ZOO 203 – MOLECULAR BIOLOGY
UNIT 1: DNA REPLICATION
(PART – III)

SANJIB KR. DAS
ASST. PROFESSOR
DEPT. OF ZOOLOGY
JHARGRAM RAJ COLLEGE

THE SPECIALIZATION OF DNA POLYMERASES

DNA Polymerases Are Specialized for Different Roles in the Cell

- E. coli has at least five DNA polymerases that are distinguished by their enzymatic properties, subunit composition, and abundance.
- **DNA polymerase III** (DNA Pol III) is the primary enzyme involved in the replication of the chromosome. Because the entire 4.6-Mb E. coli genome is replicated by two replication forks, DNA Pol III must be highly processive. Consistent with these requirements, DNA Pol III is generally found to be part of a larger complex that confers very high processivity—a complex known as the DNA polymerase III holoenzyme.
- **DNA polymerase I** (DNA Pol I) is specialized for the removal of the RNA primers that are used to initiate DNA synthesis. For this reason, this DNA polymerase has a 5' exonuclease that allows DNA Pol I to remove RNA or DNA immediately upstream of the site of DNA synthesis. Unlike DNA Pol III holoenzyme, DNA Pol I is not highly processive, adding only 20–100 nucleotides per binding event. These properties are ideal for RNA primer removal and DNA synthesis across the resulting ssDNA gap. The 5' exonuclease of DNAPol I can remove the RNA–DNA linkage that is resistant to RNase H. The low processivity of DNA Pol I readily synthesizes across the short region previously occupied by an RNA primer (<10 nucleotides) but is released before degrading and resynthesizing large amounts ofDNAthatwas primed by the RNA. Finally, when DNAPol I completes its function, only a nick is present in the DNA.
- The remaining three DNA polymerases in E. coli are specialized for DNA repair and lack proofreading activities.

TABLE 9-2 Activities and Functions of DNA Polymerases

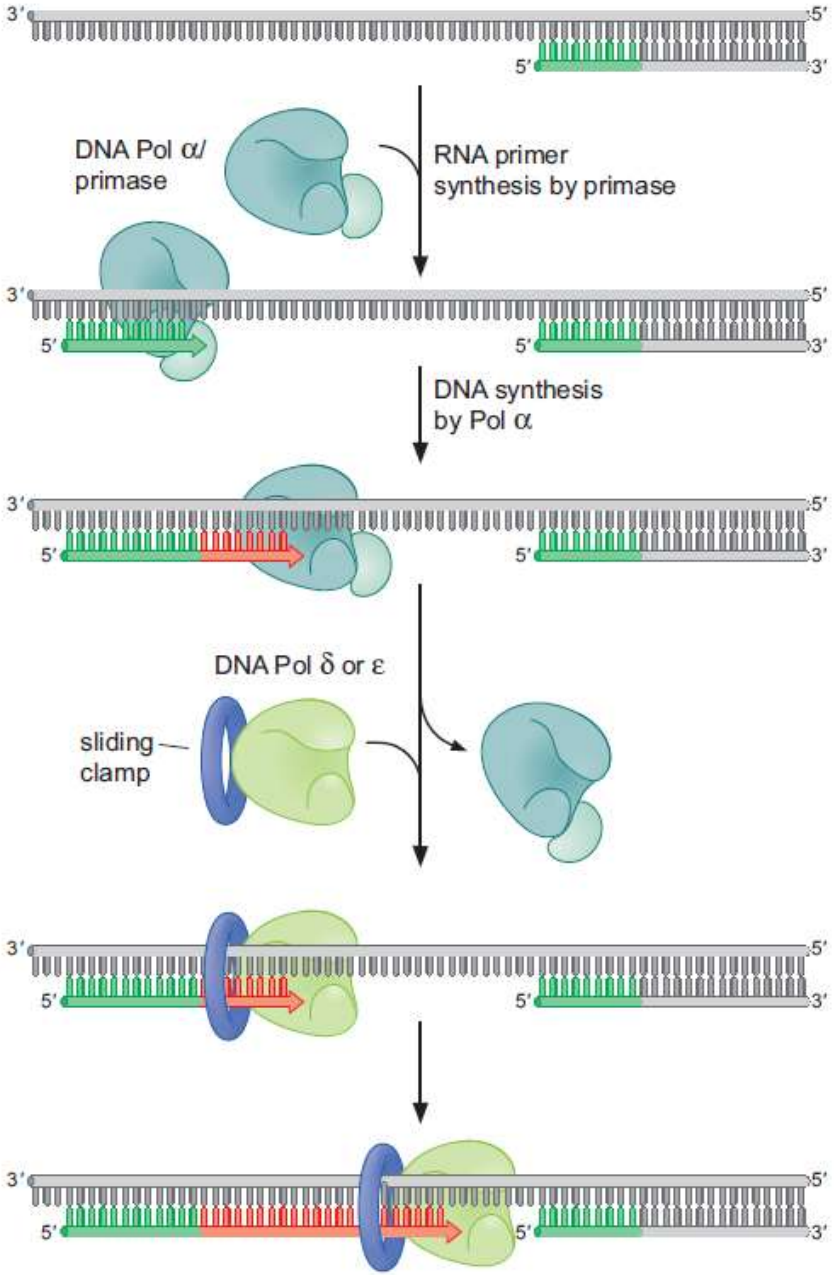
	Number of subunits	Function
Prokaryotic (*E. coli*)		
Pol I	1	RNA primer removal, DNA repair
Pol II (Din A)	1	DNA repair
Pol III core	3	Chromosome replication
Pol III holoenzyme	9	Chromosome replication
Pol IV (Din B)	1	DNA repair, translesion synthesis (TLS)
Pol V (UmuC, $UmuD_2'C$)	3	TLS
Eukaryotic		
Pol α	4	Primer synthesis during DNA replication
Pol β	1	Base excision repair
Pol γ	3	Mitochondrial DNA replication and repair
Pol δ	2–3	Lagging-strand DNA synthesis; nucleotide and base excision repair
Pol ε	4	Leading-strand DNA synthesis; nucleotide and base excision repair
Pol θ	1	DNA repair of cross-links
Pol ζ	1	TLS
Pol λ	1	Meiosis-associated DNA repair
Pol μ	1	Somatic hypermutation
Pol κ	1	TLS
Pol η	1	Relatively accurate TLS past *cis–syn* cyclobutane dimers
Pol ι	1	TLS, somatic hypermutation
Rev1	1	TLS

Data from Sutton M.D. and Walker G.C. 2001. *Proc. Natl. Acad. Sci.* **98**: 8342–8349, and references therein.

Eukaryotic DNA polymerase

- Eukaryotic cells also have multiple DNA polymerases, with a typical cell having more than 15.
- DNA Pol δ, DNA Pol ε, and DNA Pol α /primase are essential to duplicate the genome.
- **DNA Pol α /primase** is specifically involved in initiating new DNA strands. This four-subunit protein complex consists of a two-subunit DNA Pol a and a two-subunit primase. After the primase synthesizes an RNA primer, the resulting RNA primer:template junction is immediately handed off to the associated DNA Pol a to initiate DNA synthesis.
- Because of its relatively low processivity, **DNA Pol α /primase** is rapidly replaced by the highly processive **DNA Pol δ** and **Pol ε**.
- The process of replacing DNA Pol α /primase with DNA Pol δ or Pol ε is called **polymerase switching** and results in three different DNA polymerases functioning at the eukaryotic replication fork. **DNA Pol δ** and **ε** are specialized to synthesize different strands at the replication fork, with **DNA Pol ε** synthesizing the **leading strand** and DNA **Pol δ** the **lagging strand**.
- The majority of the remaining eukaryotic DNA polymerases are involved in DNA repair

DNA polymerase switching during eukaryotic DNA replication



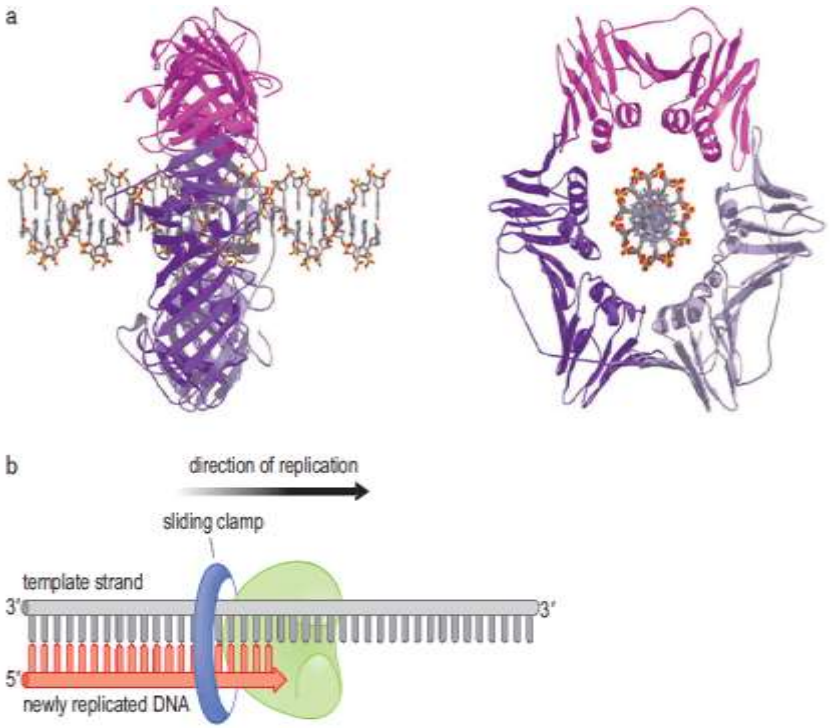
Sliding Clamps Dramatically Increase DNA Polymerase Processivity

- High processivity at the replication fork ensures rapid chromosome duplication
- High processivity of the DNA polymerases that act at replication forks is due to their association with proteins called **sliding DNA clamp** .
- These proteins are composed of multiple identical subunits that assemble in the shape of a “doughnut.”
- The hole in the center of the clamp is large enough to encircle the DNA double helix and leave room for a layer of one or two water molecules between the DNA and the protein.
- These properties allow the clamp proteins to slide along the DNA without dissociating from it. Importantly, sliding DNA clamps also bind tightly to DNA polymerases bound to primer:template junctions
- The resulting complex between the polymerase and the sliding clamp moves efficiently along the DNA template during DNA synthesis.

Structure of a sliding DNA clamp

- (a) 3D structure of a sliding DNA clamp associated with DNA. The opening through the center of the sliding clamp is 35A˚ , and the width of the DNA helix is 20A˚ . This provides enough space to allow a thin layer of one or two water molecules between the sliding clamp and the DNA. This is thought to allowthe clamp to slide along the DNA easily. (Adapted from Krishna T.S. et al. 1994. Cell 79: 1233–1243. Image prepared with MolScript, BobScript, and Raster3D. DNA modeled by Leemor Joshua-Tor.)

- (b) Sliding DNA clamps encircle the newly replicated DNA produced by an associated DNA polymerase. The sliding clamp interacts with the part of the DNA polymerase that is closest to the newly synthesized DNA as it emerges from the DNA polymerase.

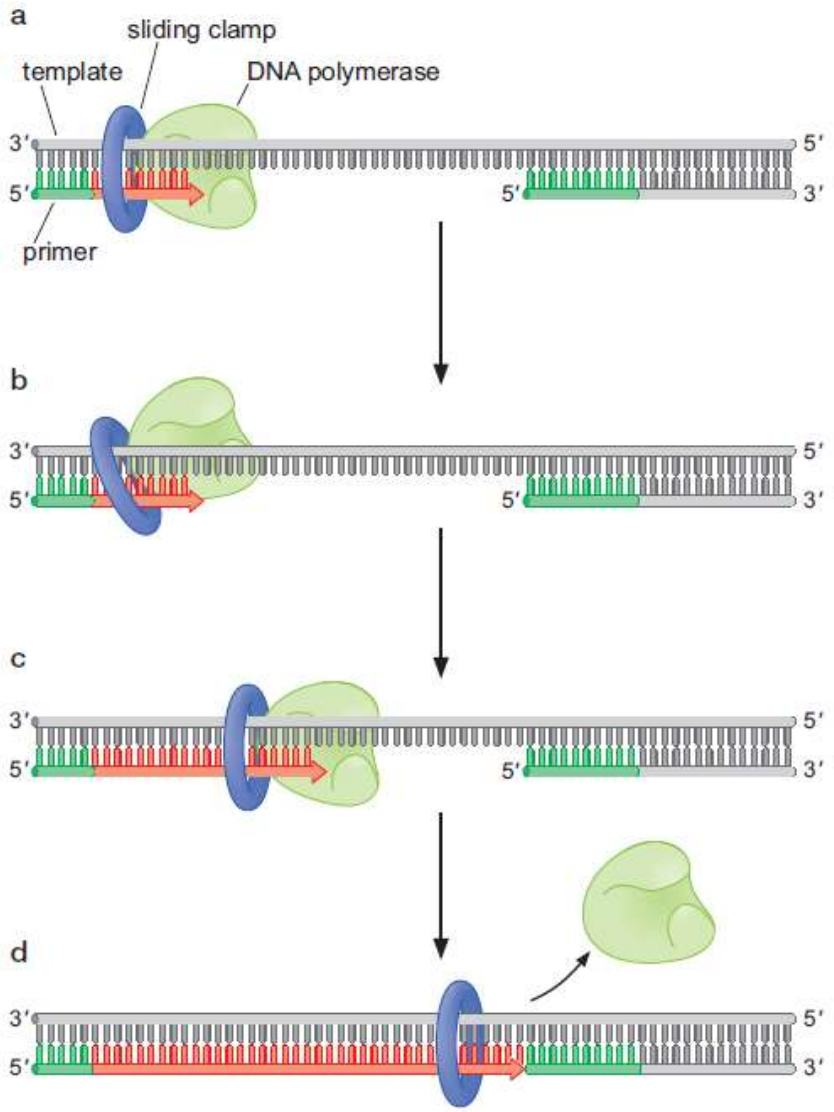


How does the association with the sliding clamp change the processivity of the DNA polymerase?

- In the absence of the sliding clamp, a DNA polymerase dissociates and diffuses away from the template DNA on average once every 20–100 bp synthesized.
- In the presence of the sliding clamp, the DNA polymerase still disengages its active site from the 3’-OH end of the DNA frequently, but th association with the sliding clamp prevents the polymerase from diffusing away from the DNA (Fig. 9-20).
- By keeping the DNA polymerase in close proximity to the DNA, the sliding clamp ensures that the DNA polymerase rapidly rebinds the same primer:template junction, vastly increasing the processivity of the DNA polymerase.

Sliding DNA clamps increase the processivity of associated DNA polymerases.

- (a) The sliding DNA clamp encircles the DNA and simultaneously binds the DNA polymerase.
- (b) The relatively low processivity of DNA polymerases leads to frequent release from the primer:template junction, but the association of the polymerase with the sliding clamp prevents diffusion away from the DNA.
- (c) The association of DNA polymerase with the sliding clamp ensures that the DNA polymerase rebinds the same primer:template junction and resumes DNA synthesis.
- (d) After DNA polymerase has completed synthesis of the template, the absence of a primer:template junction causes a change in the DNA polymerase that releases it from the sliding clamp.



MODE OF ACTION:

- Once an ssDNA template has directed synthesis of its complementary DNA strand, the DNA polymerase must release from the completed dsDNA and the sliding clamp to act at a new primer:template junction.
- This release is accomplished by a change in the affinity between the DNA polymerase and the sliding clamp that depends on the bound DNA. DNA polymerase bound to a primer:template junction has a high affinity for the clamp.
- In contrast, when a DNA polymerase reaches the end of an ssDNA template (e.g., at the end of an Okazaki fragment), the presence of dsDNA in its active site results in a change in conformation that reduces the polymerase's affinity for the sliding clamp and the DNA.
- Thus, when a polymerase completes the replication of a stretch of DNA, it is released from the sliding clamp so that it can act at a new primer:template junction.

- Once released from a DNA polymerase, **sliding clamps** are not immediately removed from the replicated DNA. Instead, other proteins that function at the site of recent DNA synthesis interact with the clamp proteins.
- Enzymes that assemble chromatin in eukaryotic cells are recruited to the sites of DNA replication by an interaction with the eukaryotic sliding DNA clamp (called “PCNA”).
- imilarlyS, eukaryotic proteins involved in Okazaki fragment repair also interact with sliding clamp proteins.
- In each case, by interacting with sliding clamps, these proteins accumulate at sites of new DNA synthesis where they are needed most.

Sliding Clamps Are Opened and Placed on DNA by Clamp Loaders

- The sliding clamp is a closed ring in solution but must open to encircle the DNA double helix.
- Special class of protein complexes, called **sliding clamp loader**, catalyze the opening and placement of sliding clamps on the DNA.
- These enzymes couple ATP binding and hydrolysis to the placement of the sliding clamp around primer:template junctions on the DNA.
- The clamp loader also **removes** sliding clamps from the DNA when they are no longer in use, although this does not require ATP hydrolysis.

What controls when sliding clamps are loaded and removed from the DNA?

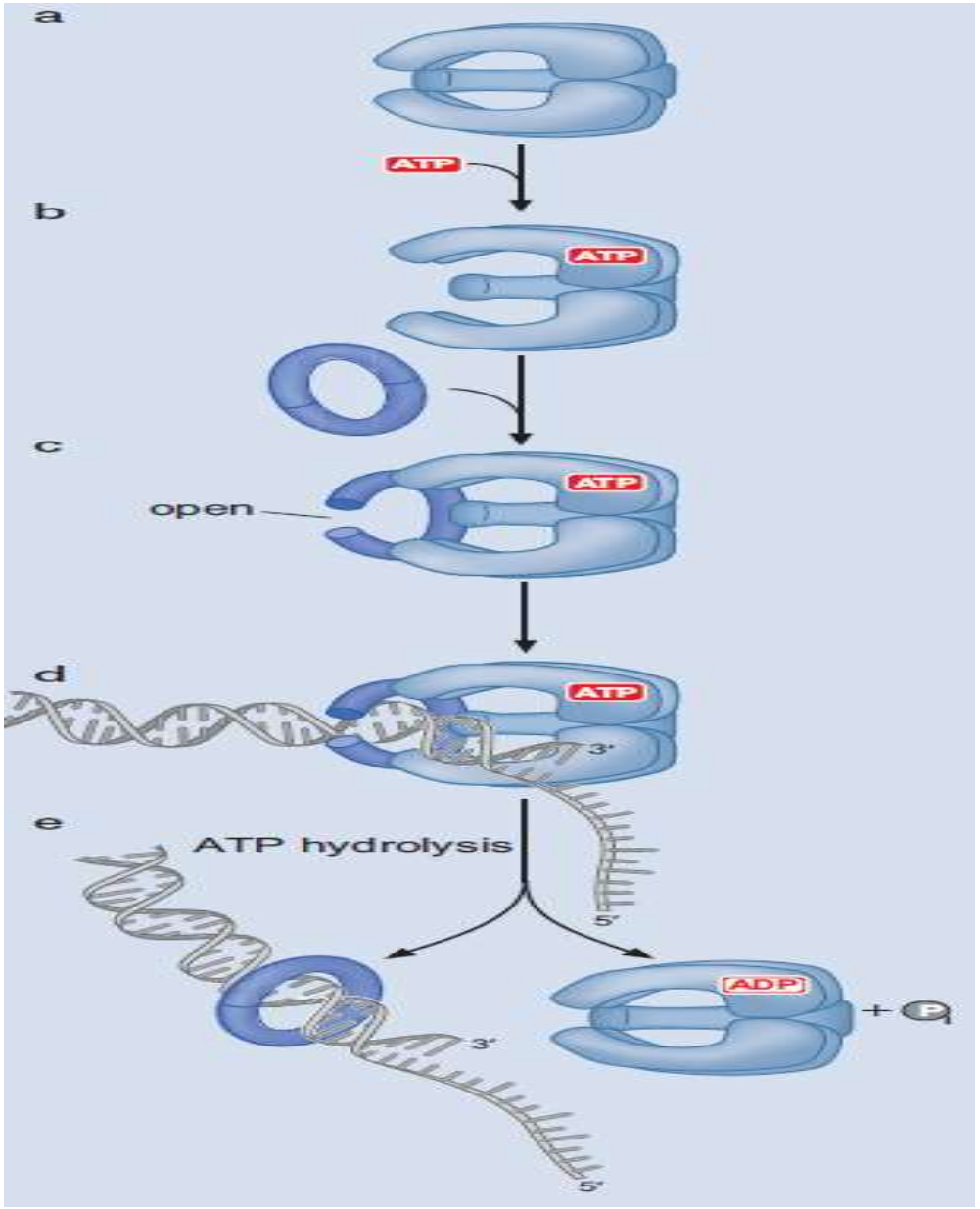
- Loading & removal of sliding clamp is done by **sliding clamp loader.**
- **Loading of a sliding clamp** occurs anytime a primer: template junction is present in the cell. These DNA structures are formed not only during DNA replication, but also during several DNA-repair events.
- A sliding clamp can only be **removed** from the DNA if it is not bound by another protein.
- Sliding clamp loaders and DNA polymerases cannot interact with a sliding clamp at the same time because they have overlapping binding sites on the sliding clamp. Thus, a sliding clamp that is bound to a DNA polymerase is not subject to removal from the DNA.
- Similarly, Nucleosome assembly factors, Okazaki fragment repair proteins, and other DNA-repair proteins all interact with the same region of the sliding clamp as the **clamp loader**.
- Thus, sliding clamps are only removed from the DNA once all of the enzymes that interact with them have completed their function.

ATP control of sliding DNA clamp loading.

- (a) Sliding clamp loaders are five-subunit protein complexes whose activity is controlled by ATP binding and hydrolysis.
- In E. coli, the clamp loader is called the **γ complex**, and in eukaryotic cells, it is called replication factor C (**RF-C**).
- b) To catalyze the sliding clamp opening, the clamp loader must be bound to ATP.
- (c) Once bound to ATP, the **clamp loader** binds the **clamp** and opens the ring at one of the subunit:subunit interfaces.
- (d) The resulting complex can now bind to DNA. DNA binding is mediated by the clamp loader, which preferentially binds to primer:template junctions.

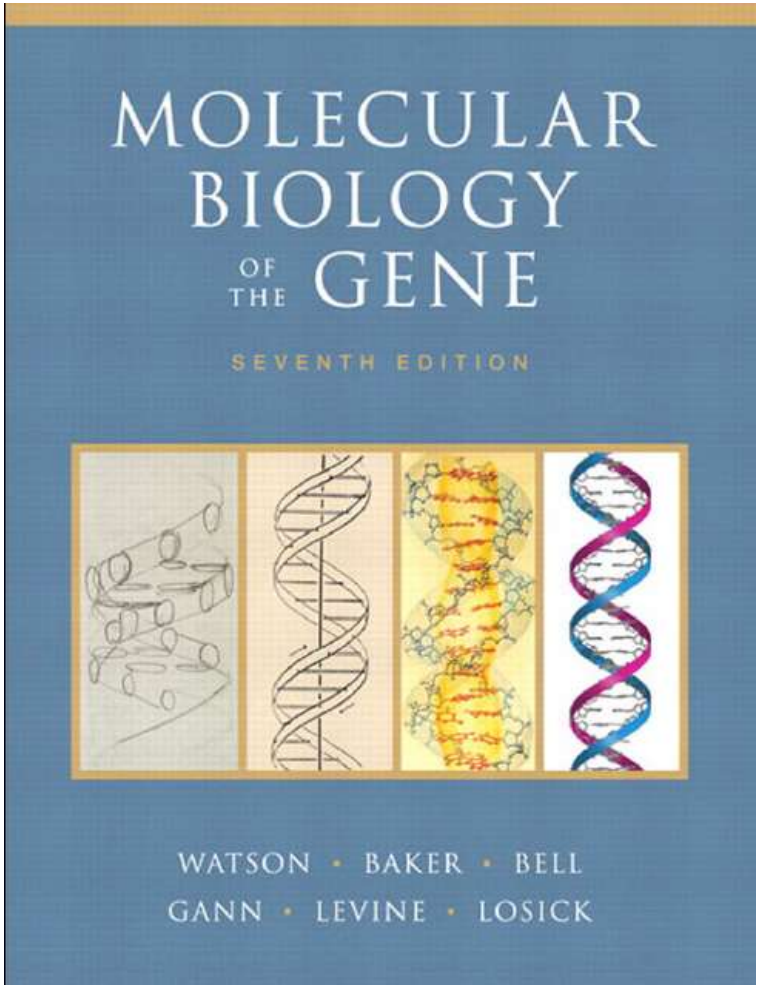
ATP control of sliding DNA clamp loading.

(Adapted, from O'Donnell M. et al. 2001. Curr. Biol. 11: R935– R946, Fig. 5.#Elsevier.)



- Correct binding to the DNA has two consequences.
- First, the opened sliding clamp is positioned so that dsDNA is in what will be the “hole” of the clamp.
- Second, DNA binding stimulates ATP hydrolysis by the clamp loader.
- (e) Because only an ATP-bound clamp loader can bind to the clamp and to DNA, the ADP form of the clamp loader rapidly disassociates from the clamp and the DNA, leaving behind a closed clamp positioned around the dsDNA portion of the primer:template junction.

REFERENCE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