Phi29 DNA polymerase: Structure and sequencing

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Bacteriophage phi29 DNA polymerase is an unusual enzyme. Unlike many other polymerases, it can efficiently displace a non-template strand and replicate with high processivity without cofactors. It can also use a protein as a primer; biologically, it initiates replication by attaching a base to a serine residue in the phage-encoded terminal protein. Crystal structures determined in Tom Steitz's lab a decade ago revealed some of the structural underpinnings of the polymerase's unusual properties [1-3].

The high processivity and strand displacement activity of phi29 polymerase have led to its use in biotechnology. For example, we have engineered variants that can efficiently utilize fluorescently modified substrates and have altered kinetics. Once these template-bound variants are immobilized on a chip, the sequential binding of nucleotides by individual polymerases through tens of thousands of cycles of incorporation can be tracked, thereby enabling genomic sequencing [4]. The kinetic information in these sequencing traces can also be used to determine the presence of DNA modifications [5]. Here we will present structural data and a simple model that explain the altered kinetics observed when a polymerase encounters 6-methyladenine or 4-methylcytosine in a template strand. (Fig. 1).

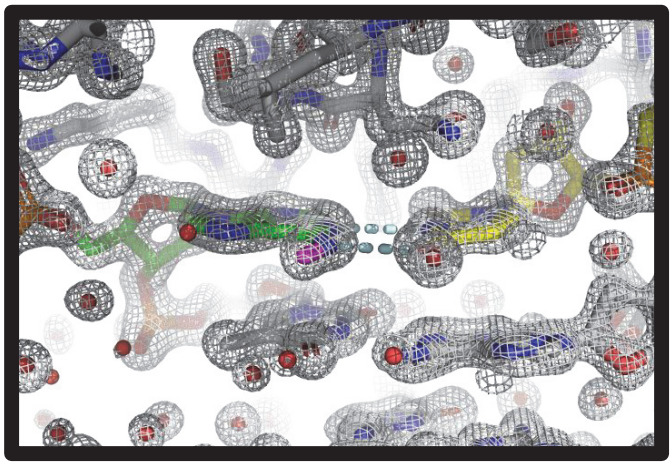

Fig. 1. A templating 6-methyladenine in the active site of Phi29 DNA polymerase (1.6 Å resolution). The carbon atoms of the incoming deoxynucleoside triphosphate are colored yellow, those of the templating nucleotide are green, with the 6-methyl moiety indicated in magenta.

[1] Kamtekar S, *et al.* (2004). *Molecular Cell* **16**, 609-618.

[2] Kamtekar S, *et al.* (2006). *EMBO J.* **25**, 1335-1343.

[3] Berman AJ, *et al.* (2007). *EMBO J.* **26**, 3494-3505.

[4] Wenger AM, *et al.* (2019) https://www.biorxiv.org/content/10.1101/519025v2

[5] Flusberg BA, *et al.* (2010). *Nat. Methods.* **6**, 461-465.