in the absence of DNA, and dATP and dGTP induced a unique polymerase conformation that was not detected in the presence of pyrimidine triphosphates. In contrast, kinetic analysis of dNTP binding in the presence of DNA indicates that the incoming nucleotide has very low affinity in the formation of Watson-Crick base pairs ($K_d$ > 230 μM)[8] and the ranking of these affinities parallel those reported by Maciejewski *et al.*[2] (dGTP > dCTP > dATP > dTTP). Interestingly, the affinities increase for purine nucleotides ($K_d$ ~50 μM) and generally decrease for pyrimidine nucleotides in the formation of mismatches[8]. Thus, DNA seems to interfere with dNTP binding and pol X does not generally utilize the dNTP binding step to enhance fidelity. On the contrary, it appears to utilize the binding of deoxypurine triphosphates to enhance its infidelity (see below).

As noted above, the C-terminal subdomain of pol β closes around the nascent base pair upon binding the correct dNTP. Superimposing the catalytically conserved active site carboxylates of the pol X structure reported by Maciejewski *et al.*[2] with those of human pol β[9] suggests that their pol X apo enzyme is in a closed conformation (Fig. 2). Recently reported crystal structures of apo forms of polymerases belonging to the B-family[10] and Y-family of DNA polymeraes[11,12] also appear to be

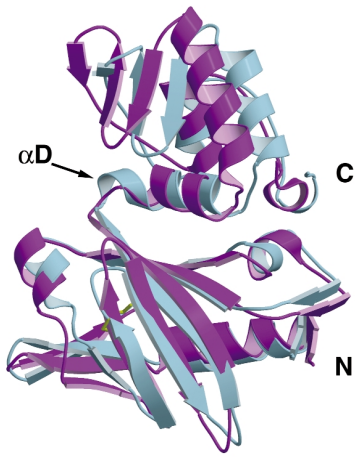


**Fig. 3** Comparison of independently determined solution structures of ASFV pol X. Ribbon representation of the superimposed structures of pol X reported by Maciejewski *et al.*[2] (purple) and Showalter *et al.*[3] (light blue). The backbone atoms of the N-terminal catalytic subdomains (residues 1–105) were used to superimpose these structures (r.m.s. deviation of 2.3 Å). The C-terminal subdomain in the structure reported by Maciejewski *et al.*[2] is rotated about an axis (αD) toward the N-terminal catalytic subdomain, relative to the position of the C-terminal subdomain observed by Showalter *et al.*[3].

in a closed conformation. Thus, this subdomain opens and closes independent of dNTP binding. This observation is consistent with the idea that this subdomain can reposition itself rapidly so that this interconversion is not a rate limiting step in the polymerase's reaction pathway[13,14]. A conformational change in the polymerase–substrate complex, prior to nucleotide insertion (that is, the chemical step), has been postulated to provide an important opportunity for DNA polymerases to enhance their fidelity.

## Function

Pol X exhibits low fidelity (but see also ref. 1 for contrasting results for assays performed under different conditions), and, as with other DNA polymerases that exhibit low fidelity (for examples, DNA polymerase η and ι), this is primarily due to the inefficient formation of Watson-Crick base pairs[8]. However, in contrast to these Y-family polymerases, pol X has a high propensity ($k_{cat}/K_m$) to form G:G mispairs that is four times greater than any other DNA polymerase examined.

The sequence and structural similarity to pol β coupled with its low fidelity suggests that pol X may be involved in a mutagenic repair pathway that could lead to viral heterogeneity. The observation that pol X binds avidly to base excision repair intermediates is consistent with this proposal[2,3] (see ref. 15 for a recent review of base excision repair). Likewise, the ASFV also codes for a protein predicted to be a class II apurinic/apyrimidinic endonuclease[1] that incises DNA on the 5′ side of an abasic site leaving a 3′ hydroxyl and 5′ deoxyribose phosphate in the single-nucleotide gap. Since pol X lacks the N-terminal pol β domain that contributes the lyase activity that removes the 5′-deoxyribose phosphate, another enzyme would need to provide this activity. The repair of oxidative DNA lesions is often initiated with a bifunctional DNA glycosylase that has an associated lyase activity (incises on the 3′ side of an abasic site), so the ASFV endonuclease may contribute a 3′ tailoring activity (for example, 3′ phosphodiesterase) rather than an endonuclease function and a 5′ processing activity would no longer be required.

The increased ability to produce G:G mispairs would predict a bias in the mutation spectrum for G→C transversions. It remains to be determined if such a bias exists. Although mutagenic repair may seem like a contradiction in terms, it may be necessary to repair oxidative DNA lesions in the viral genome. These lesions could result from a cellular response to infection that increases the production of reactive oxygen species through mitochondrial dysfunction or metabolic alterations. Left unrepaired, these lesions could stall a replication complex. In this context, pol X has biochemical characteristics similar to some members of the Y-family of translesional polymerases (for example, very low fidelity and inserts only a limited number of nucleotides during each DNA binding event) and may therefore assist viral replication that is stalled at a DNA lesion. It remains to be determined if pol X has DNA translesional activity.

## Concluding remarks

ASFV pol X does not utilize a hand-like architecture to perform DNA synthesis. Instead, it has evolved to utilize a minimal structural scaffold similar to the C-terminal portion of pol β with specific alterations to perform its physiological role. The successful application of NMR to structural determination and ligand binding of DNA polymerases bodes well for the future of this approach to complement mechanistic studies. This approach has the potential to accelerate our understanding of templated and untemplated nucleotide binding, divalent metal binding, substrate-induced conformational changes, chemistry, and side-chain dynamics related to these important events during catalytic cycling.

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