DNA REPLICATING ENZYMES

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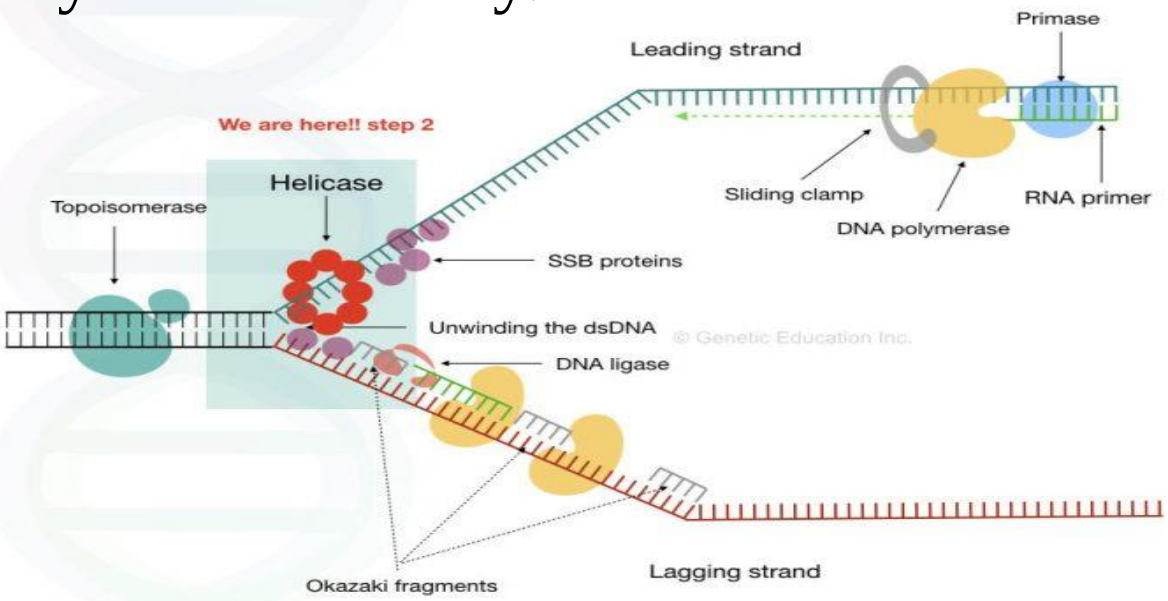
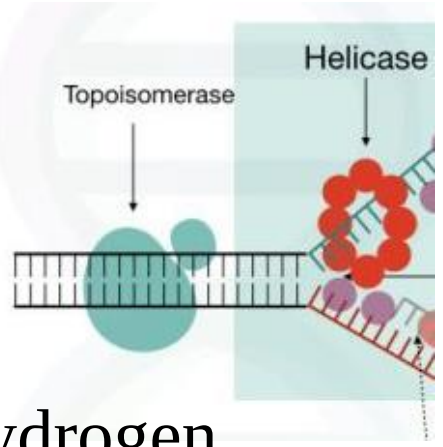
Enzymes and proteins:

- Cells need to copy their DNA very **quickly**
- With very **few errors** (or risk problems such as cancer).
- They use a variety of enzymes and proteins, which work together to make sure DNA replication is performed smoothly and accurately.

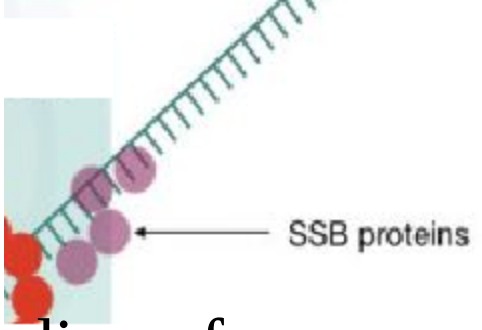


1. Helicases



- They translocate along strands of DNA
- They use energy: ATP hydrolysis to break hydrogen bonds
- Unwind DNA ahead of the replication fork and separate duplex molecules.
- They translocate: one direction only along DNA
- In topologically constrained DNA, helicase work together with a topoisomerase to relieve torsional strain.
- Primase and helicase form a functional complex called the **primosome.**

2. Single-strand binding proteins



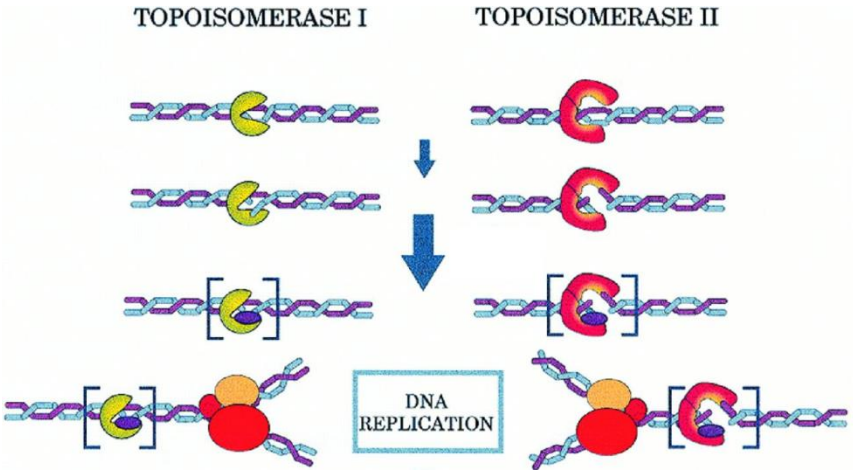
- They coat the DNA around and prevent rewinding of the DNA.
- They **lack enzymatic activity**
- SSB functions in the cell concerning the stability of single stranded regions of DNA.
- In replication,
  - This sustain the activity of helicases, removing the secondary structures from the DNA template and
  - The inhibition of the nuclease activity.
- SSB interacts with primase and components of the primosome to facilitate specific activity.

3. Topoisomerase

- Works at the region ahead of the replication fork
- They catalyze
  - the reversible breakage and
  - join the DNA strands.
- Required: to relax the torsional strain
- They perform similar functions during transcription and recombination.
- These enzymes remove knots and resolve catenate (interlocked circles) which arise during replication and recombination.

Topoisomerase types

- **Topoisomerases are divided into two functional classes.**
  - **Class I topoisomerase:**
    - They cleave only one strand and
    - Catenate/decatenate only substrates containing a nick.
  - **Class II topoisomerase:**
    - Cleave both strands and
    - can catenate/decatenate covalently closed circles.



4. Primase

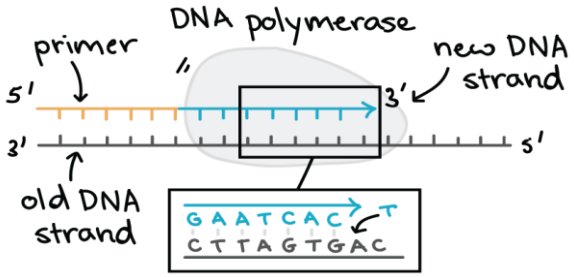
- Synthesizes RNA primers
- They mitigate leading strand synthesis at the origin
- Facilitate the **repetitive initiation** of *Okazaki fragments* during elongation.
- In E. coli,
  - DNA primase is encoded by the *dnaG* locus,
  - The primase depends on the helicase (DnaB) for active primary activity.
- In eukaryotes
  - two polypeptides associated with DNA polymerase α possess primase activity.

5. DNA polymerase

- One of the key molecules in DNA replication is the enzyme DNA polymerase.
- Responsible for synthesizing DNA:
- They add nucleotides one by one to the growing DNA chain
- Incorporating only those that are **complementary** to the template.
- Here are some key features of DNA polymerases

Features of DNA polymerases

- Always need a **template**
- Can only add nucleotides to the **3' end of a DNA strand**
- They **can't start** making a DNA chain from scratch,
- Require a pre-existing chain or short stretch of nucleotides called a ***primer***
- They **proofread** or check their work,
  - removing the vast majority of "wrong" nucleotides
- The addition of nucleotides requires energy.
- Comes from three phosphates attached to them (much like the energy-carrying molecule ATP).
- When the bond between phosphates is broken, the energy released is used to form a bond between the incoming nucleotide and the growing chain.



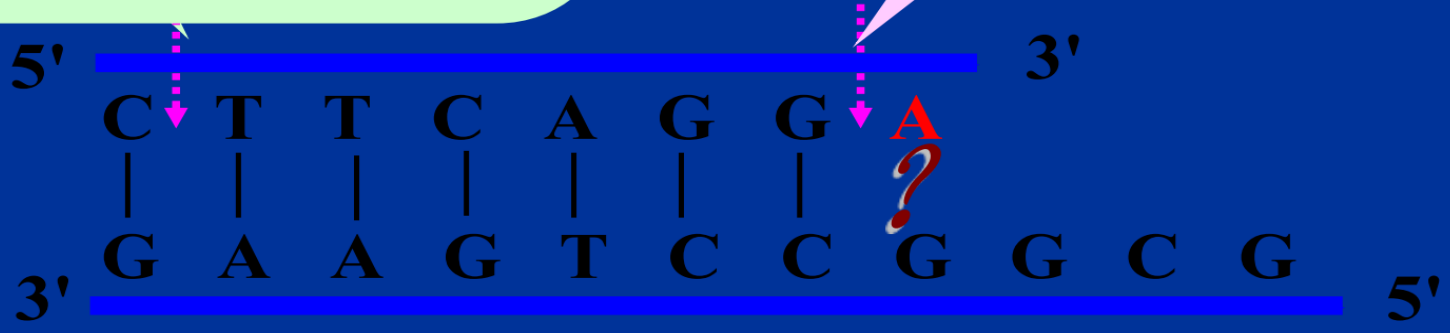
Comparison of *DNA Polymerases* of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC* (*dnaE*)
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16-20	40	250-1,000
Processivity (nucleotides added before polymerase dissociates)	3-200	1,500	≥500,000

Exonuclease functions

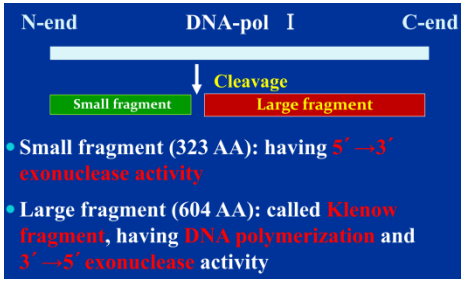
5′ →3′ exonuclease activity removes primer or excise mutated segment

3′ →5′ exonuclease activity excise mismatched nuleotides



Prokaryotic DNA polymerases: DNA Pol I

- This enzyme is coded by a ***polA gene*** and
- Molecular weight of 1,09,000 d and
- Consists of a single polypeptide chain.
- This enzyme is known to be a repair **enzyme**, rather than a replication enzyme.
- The enzyme has **5' to 3' polymerization** and
- Both 5' to 3' and 3' to 5' **exonuclease** activity.
- The enzyme **removes RNA primers** from *Okazaki fragments* and
- Fills up the gaps due to its 5' to 3' polymerization capacity.
- DNA pol I consists of two fragments:
  - a larger fragment called *Klenow fragment* having *3' to 5' exonuclease* activity and 5' to 3' *polymerization activity* and
  - a small fragment having 5' to 3' exonuclease activity.
- The activity of Klnew fragment in using nicked DNA *in vitro* is unique and
- Used to level DNA molecules with radioactive nucleotides *(nick translation).*

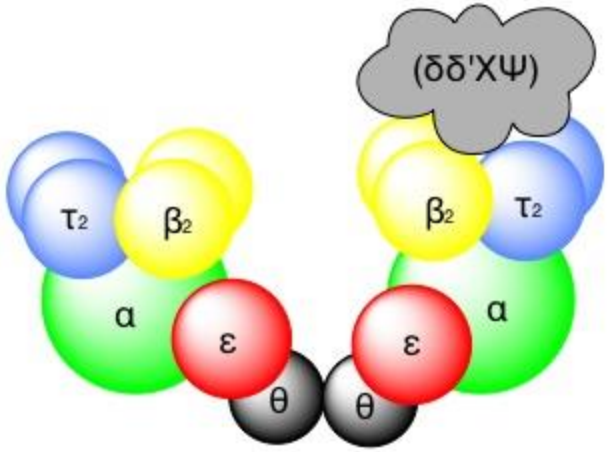


DNA polymerase II

- Coded by *polB* gene,
- Has a molecular weight of 1,20,000 d and
- Contains single polypeptide.
- It has 5’ to 3’ polymerization activity using 3’ –OH groups but mainly uses duplexes (unlike DNA polymerase I).
- It has 3’ to 5’ exonuclease activity,
- It lacks 5’ to 3’ exonuclease action.
- It is also a repair enzyme.

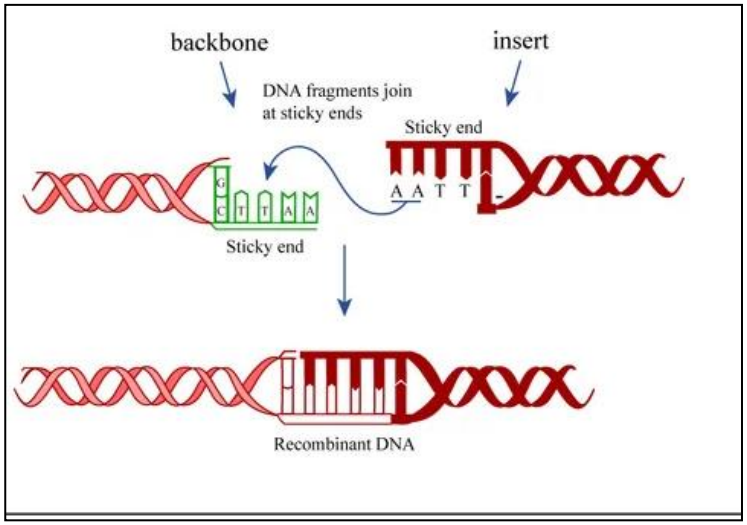
DNA polymerase III

- It is a **heterodimeric** enzyme with 10 units having a mass of a 9,00,000 d.
- The enzyme is an **asymmetric dimer**
- Both strands of the parental DNA of the parent DNA is replicated at the same time.
- The leading strand and lagging strands are **synthesized differently**
- The core of each branch consists of an **α ε θ complex**.
- A β_2 subunit is associated with one branch of the holoenzyme and γ_2 and $(\delta\ \delta\ \chi\ \psi)_2$ with the other.
- The **core** of each branch is same, an α ε θ complex.
- The ***α subunit*** (coded by *polC* or *dnaE* gene) has **5' to 3' synthesis activity** and
- The ***ε subunit*** has **3' to 5' exonuclease** proofreading activity.
- The ***β subunit*** makes the holo enzyme **highly processive** by encircling the template DNA.
- The β_2 dimer forms a **star shaped ring**.
- The **35 A^0 diameter** hole in centre readily accommodate a duplxes DNA molecule.
- The template is trapped but enough space between the DNA and the protein allows rapid sliding and turning during replication.
- Thus β_2 subunit plays a key role in replication serving as a **DNA clamp**.
- The adds 1000 nucleotide per second.



6. DNA ligase:

- Catalyzing the phosphodiester bond formation between adjacent nucleotides in double stranded DNA.
- The 5’ nucleotide must have an intact phosphate group and the 3’ end nucleotide an intact hydroxyl group.
- The gaps between DNA fragments are sealed by DNA ligase.
- In bacteria, DNA ligase needs a NAD factor,
- Eukaryotes and archaea ligase needs ATP.



DNA ligase function