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Structure of DNA polymerase ζ : capturing the getaway driver

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Abstract

During translesion synthesis, eukaryotic DNA polymerase ζ (zeta) carries out extension from a wide range of DNA lesions. In this issue, Malik et al. (2020) (1) present the cryo-EM structure of polymerase ζ and show how it catalyzes the extension step of translesion synthesis.

Translesion synthesis (TLS) is a mostly error-prone pathway used by cells to bypass DNA damage that blocks normal DNA replication (2–7). During TLS, the stalled, replicative DNA polymerase is replaced by one or more specialized TLS polymerases, which carries out replication through the DNA lesion. In many cases, TLS requires two specialized polymerases: an “insertor” to incorporate a nucleotide across from the DNA lesion and an “extender” to catalyze further extension. The primary “extender” polymerase in eukaryotes is DNA polymerase (pol) ζ (zeta) (8). While this enzyme was first purified nearly twenty-five years ago (9), structural studies of it have been unsuccessful due in part to the inability to obtain sufficient quantities of the protein for X-ray crystallography. In this issue, Malik et al. (2020) (1) report the cryo-EM structure of pol ζ and describe the structural features that allow it to catalyze the extension step of TLS.

A number of eukaryotic DNA polymerases have been shown to be able to incorporate nucleotides across from DNA damage. Some of these polymerases have a major role in DNA repair pathways such as base-excision repair and non-homologous end joining and perhaps have a minor role in TLS. Examples of these are the A-family polymerases pol θ (theta) and pol ν (nu) as well as X-family polymerases pol β (beta), pol λ (lambda), and pol μ (mu). By contrast, five eukaryotic polymerases have a major role in TLS. These are the Y-family polymerases pol η (eta), pol ι (iota), pol κ (kappa), and Rev1 as well as the B-family polymerase pol ζ . Of the five major TLS polymerases, only pol ζ , pol η , and Rev1 are found in all eukaryotes.

The Y-family polymerases generally function as insertors during translesion synthesis. Each of these polymerases has one or more cognate lesions that the polymerase has evolved to accommodate. Structural studies of the Y-family polymerases have provided tremendous insights into how these enzymes accommodate their cognate lesions. For example, the cognate lesions of pol η include thymine dimers and 8-oxoguanines, and the cognate lesions of pol κ include minor-groove guanine adducts. Like replicative polymerases, the active sites of pol η and pol κ facilitate the formation of Watson-Crick base pairs between the incoming

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dNTP and the damaged template base. These enzymes, however, have more space in their active sites in order to accommodate these lesions (10, 11). The cognate lesions of pol η include minor-groove purine adducts and exocyclic guanine adducts not capable of forming Watson-Crick base pairs. To accommodate these lesions, the active site of this enzyme favors the formation of Hoogsteen base pairs between the incoming dNTP and the template residue (12). Finally, the cognate lesions for Rev1 include abasic sites as well as minor-groove and exocyclic guanine adducts. To accommodate these lesions, this polymerase forces the damaged template out of the DNA double helix and into a pocket within the active site. An arginine side-chain then acts as a template to bind the incoming dCTP and position it for catalysis (13).

Unlike these other major TLS polymerases, pol ζ belongs to the B-family of DNA polymerases. The other members of this family, which function primarily in normal DNA replication, include: pol α (alpha), which initiates Okazaki fragments on the lagging strand; pol δ (delta), which synthesizes the lagging strand and functions in multiple DNA repair pathways; and pol ϵ (epsilon), which synthesizes the leading strand (14). Pol ζ differs from these other B-family polymerases in that it is required for nearly all damage-induced mutations and over half of spontaneous mutations (15). This is important because it shows that pol ζ is essential for most error-prone TLS in cells. When pol ζ was initially purified, the field widely believed that it was comprised of one Rev3 catalytic subunit and one Rev7 non-catalytic subunit. Biochemical studies of pol ζ lead to two important discoveries. First, pol ζ was shown to extend efficiently from primer-termini containing mismatches and template DNA lesions (8). This led to the notion that pol ζ functions as the extender in most error-prone TLS. Second, pol ζ was shown to have two additional non-catalytic subunits: Pol31 and Pol32 (16–18). This is remarkable, because Pol31 and Pol32 are also non-catalytic subunits of replicative pol δ .

Despite pol ζ being the first eukaryotic polymerase discovered and purified, its structure has only now been determined (1). Overall, pol ζ forms a pentameric ring comprised of one catalytic Rev3 subunit, one Pol31 subunit, one Pol32 subunit, and, surprisingly, two Rev7 subunits (Fig. 1) (1). The positions and orientations of the Pol31 and Pol32 subunits are similar between pol δ and pol ζ , except that in pol ζ these subunits rotate slightly to accommodate the two additional Rev7 subunits (Fig. 1) (1, 19). The two Rev7 subunits are arranged in a head-to-tail fashion, and both subunits bind to an extended, disordered region of the Rev3 subunit. The Rev7 subunits are important because they interact with other TLS proteins, most notably the TLS polymerase Rev1 (20). Modeling based on the structure of pol ζ suggests that only one of the two Rev7 subunits is capable of binding Rev1.

The folds of the Rev3 catalytic subunit of pol ζ and the Pol3 catalytic subunit of pol δ are similar (1, 19). These subunits both have an N-terminal domain (NTD), an exonuclease domain, and a polymerase domain comprised of fingers, palm, and thumb subdomains. Like pol δ , the DNA substrate binds only to the catalytic subunit of pol ζ (Fig. 1). Residues in the palm coordinate two metal ions suggesting a chemical mechanism similar to that of other polymerases. Moreover, several α -helices of the fingers contact the nascent base pair. These steric constraints provide the structural basis for the moderate to high fidelity of pol ζ as well as its inability to incorporate nucleotide opposite template lesions. Unlike pol δ , the

exonucleases domain is inactive, which prevents the removal of residues from aberrant primer-terminal base pairs containing mismatches or lesions. Furthermore, the conformation of the linker between the NTD and the palm is different. In pol δ , this linker contacts the primer-terminal base pair ensuring that pol δ does not efficiently extend from aberrant primer termini. In pol ζ , this linker does not contact the primer-terminal base pair. This provides the structural basis of the extension ability of pol ζ during TLS.

Error-prone TLS by the concerted effort of two polymerases, an inserter and extender, has been likened to a pair of criminals robbing a bank. One polymerase incorporates the nucleotide opposite the template DNA lesion; this is akin to the bank robber giving the teller a stick up note and running out of the bank with a bag of cash. The other polymerase catalyzes extension beyond the DNA lesion; this is akin to driving the getaway car. Much of the last twenty years has focused on structural and mechanistic studies of the inserters – *i.e.*, apprehending and interrogating the bank robbers. With the work of Malik et al. (1), the getaway driver has now been captured. Certainly many more secrets regarding the mechanism of pol ζ will be revealed in the interrogations to come.

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Figure 1. Comparison of the structures of pol δ and pol ζ .

The structure of pol δ (*left*) was reported by Jain et al. (19), and the structure of pol ζ (*right*) was reported by Malik et al. (1). Pol δ is comprised of one Pol3 catalytic subunit (*blue*), one Pol31 non-catalytic subunit (*green*), and one Pol32 non-catalytic subunit (*yellow*). Pol ζ is comprised of one Rev3 catalytic subunit (*light blue*), one Pol31 non-catalytic subunit (*green*), one Pol32 non-catalytic subunit (*yellow*), and two Rev7 non-catalytic subunits (*pink*).