Structural and functional analysis through molecular modeling of DNA polymerase III α-subunit

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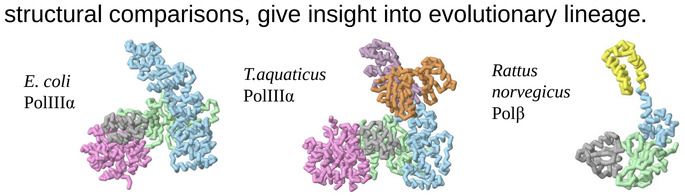
Abstract

DNA polymerases function in the replication and repair of chromosomes. Bacterial chromosome replication is performed by the replisome, a multi-subunit holoenzyme complex, comprised of a core polymerase complex (α, ε, θ), a clamp loader complex (γ, τ_2, δ, δ, χ, ψ), and a β-sliding clamp. A crystal structure of the DNA polymerase III α-subunit (PolIIIα) of *Thermus aquaticus* (Taq) with a template DNA fragment and a deoxynucleotide has been recently determined. In the present project a physical model of PolIIIα was created. The Taq α-subunit has the similar "hand-like" structure of PolIIIα of *E.coli* and is composed of six subdomains: palm, fingers, thumb, histidinol phosphatase (PHP), β-binding, and a C-terminal domain (CTD). The catalytic palm domain is similar to the eukaryotic DNA repair polymerase, Polβ, and not eukaryotic replicative DNA polymerases. Three highly conserved aspartate residues (D463, D465, D618) within the palm domain coordinate divalent metals involved in catalysis. A conserved glycine-serine motif (G425, S426) and four conserved arginine residues (R452, R458, R766, R767) participate in incoming nucleotide binding. A lysine residue (K616) near the catalytic site positions the 3'-terminal phosphate of the primer strand for dNTP addition. Incoming nucleotides undergo deoxyribose selection and pre-catalytic positioning in an entry channel through the fingers domain. Upon binding DNA, the α-subunit undergoes a conformational shift allowing interactions with the sugar-phosphate backbone. Several subdomains contribute to forming a DNA binding pocket. Interaction of a loop from the β-binding site with a cleft within the β-clamp supports a model for polymerase switching.

Introduction

DNA polymerases are molecular complexes crucial for maintenance of DNA through multiple functions including editing, repair, and replication. This model displays the α-subunit crystal structure of *Thermus aquaticus* DNA polymerase III (Taq PolIII). There are six families of DNA-dependent DNA polymerases (Bailey et al., 2006; Wing et al., 2008). Family C, to which Taq PolIII belongs, and family X make up the Polβ-like nucleotidyl-transferase superfamily characterized by the structure of the palm domain's catalytic site (Lamers et al., 2006). All polymerases, regardless of family, use the same catalytic mechanisms despite structural differences. The most thorough method to observe relationships between structure and functions is through X-ray crystallography of a ternary complex which includes enzyme, DNA, and incoming NTP. The structurally similar *E. coli* PolIIIα subunit has been crystalized; however, the protein is truncated as well as lacking DNA and NTP (Lamers et al., 2006). The ternary complex is useful for observing DNA-influenced conformational changes. These observations, along with other structural comparisons, give insight into evolutionary lineage.

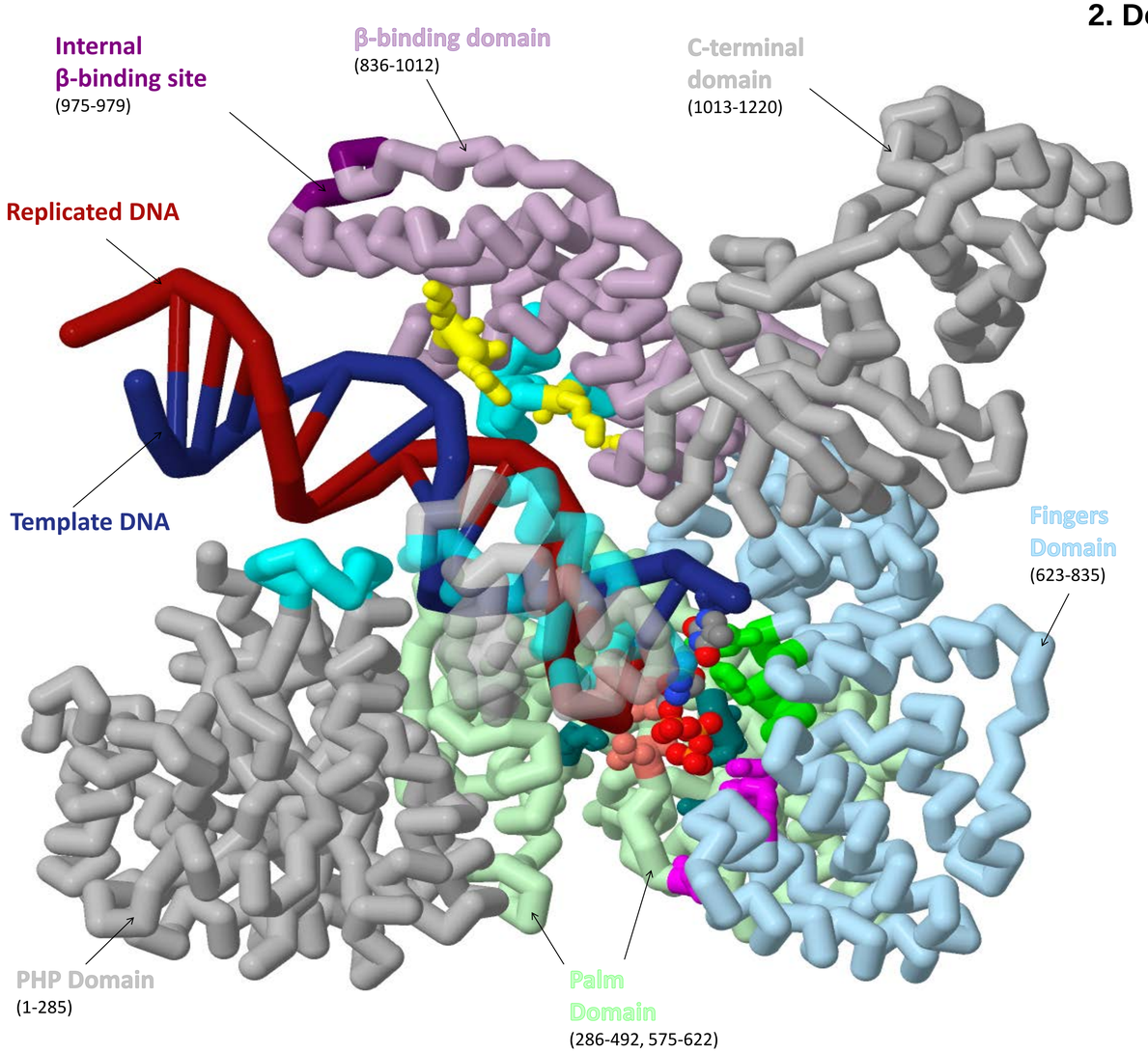


Domain	Function
PHP	Exit channel, possible 3'-5' exonuclease
Palm	Catalysis, incoming NTP positioning
Thumb	Exit channel
Fingers	NTP positioning, deoxyribose selection
β-binding	β-clamp binding, dsDNA positioning
C-Terminal	ssDNA binding, τ-subunit interaction

Mechanism

1. Initial DNA Binding

DNA polymerase III α-subunit undergoes conformational changes upon binding DNA. The fingers, thumb, β-binding and PHP domains rotate to grip the DNA backbone. This motion brings the helix-hairpin-helix DNA binding motif (HhH) into proximity with the DNA, as well as facilitating electrostatic interactions with the remainder of the DNA binding pocket (see supplemental movie).



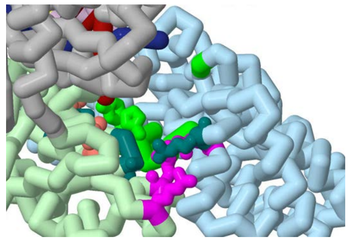
*Thumb Domain Transparent (493-574)

2. Domains and DNA Interactions

DNA binding pocket consists of:
- thumb: 500-511 and 515-535
- β-binding domain: the HhH motif 892-910
- PHP domain: 232-241

Amino Acid Contacts:
- K848, K932, R933 and R895

3. Nucleotide Entrance



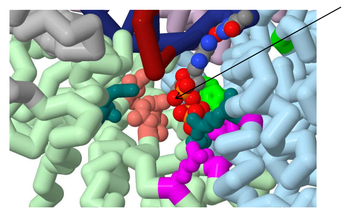
Nucleotides enter the catalytic zone through a gap in the palm domain.

Arginine residues involved in pre-positioning of dNTP:
- R452, R458 and R766
- Contact in a relay-like manner

Nucleotide binding pocket:
- H817 and Y821
- Select for deoxyribose triphosphates

4. dNTP Addition

α-phosphate of dNTP



Positioning of dNTP for catalysis:
- G425 and S426 act on β-phosphate
- R767 acts on γ-phosphate
- α-phosphate aligns near 3' hydroxyl
- Pyrophosphate group later released

Active site aspartates:
- D463, D465, and D618
- Coordinates two Mg^{2+} catalysis

Summary

- A model of *T. aquaticus* DNA PolIIIα was created. This is the first crystallized structure of a ternary complex including enzyme, DNA, and an incoming nucleotide.
- The homology of this enzyme with eukaryotic DNA Polβ, a DNA repair polymerase, shows that *T. aquaticus* and *E. coli* PolIIIα, a replicative polymerase, may possibly share an evolutionary link. This concept is contrary to the prior hypotheses in which eukaryotic and bacterial replicative enzymes share an ancestral lineage.
- Future research will include a resource page (Proteopedia.com) and possibly creation of more molecular models once future crystallizations of the entire DNA replication holoenzyme or replisome complex become available.

References

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Lamers MH, Georgescu RE, Lee SG, O'Donnel M, Kuriyan J. Crystal structure of the catalytic α subunit of *E. coli* replicative DNA polymerase III. *Cell*. 2006; 126: 881-892.
Sawaya MR, Pelletier H, Kumar A, Wilson SH, Kraut J. Crystal structure of rat DNA polymerase β: Evidence for a common polymerase mechanism. *Science.* 1994; 264: 1930-1935.
Wing RA, Bailey S, Steitz TA. Insights into the replisome from the structure of a ternary complex of the DNA polymerase III α-subunit. *J Mol Biol*. 2008; 382:859–869..

PDB Files
Model: 3E0D - *T. aquaticus* DNA polymerase III α (terinary complex)
Image: 2HQA - *E. coli* PolIIIα
Image: 2HPI - *T. aquaticus* PolIIIα (uncomplexed)
Image: 1BPD - *Rattus norvegicus* Polβ

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