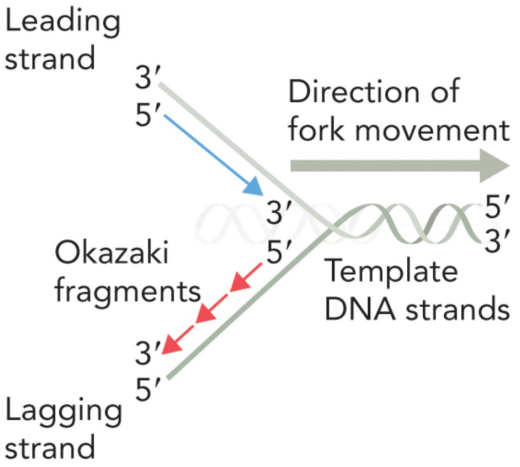
# BIOC 385: M10.T01-Miesfeld

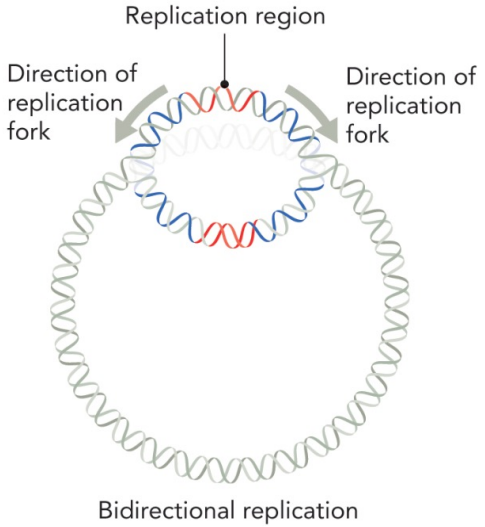
Assigned Reading: *Biochemistry* Chapter 23.1bc

# Structure and Function of DNA Replication Proteins

# The Big Picture

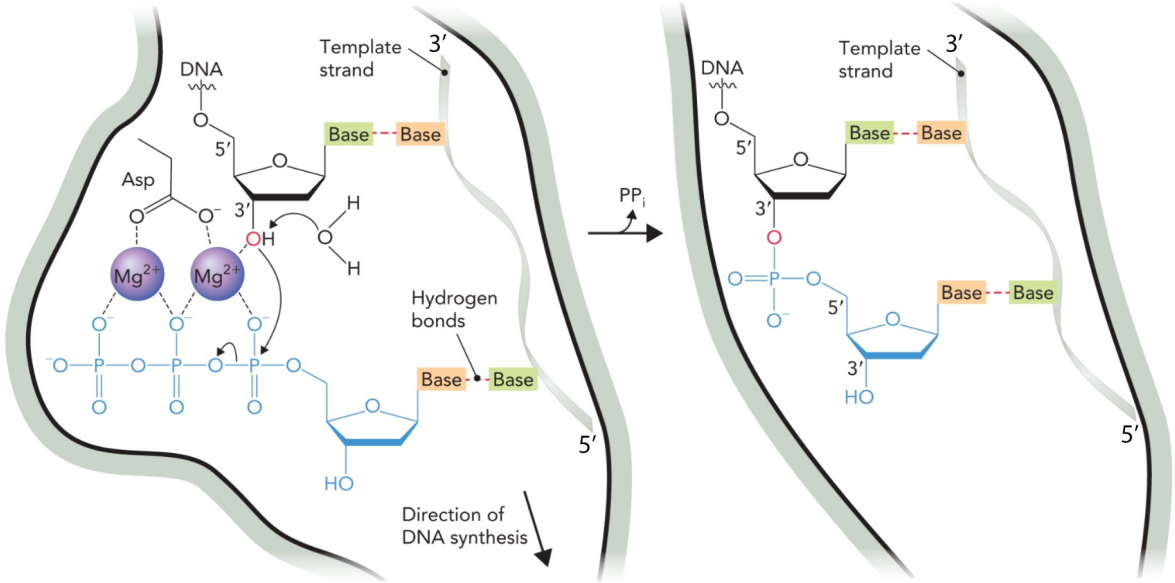
- DNA replication requires a coordinated set of enzymes and proteins that ensure genome duplication is both efficient and accurate.
- Understanding the proteins of the replisome clarifies how nucleotide addition, proofreading, helicase unwinding, priming, and strand coordination occur *in vivo*.



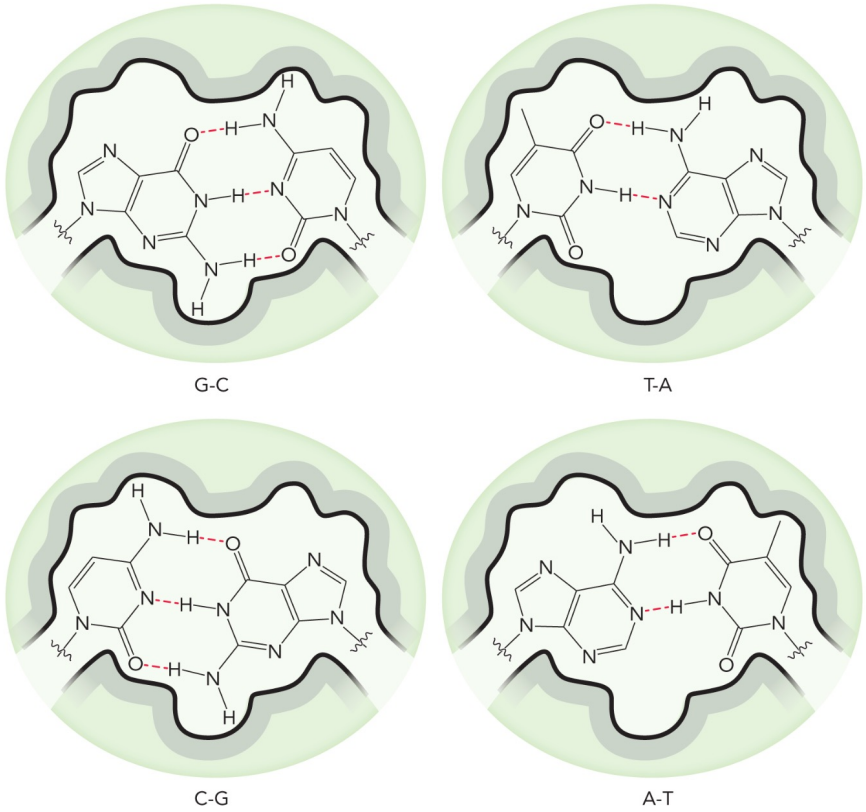
# Catalytic Mechanism of DNA Polymerase

- DNA polymerase extends DNA at the 3′ end, with $Mg^{2+}$ ions facilitating 3′-OH deprotonation and stabilizing the incoming dNTP during nucleophilic attack.
- Pyrophosphate hydrolysis drives the reaction irreversibly, ensuring efficient chain elongation during replication.



# Nucleotide Binding to the Active Site

- The DNA polymerase active site accommodates correct A–T and G–C pairs, while mismatches are excluded due to size and geometry constraints.
- This structural fidelity reduces error rates, though occasional mismatches require correction by proofreading activities.



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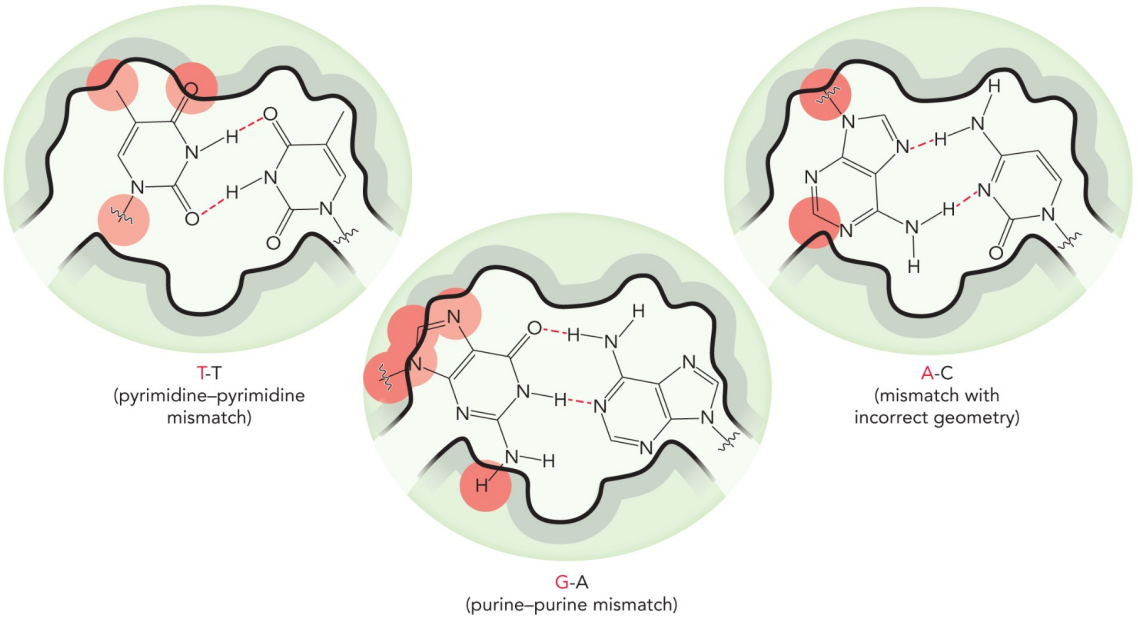


# Bacterial DNA Polymerases

- Pol III holoenzyme is the primary replicative enzyme, with high speed (~1000 nt/s), unlimited processivity, and proofreading activity.
- Pol I removes primers and repairs DNA with both 3′→5′ and 5′→3′ exonuclease activities, while Pol II primarily functions in DNA repair.

| | Pol I | Pol II | Pol III |
|---|---|---|---|
| **Major function(s)** | Proofreading, repair, primer removal | Repair | Main polymerizing enzyme |
| **5'→3' exonuclease** | Yes | No | No |
| **3'→5' exonuclease** | Yes | Yes | Yes |
| **Molecules per cell** | ~400 | Unknown | ~10–20 |
| **Mass (kDa)** | 103 | 90 | 167[a] |
| **Nucleotide polymerization rate (nucleotides per second)** | 10 | 0.5 | ~1000 |
| **Processivity (nucleotides)** | 10–20 | 1500 | Unlimited[b] |
| **Conditional lethal mutant** | Yes | No | Yes |

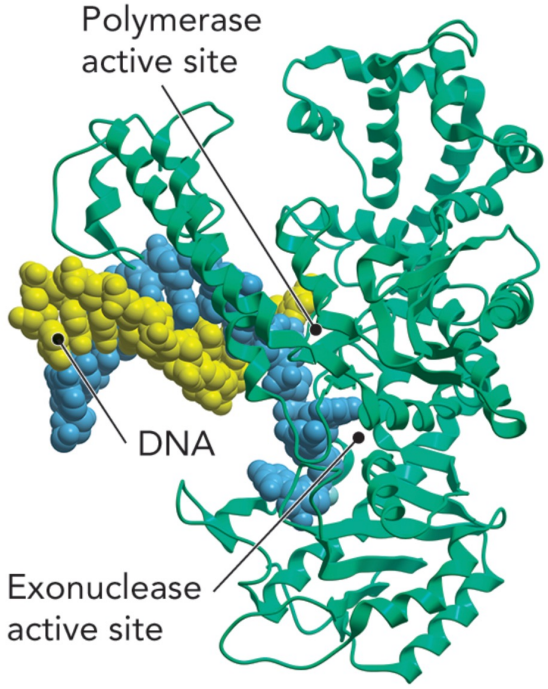
# Eukaryotic DNA Polymerases

- Pol ε synthesizes the leading strand, Pol δ the lagging strand, and Pol α initiates synthesis with primase; δ and ε rely on PCNA for processivity.
- Proofreading by δ and ε enhances accuracy, while Pol α's low processivity and lack of proofreading limit its role to primer extension.

| | Pol δ | Pol ε | Pol α |
|---|---|---|---|
| **Major function(s)** | Lagging strand synthesis | Leading strand synthesis | Initial synthesis from primer, primase |
| **3'→5' exonuclease** | Yes | Yes | No |
| **Processivity (nucleotides)** | High | High | Low |
| **PCNA association** | Yes | Yes | No |

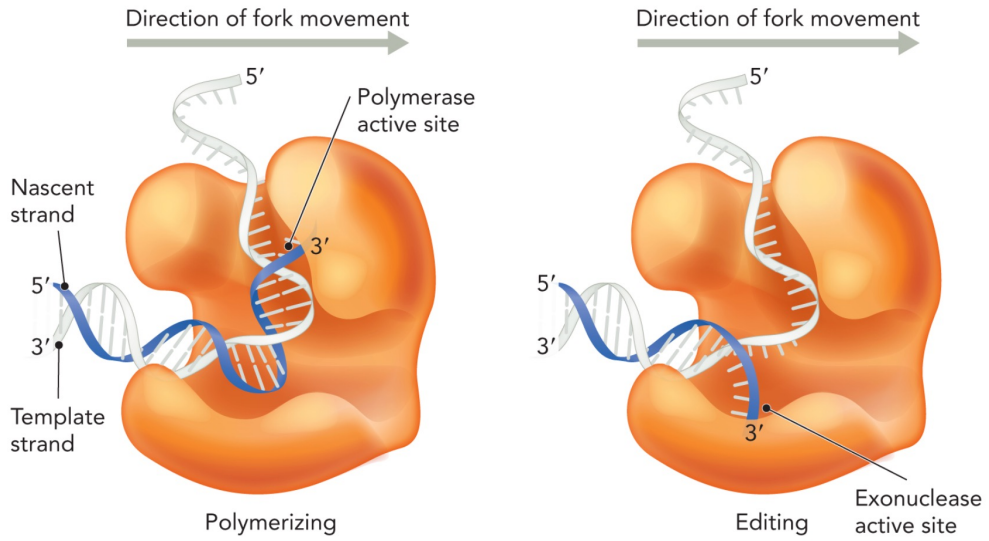
# Bacterial Klenow Fragment of DNA Pol I

- Proteolysis of Pol I produces the Klenow fragment with separate polymerase and exonuclease domains ~25–35 Å apart.
- $Mg^{2+}$ ions and positively charged residues at the polymerase active site optimize substrate positioning and catalysis.



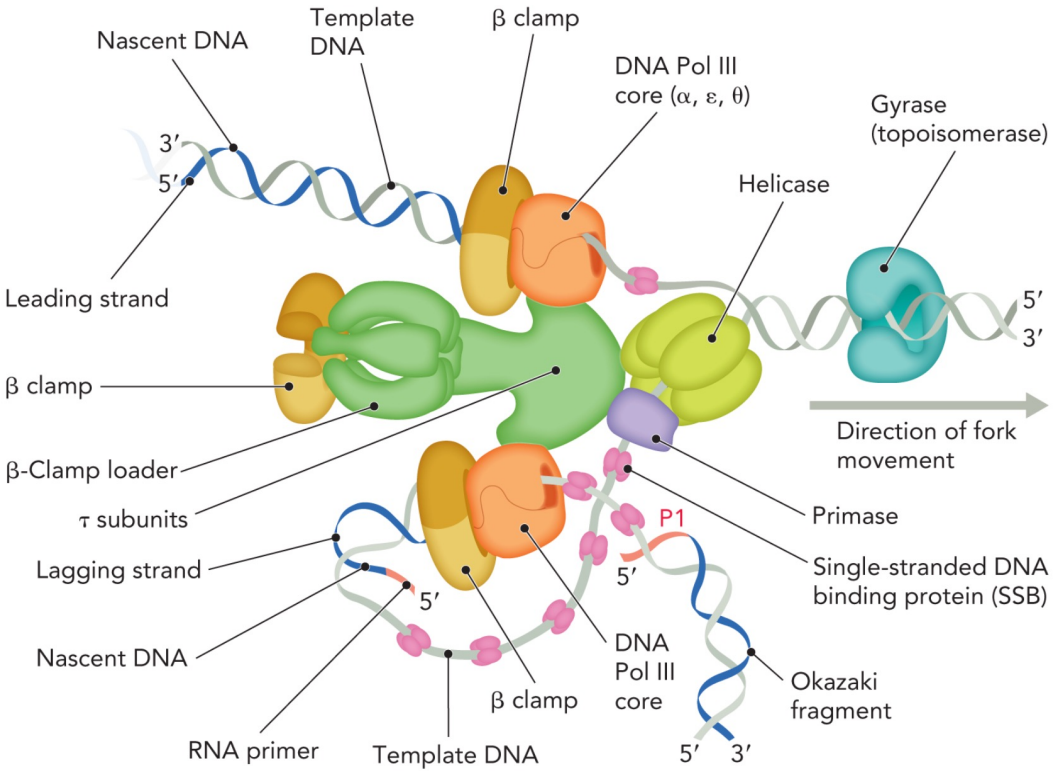
# Proofreading Activity Prevents Mutations

- Mismatched bases are corrected when the nascent strand flips into the 3′→5′ exonuclease site for excision.
- The corrected strand then returns to the polymerase site, ensuring replication fidelity.



# Proteins at the Replication Fork

- Helicase unwinds DNA, gyrase relieves supercoiling, SSB prevents strand reannealing, and primase generates RNA primers.
- Pol III synthesizes new DNA while being tethered to DNA by the β clamp, ensuring coordinated leading and lagging strand synthesis.



# Six Essential Proteins at the Replication Fork

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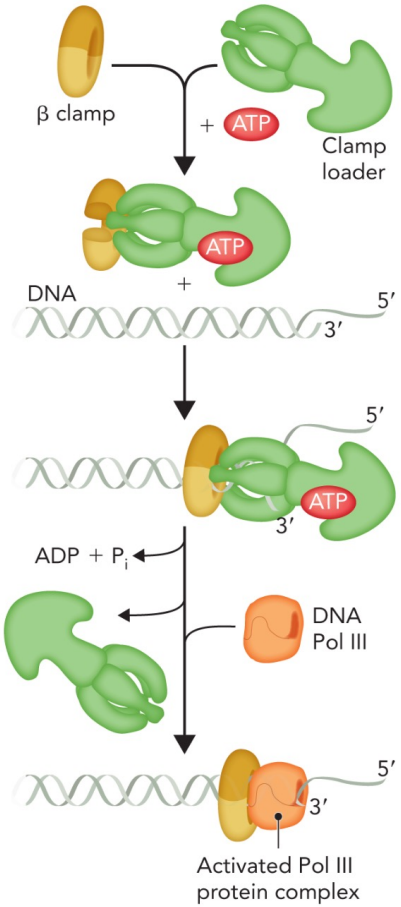
| Replication fork proteins | Function in DNA replication |
|---|---|
| DNA polymerase | Synthesizes nascent DNA on leading and lagging strands |
| DNA helicase | Unwinds the DNA double helix ahead of the replicating complex |
| Primase | Synthesizes RNA primers on the lagging strand |
| Single-stranded DNA binding protein | Binds to single-stranded DNA to prevent reannealing of the DNA double helix |
| DNA gyrase | Relieves torsional stress in front of the fork (a type II topoisomerase) |
| β clamp | Prevents DNA polymerase III from dissociating from the DNA |

# Function of the β Clamp and Clamp Loader

- The β clamp encircles DNA to confer processivity, while the clamp loader opens and closes the clamp using ATP.
- This dynamic system allows Pol III to efficiently engage and disengage DNA during replication.



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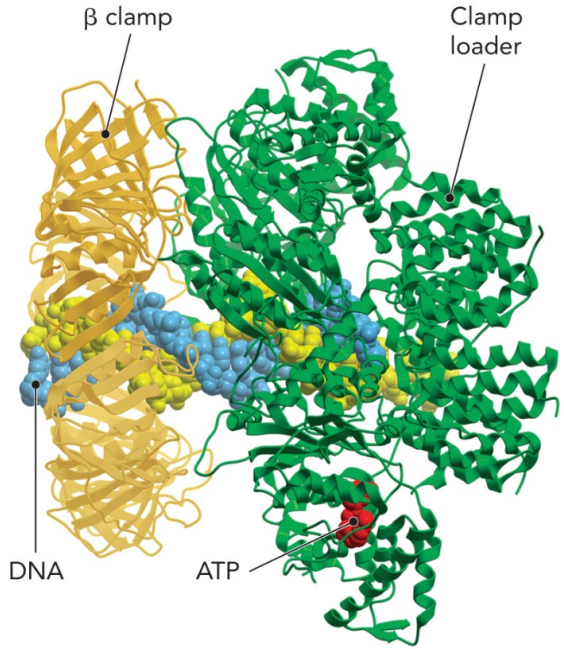
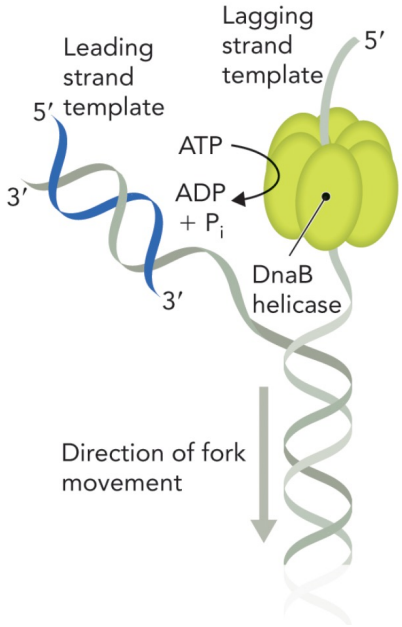
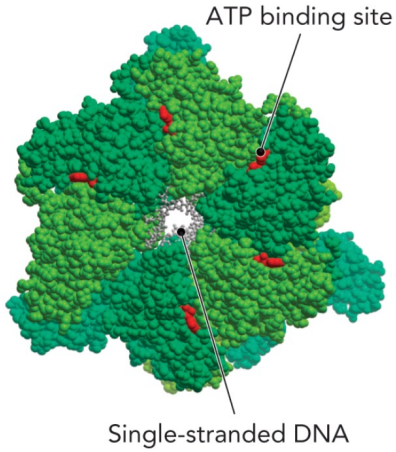


# Strand Separation by the Helicase Protein

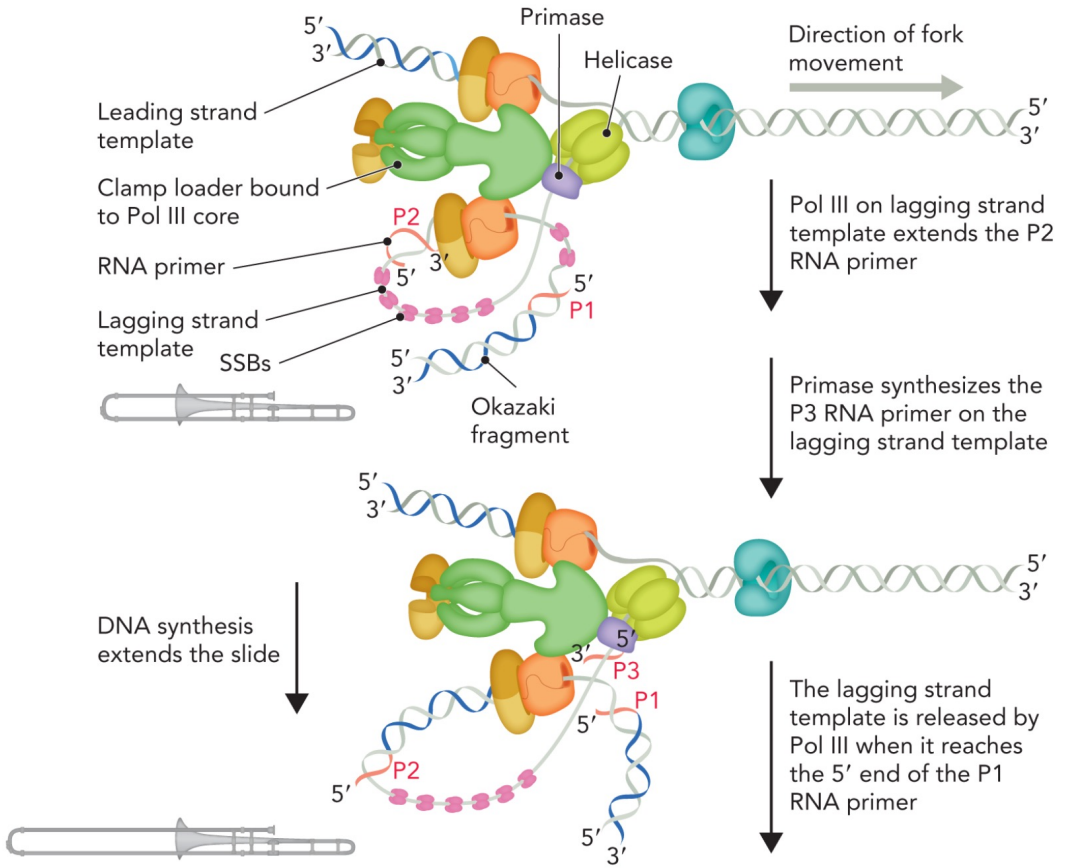
- DnaB helicase forms a hexameric barrel, translocating along DNA with ATP hydrolysis to separate strands.
- The ATP-driven step of each monomer advances the helicase by two nucleotides, functioning as a wedge to disrupt base pairing.



# The Trombone Model of DNA Synthesis

- According to the “trombone model” of DNA synthesis at the fork, the Pol III core on the lagging strand template alternates between bound and unbound forms.
- The Pol III core on the lagging strand template synthesizes an Okazaki fragment from the 3′ end of one RNA primer until it reaches the 5′ end of the RNA primer farther down the lagging strand template.



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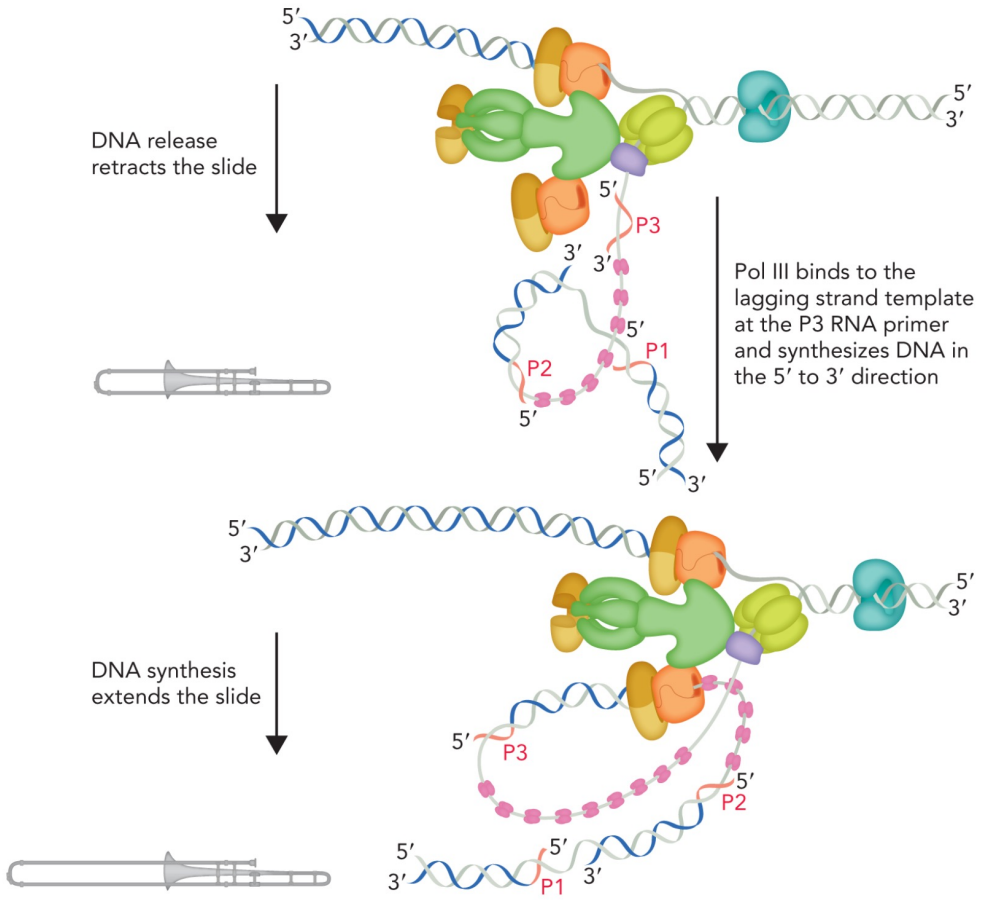

# Key Concepts to Guide Your Learning

- In prokaryotes, DNA polymerase III is the primary polymerizing enzyme; in eukaryotes, DNA polymerase δ and ε fulfill that role; secondary DNA polymerases remove primers, repair DNA, and synthesize mitochondrial DNA.
- The prokaryotic replication fork requires topoisomerase, DNA helicase, primase, single-stranded DNA binding protein, the core polymerase, and the β.
- DNA polymerases discriminate by proper base pairing such that only A-T and G-C base pairs fit the geometry of the active site, in addition the 3’ to 5’ proofreading function of DNA polymerases removes incorrect nucleotides.
- The trombone model proposes coordinate polymerizing activity on the leading and lagging DNA strands to ensure that the replication fork moves in the forward direction; the model proposes that the lagging strand DNA template is alternately bound and released as each Okazaki fragment is synthesized.