This report features the work of Rey-Ting Guo and his co-workers published in Angew. Chem. Int. Ed. ***55****, 4716 (2016) .*

TLS 13B1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography
- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Sciences

| References |

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How the Fidelity of DNA Repair Is Modulated in Human Beings

The crystal structures of DNA Polymerase λ in various states were determined to elucidate the structural mechanism for the fidelity modulation.

The mechanism of DNA polymerase (pol) fidelity is of fundamental importance in chemistry and biology. Whereas the pols responsible for DNA replication are required to perform catalysis with great fidelity, those involved in DNA repair or mutagenic functions typically exhibit less fidelity. Although high-fidelity pols have been well studied, much less is known about how some pols achieve medium or low fidelity with functional importance. A structural basis for an atypical dG:dGTP mismatch (GG mismatch) incorporated catalyzed by the most error-prone DNA polymerase from Africa swine fever virus (Pol X) was reported.[1] Pol X catalyzes the dG:dGTP mismatch on prebinding syn-dGTP in the absence of DNA; the syn-dGTP then form an anti:syn dG:dGTP Hoogsteen base pair with the template dG of the DNA. His115 was found to be the critical residue in stabilizing the syn-dGTP; mutation of His115 to an alanine residue abolished the syn-dGTP (only anti-dGTP observed), and resulted in a fidelity increase by 330 fold. This unprecedented finding of pre-binding MgdNTP was in contrast to the paradigm in DNA polymerases, which states that DNA binding precedes that of MgdNTP.

Wen-Jin Wu, Ming-Daw Tsai and their co-workers of Academia Sinica extended their investigation to human DNA polymerase λ Pol λ.[2] They examined how Pol λ achieves its moderate fidelity by determining 12 crystal structures of *apo*-Pol λ, MgdTNP binary complexes, MnMgdNTP binary complex (in which dNTP refers to dGTP dATP, dTTP and dGTP), dG:dATP mismatched ternary complex, *apo*-L431A mutant, binary complexes of L431A:MgdCTP, L431A:MgdTTP and L431A:dGTP, dG:dCTP matched ternary complex but with L431A mutant. X-ray diffraction data were collected at **TLS 15A1**, **TLS 13B1**, **TLS 13C1** and **SP 44XU**. The authors also performed pre-steady-state kinetic analyses to determine the rate of dNTP incorporation, apparent K_d values of incoming dNTP to Pol-DNA binary complexes, catalytic specificity and fidelity. They showed that *apo*-Pol λ already exists in the closed conformation (**Fig. 1(a)**), unprecedentedly with a preformed MgdNTP binding pocket (**Figs. 1(c)-1(e)**), and binds MgdNTP readily in the active conformation in the absence of DNA (**Fig. 1(d)**). A large conformational change occurs upon the binding of the gapped DNA substrate containing a 5′-phosphate in the downstream primer (**Fig. 1(f)**).

The structure of Pol λ:MgdNTP binary complexes revealed that the tight MgdNTP binding was contributed in part from partial stacking between the Tyr505 side chain and the incoming dNTP. The MgdNTP affinity was decreased significantly in the Y505A mutant

and moderately decreased in the Y505F mutant. As prebinding of MgdNTP might produce little fidelity for the Pol X case,[1] it is attenuated in Pol λ with a hydrophobic core including Leu431, Ile492 and the Tyr505/Phe506 motif. The authors then predicted and demonstrated that L431A mutation enhances MgdNTP prebinding and decreases the fidelity. They also hypothesized that the MgdNTP-prebinding ability could stabilize a mismatched ternary complex and destabilize a matched ternary complex, and further provided structural evidence of both forms. Their results demonstrate that, whereas high-fidelity pols follow a common paradigm, Pol λ has developed specific conformations and mechanisms for its moderate fidelity. Structural comparison with other pols indicates that pols likely use varied conformational changes and microscopic mechanisms to achieve their catalytic functions with varying fidelities (**Fig. 2**). (Reported by Chun-Jung Chen)

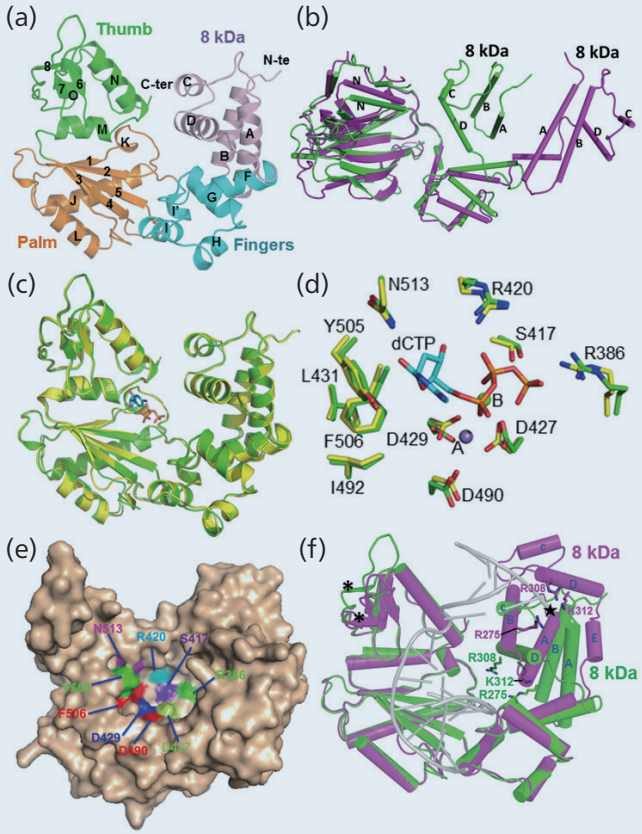

Fig. 1: Comparison of *apo*-Pol λ with other relevant structures. (a) Structure of *apo*-Pol λ. (b) Comparison between *apo*-Pol λ (green) and *apo*-Pol β (magenta). (c) Overlay of *apo*-Pol λ (green) and Pol λ:MnMgdCTP binary complex (yellow, with dCTP shown in sticks). (d) Expansion of (c) with the dCTP binding residues shown. (e) Surface representation of *apo*-Pol λ with dCTP binding residues shown in (d) colored and labeled. (f) Overlay of *apo*-Pol λ (green) and Pol λ:DNA binary complex (protein magenta, DNA grey) showing a large conformational change upon substrate-gapped DNA binding. Asterisk symbols indicate the β-strand 8 location; a black star indicates the location of downstream primer 5′-phosphate. [Reproduced from Ref. 2]

This report features the work of Wen-Jin Wu, Ming-Daw Tsai and their co-workers published in J. Am. Chem. Soc. ***138****, 2389 (2016).*

TLS 13B1 SW60 – Protein Crystallography
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TLS 15A1 Biopharmaceuticals Protein Crystallography
SP 44XU Macromolecular Assemblies

- Protein Crystallography
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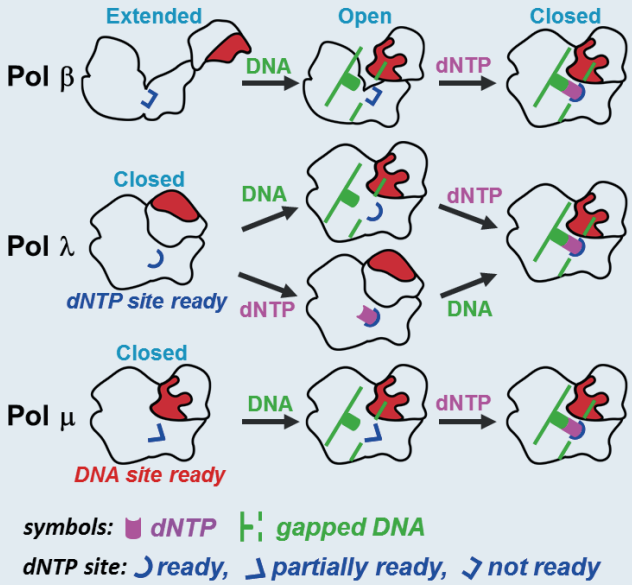

Fig. 2: X-family DNA polymerases utilize varied conformations to perform their specific functions and to regulate the DNA repair fidelity. For example, the dNTP-binding site is not formed in *apo*-Pol β; DNA-binding is required to form a dNTP-binding site. In contrast, the dNTP-binding site is ready in *apo*-Pol λ, and it can follow either the canonical DNA-binding first path or our newly discovered dNTP-binding first path. *Apo*-Pol μ adopts a closed form with a DNA-binding site ready, which is absent in *apo*-Pol β or Pol λ. For *apo*-Pol β, neither dNTP site nor DNA site is ready. [Reproduced from Ref. 2]