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Structure and Mechanism of the DNA Polymerase Processivity Clamp Loader

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Beamline(s): X25

Introduction: Chromosomal replicases from prokaryotes to eukaryotes are organized around three functional components, a DNA polymerase, a ring-shaped processivity clamp, and a clamp-loading machine. DNA polymerases catalyze the addition of nucleotides to primed template DNA and, in isolation, are poorly processive enzymes (3, 8, 11). Presence of the processivity clamp, dramatically increase processivity. Processivity clamps are ring shaped proteins composed of 2 (eubacteria) or 3 (phage, archae, eukaryote) subunits (4). The clamps confer processivity by encircling DNA and physically tethering the polymerase subunit to the DNA as it tracks along the template (9). Assembly of sliding clamps on DNA is performed by the clamp loader assembly in a reaction fueled by ATP (10). In bacteria, the clamp loader is composed of 7 unique subunits (γ , δ , δ' , χ , ψ) and the β_2 dimer is the processivity clamp. The clamp loader is an AAA+ ATPase and is the bacterial homologue of the eukaryotic replication factor C (RFC) (5, 6). We have determined the structure of the bacterial clamp loader $\gamma_3\delta\delta'$ and that of the δ wrench in complex with a clamp subunit ($\beta:\delta$). These structures were solved using data measured at the National Synchrotron Light Source (X-25) at the Brookhaven National Laboratory, at the Structural Biology Center at the Advanced Photon Source (ID-19), the Stanford Synchrotron Radiation Laboratory (9.2), and the Advanced Light Source (5.02) at the Lawrence Berkeley Laboratory.

Results: The $\beta:\delta$ assembly. The crystal structure of the $\beta:\delta$ assembly revealed that the δ subunit binds to the C-terminal side of the processivity clamp at a location near to, but not at the dimer interface (Figure 1) (2). We used a mutated (I272A, L273A) form of β (β_1), which exists as a stable monomer in solution and which binds to the wrench subunit (δ) with a 50 fold higher affinity than wild type (7). The δ subunit, which adopts the same fold as the other clamp loader subunits, places its N-terminal domain (β -interacting element) into a binding site composed of two domains (2 & 3) on β_1 . Two highly conserved hydrophobic δ residues (F73, L74) are wedged into a hydrophobic pocket on the clamp. Binding of the δ subunit requires a conformational change in β that renders the clamp interface incapable of closing. With respect to its structure in the dimeric clamp, β from the $\beta:\delta$ complex adopts a conformation of reduced curvature. This observation along with molecular dynamics simulations suggest a spring-loaded mechanism in which the β ring opens spontaneously once a dimer interface is perturbed by the δ wrench.

The $\delta:\gamma_3:\delta$ assembly. The 2.7/3.0 Å crystal structure of the clamp loader revealed a pentameric arrangement of subunits, with a stoichiometry $\delta':\gamma_3:\delta$ (Figure 2) (1). The C-terminal domains of the subunits form a circular collar that supports an asymmetric arrangement of the N-terminal ATP binding domains of the γ motor and the structurally related domains of the δ' stator and the δ wrench. The nucleotide free clamp loader crystallized with the β -interacting element of the δ wrench accessible to solvent and not buried within the assembly. This observation was unexpected since the clamp loader has no detectible interaction with the clamp in the absence of nucleotide. The 3 ATP binding sites on the γ motor subunits are located near clamp loader subunit interfaces. The structure crystallized with only 2 of 3 sites available for ATP binding; the third site is blocked by the presence of structural elements from a neighboring subunit. We hypothesized that the structure in the crystal represents an intermediate in the clamp loading reaction. Nucleotide hydrolysis likely involves a conserved arginine residue from a neighboring subunit in a manner reminiscent to mechanisms proposed for other AAA+ ATPases (e.g. p97, NSF). The structure suggests a mechanism by which the γ complex switches between a closed state, in which the β -interacting element of δ is hidden by δ' , and an open form similar to the crystal structure, in which δ is free to bind to β .

References:

1. Jeruzalmi, D., M. O'Donnell, and J. Kuriyan, "Crystal structure of the processivity clamp loader gamma (gamma) complex of E. coli DNA polymerase III". *Cell*, 2001. **106**, 429-41.
2. Jeruzalmi, D., et al., "Mechanism of processivity clamp opening by the delta subunit wrench of the clamp loader complex of E. coli DNA polymerase III". *Cell*, 2001. **106**, 417-28.
3. Kelman, Z. and M.O. Donnell, "DNA Polymerase III Holoenzyme: Structure and Function of a Chromosomal Replicating Machine". *Annual Reviews of Biochemistry*, 1995. **64**, 171-200.
4. Kuriyan, J. and M. O'Donnell, "Sliding clamps of DNA polymerases". *J. Mol. Biol.*, 1993. **234**, 915-925.
5. Neuwald, A.F., et al., "AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes". *Genome Res*, 1999. **9**, 27-43.
6. O'Donnell, M., et al., "Homology in accessory proteins of replicative polymerases—E. coli to humans". *Nucleic Acids Res*, 1993. **21**, 1-3.
7. Stewart, J., et al., "Mechanism of beta Clamp Opening by the delta Subunit of Escherichia coli DNA Polymerase III Holoenzyme". *J Biol Chem*, 2001. **276**, 19182-9.
8. Stillman, B., "Smart machines at the DNA replication fork". *Cell*, 1994. **78**, 725-8.
9. Stukenberg, P.T., P.S. Studwell-Vaughan, and M. O'Donnell, "Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme". *J Biol Chem*, 1991. **266**, 11328-34.
10. Turner, J., et al., "The internal workings of a DNA polymerase clamp-loading machine". *EMBO J*, 1999. **18**, 771-83.
11. Young, M.C., M.K. Reddy, and P.H. von Hippel, "Structure and function of the bacteriophage T4 DNA polymerase holoenzyme". *Biochemistry*, 1992. **31**, 8675-90.

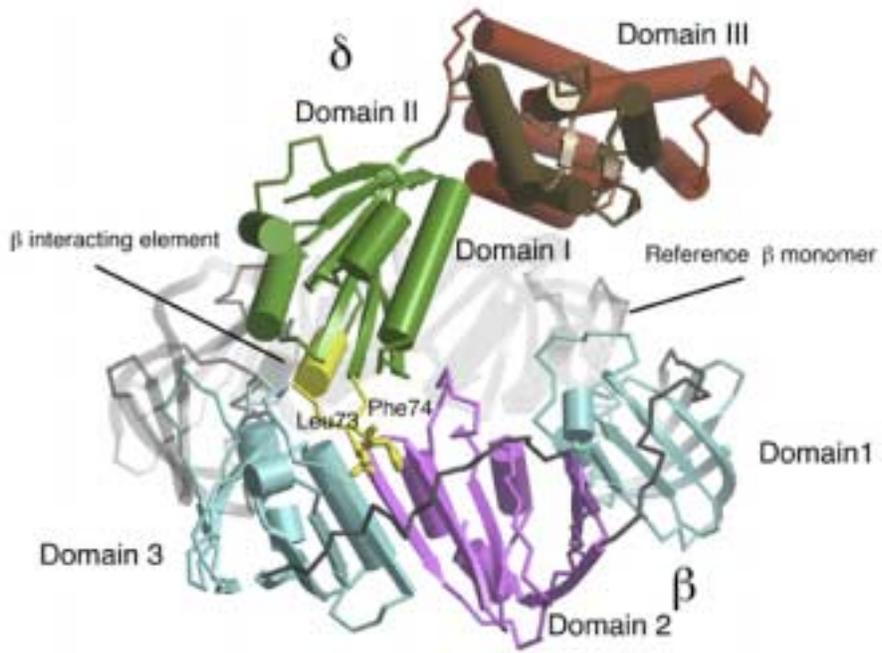


Figure 1. Structure of the β : δ complex

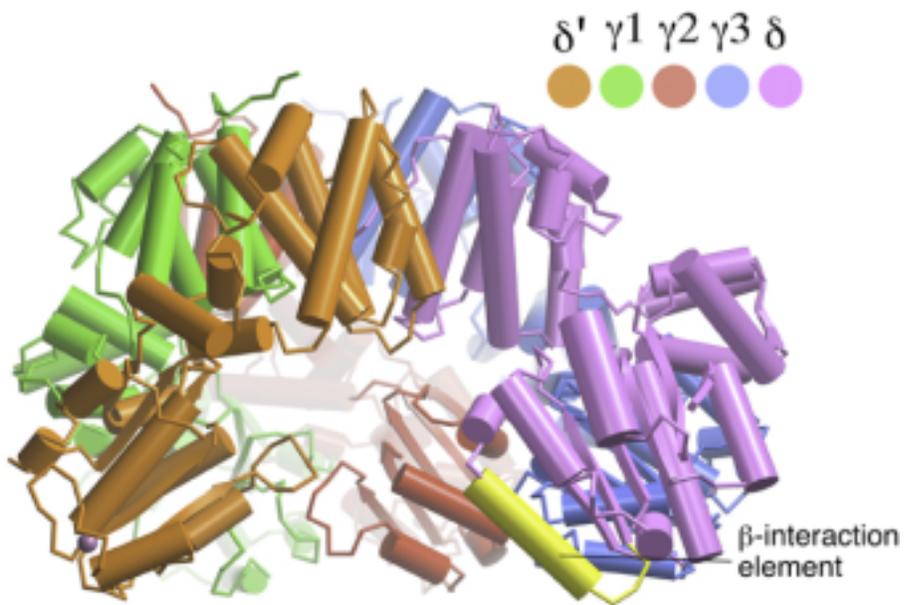


Figure 2. Structure of the γ complex.