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New structural snapshots provide molecular insights into the mechanism of high fidelity DNA synthesis

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ABSTRACT

Time-lapse X-ray crystallography allows visualization of intermediate structures during the DNA polymerase catalytic cycle. Employing time-lapse crystallography with human DNA polymerase β has recently allowed us to capture and solve novel intermediate structures that are not stable enough to be analyzed by traditional crystallography. The structures of these intermediates reveals exciting surprises about active site metal ions and enzyme conformational changes as the reaction proceeds from the ground state to product release. In this perspective, we provide an overview of recent advances in understanding the DNA polymerase nucleotidyl transferase reaction and highlight both the significance and mysteries of enzyme efficiency and specificity that remain to be solved.

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1. Introduction

DNA polymerases play an essential role in DNA repair and replication. Accordingly, they are principal targets for chemotherapies and for understanding the efficiency and accuracy of DNA repair and replication. Additionally, DNA polymerases play a vital role in genetic engineering and the biotechnology industry. For these reasons, an enhanced view of their structure/function is a prerequisite for rational drug and protein design. Human cells have at least 16 DNA polymerases, and based on primary sequences have been categorized into four families (A-, B-, X-, and Y-families). DNA polymerases often include an accessory domain that complements its biological function (e.g., proofreading exonuclease or processivity).

DNA polymerase (pol) β (X-family) is the smallest human cellular polymerase and contributes two-enzymatic activities during base excision repair of simple DNA lesions. In addition to its metal-dependent DNA synthesis activity, a deoxyribose phosphate lyase activity is found on the amino-terminal lyase 8-kDa domain. The lyase activity is required to cleanse the 5′-end of a DNA gap to generate a substrate that can be ligated to complete repair. DNA polymerase β has been extensively characterized structurally, kinetically, computationally, and biologically [1,2]. Consequently, pol β serves as a model DNA polymerase to explore the nucleotidyl transferase reaction as well as examine how polymerases gain specificity by choosing right from wrong nucleotide substrates. Here, we review recent structural studies that provide a new framework to analyze the nucleotidyl transferase chemical reaction and substrate discrimination.

2. Structural characterization of DNA polymerase β

2.1. Approach

The first mammalian crystal structure of pol β was deposited in 1994; and to this day pol β remains a model DNA polymerase in deciphering the DNA polymerase chemical mechanism. As of November 2014, more than 240 X-ray crystallographic structures of pol β have been deposited in the protein data bank. These structural snapshots were obtained using multiple approaches ranging from modified protein or substrates to the recently developed time-lapse crystallography that employs natural substrates. In this section we will highlight several approaches utilized to capture key intermediate structures of pol β.

2.1.1. Modified substrates

The first approach to successfully capture a crystal structure of pol β bound to DNA utilized a modified DNA substrate to form

Abbreviations: 1-nt, 1-nucleotide; AP, apurinic/apyrimidinic; BER, base excision repair; dNTP, deoxynucleoside triphosphate; dRP, deoxyribose phosphate; M_c, catalytic metal; M_g, ground state metal; M_n, nucleotide or transition state metal; M_p, product metal; PP_i, pyrophosphate; pol, DNA Polymerase.

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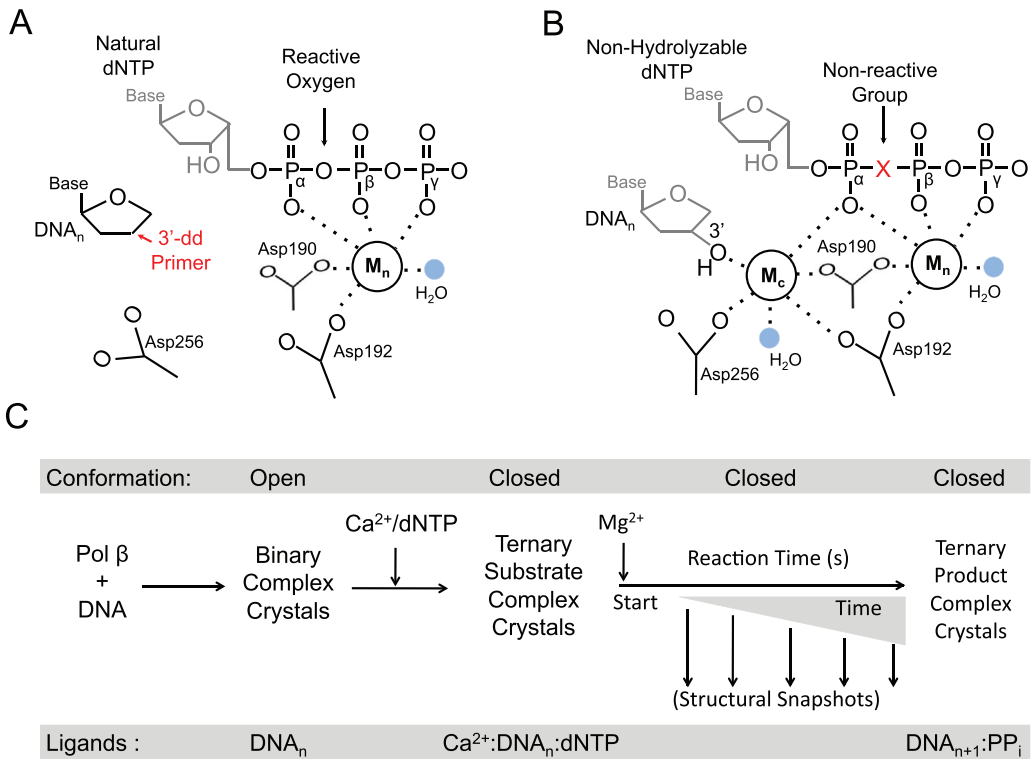

Fig. 1. Structural approaches for capturing ternary pol β structural snapshots. A diagram of the pol β active site with either a (A) dideoxy-terminated primer or (B) a non-hydrolysable deoxynucleotide analog are shown. The active site aspartic acid residues, catalytic metal (M_c), nucleotide metal (M_n), and coordinating water molecules (blue) are indicated. The metal coordination is shown with dashes. In panel A, the position of the missing O3′ of the dideoxy-terminated primer is indicated in red. Additionally, the reactive oxygen group between Pα and Pβ of the triphosphate of a natural dNTP is indicated. The use of a dideoxy-terminated primer hinders catalytic metal binding and proper Asp256 coordination. In panel B, the 3′—OH for the primer terminus, proper orientation/coordination of Asp256, and M_c are shown. The bridging position of the non-hydrolysable deoxynucleotide analog is indicated with a red X. This position commonly contains either a non-reactive carbon or nitrogen group to prevent catalysis. (C) The time-lapse crystallography schematic for correct insertion is shown going from left to right. The global conformation of pol β is indicted at the top and the bottom corresponds to the ligands in the pol β active site at each step. The transfer to a solution containing $MgCl_2$ starts the reaction (i.e., $t = 0$). The structural snapshots are captured by freezing the reaction at various time points (downward facing arrows). Correct insertion results in a closed ternary product complex.

an inactive pre-insertion complex. The ternary complex (+ddCTP) of rat pol β bound to DNA with an incoming nucleotide was determined to 2.9 Å using a dideoxy-terminated primer [3]. The dideoxy-terminated primer does not support catalysis due to the missing 3′—OH (Fig. 1A). Using this approach, a general pol β catalytic mechanism (see below) and key active site residues were identified. A subsequent study using human pol β with the preferred 1-nucleotide (1-nt) gapped DNA containing a dideoxy-terminated primer diffracted to ~3.0 Å. These structures identified key conformational changes that occur upon pol β binding to the DNA and an incoming dNTP [4]. The use of a dideoxy-terminated primer was instrumental in capturing these early pre-insertion structural snapshots of pol β and remained the standard until non-hydrolysable deoxynucleotide analogs became readily available.

A limitation of using a dideoxy-terminated primer is that the catalytic metal binding site is distorted by the absence of a key coordinating ligand (i.e., primer sugar O3′; Fig. 1A). To overcome this limitation, ternary complex pre-insertion structures with a non-hydrolysable deoxynucleotide analog (2′-deoxyuridine 5′-α,β-imido-triphosphate) were determined for pol β in 2006 [5]. This analog contains nitrogen at the bridging oxygen position between the α- and β-phosphates to prevent catalysis while allowing the utilization of a natural primer terminus with a 3′—OH (Fig. 1B). Soaking binary DNA pol β crystals in a solution containing the non-hydrolysable analog results in a ternary complex that routinely diffracts well past 2.0 Å [5]. The first structural snapshots using non-hydrolysable analogs provided direct evidence that the 3′—OH and the catalytic Mg^{2+} ion are required to achieve the proper geometry necessary for an in-line nucleophilic attack of O3′ (primer terminus) on the α-phosphate of the incoming nucleotide. The availability of non-hydrolysable nucleotide analogs has allowed for subsequent studies looking at polymerase fidelity and lesion bypass [6–9].

2.1.2. Site-directed mutagenesis

Prior to 2012, the main approach for capturing structural snapshots utilized wild-type pol β with modified DNA or nucleotides. A caveat with this approach is that it only captures a closed pre-insertion complex. To overcome this limitation and trap structural intermediates prior to the closed pre-insertion complex, selective mutations within the pol β active site have been utilized to alter the equilibrium between the open and closed states that occur upon substrate binding (see below). For example, Arg283 contacts the DNA minor groove in the active site and stabilizes the closed ternary complex. A R283K point mutant shifts the pol β equilibrium to favor the open conformation thereby providing an opportunity to capture structural intermediates prior to polymerase closure [6]. These snapshots revealed that Watson–Crick hydrogen bonding of the nascent base pair is assessed prior to enzyme closing around the nascent base pair. The closed/open equilibrium is also altered by another mutation, the E295K variant. Interestingly, this enzyme has been associated with some gastric carcinomas and has a dramatically lower polymerase activity [1,7]. Structural studies with this protein provided insight into the structural rearrangements that occur during nucleotide insertion and provide structural evidence that alternate conformations modulate insertion efficiency and discrimination. Together, the R283K and E295K variant enzymes are examples of using site directed mutagenesis to alter the

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