RAMA UNIVERSITY

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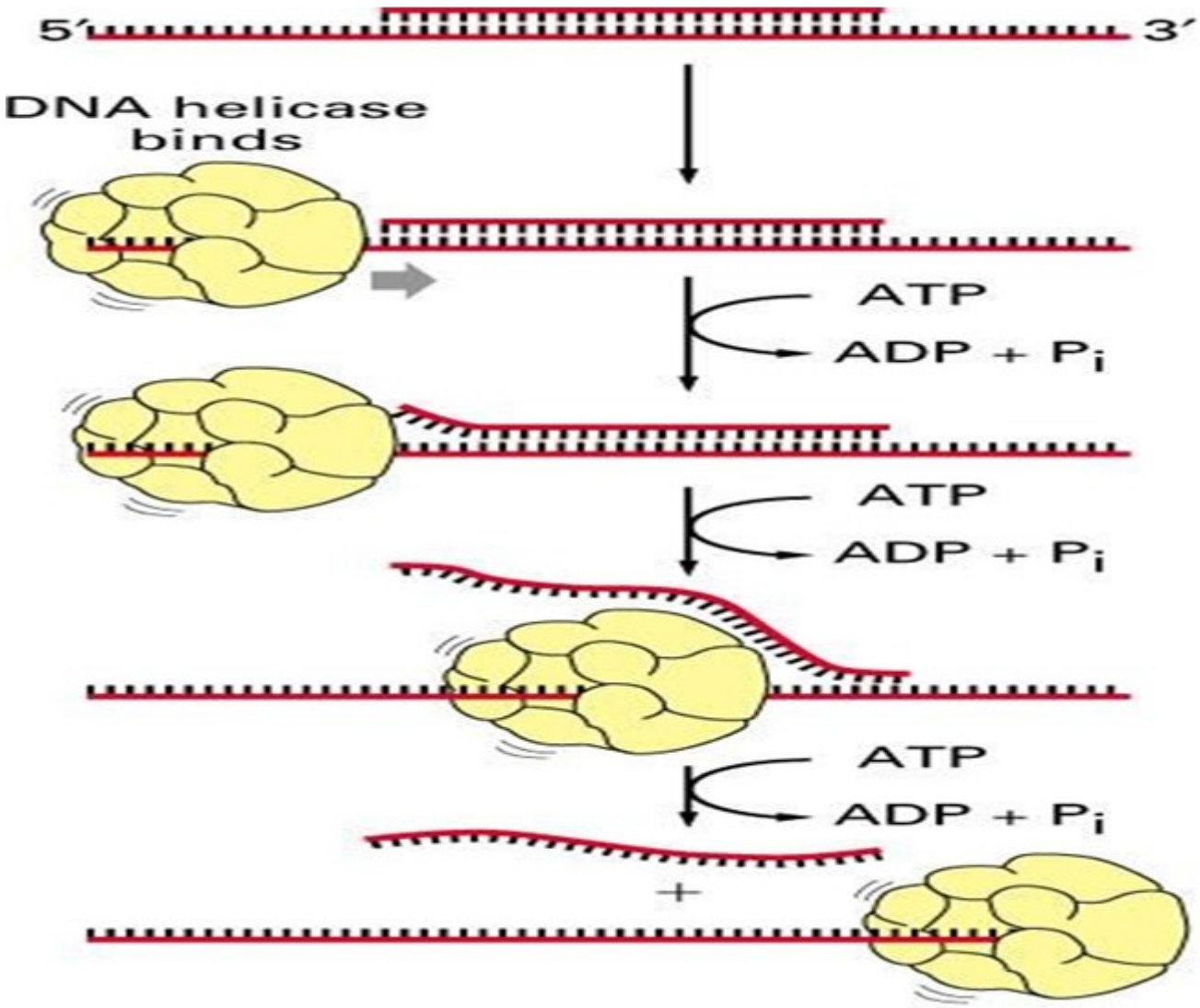
FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

- **Helicases s**eparate double stranded DNA to single stranded DNA during replication and the energy derived from ATP hydrolysis .
- **Helicases** unwind the DNA duplex just ahead of the replication fork at a rate of 1000 bp/s. Two DNA Helicases unwind DNA at a replication fork moving in opposite directions ,one on the leading strand template and another on the lagging strand template.

❖ **Functions** of **Helicases:**

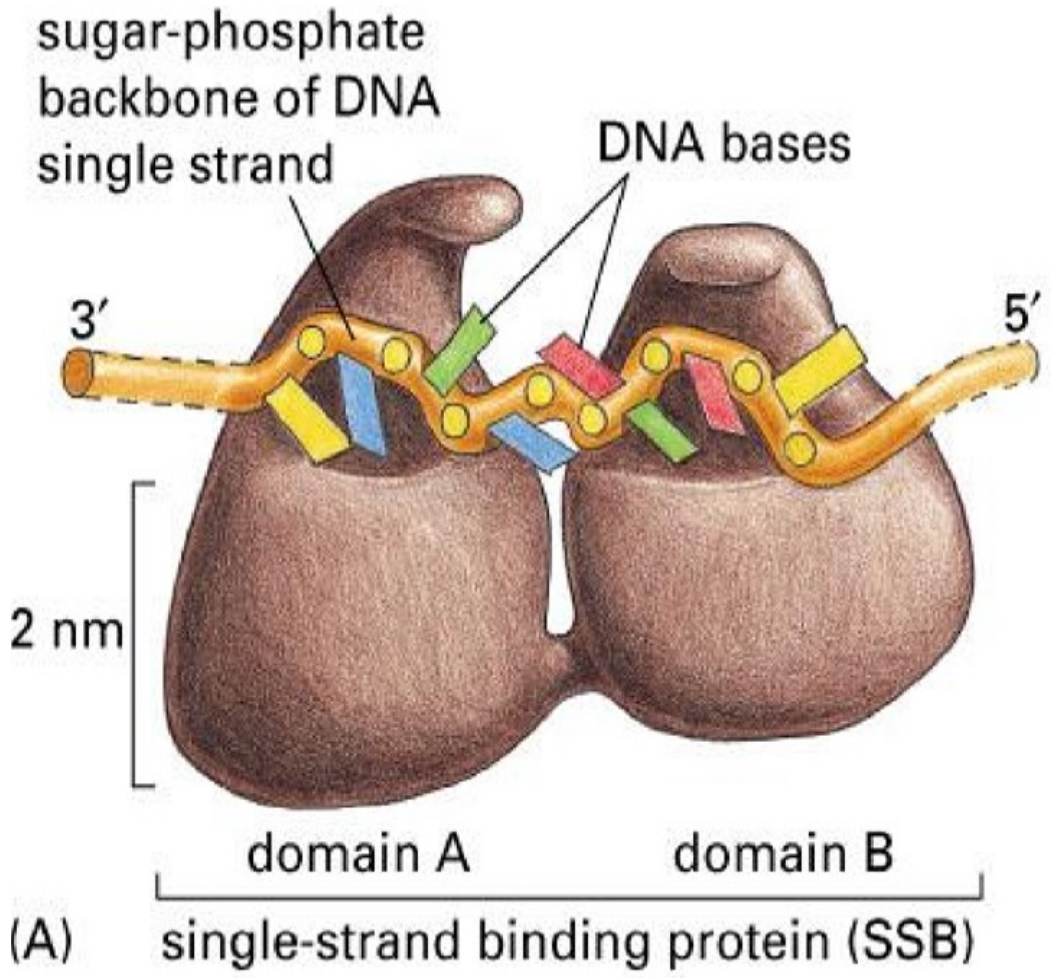
1. DNA unwinding occurs during replication. Helicases in conjunction with topoisomerase relieve torsional stain .
2. It functions in homologous recombination, nucleotide excision repair , transcription termination and conjugation.

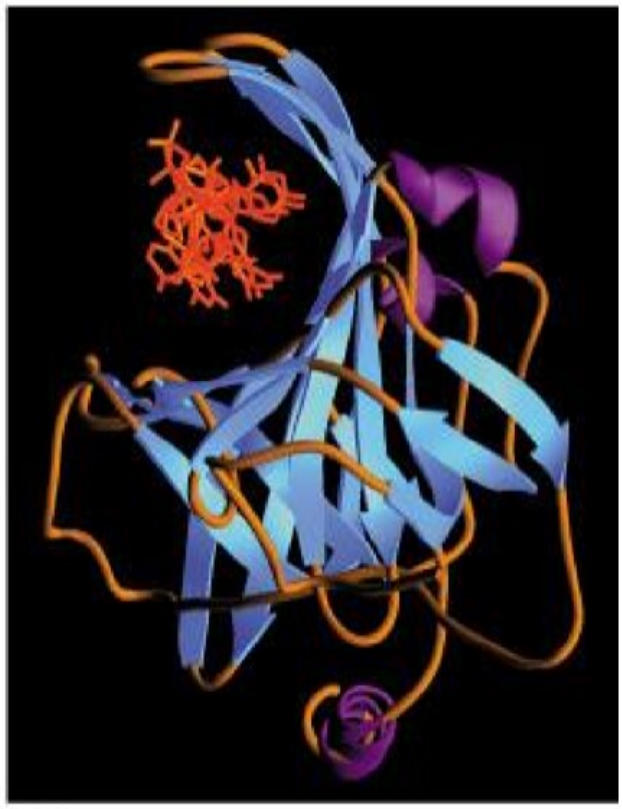

5′
3′
DNA helicase
binds
ATP
ADP + Pi
ATP
ADP + Pi
ATP
ADP + Pi
+

SSB Protein

- **Single Stranded binding proteins(SSB):** also known as **DNA helix destabilizing proteins or Single stranded DNA binding proteins. They have no enzyme activity.**
- **Functions of SSB Proteins during DNA Replication:**

1. Keep two strands of DNA separate(separated by helicases).
2. Bind tightly in a co-operative manner to single stranded DNA (separated strands) and makes it available as a template for DNA Replication/ synthesis.
3. Stabilize DNA in a single strand state and prevent base pairing.
4. Protect single stranded DNA degradation by nucleases.

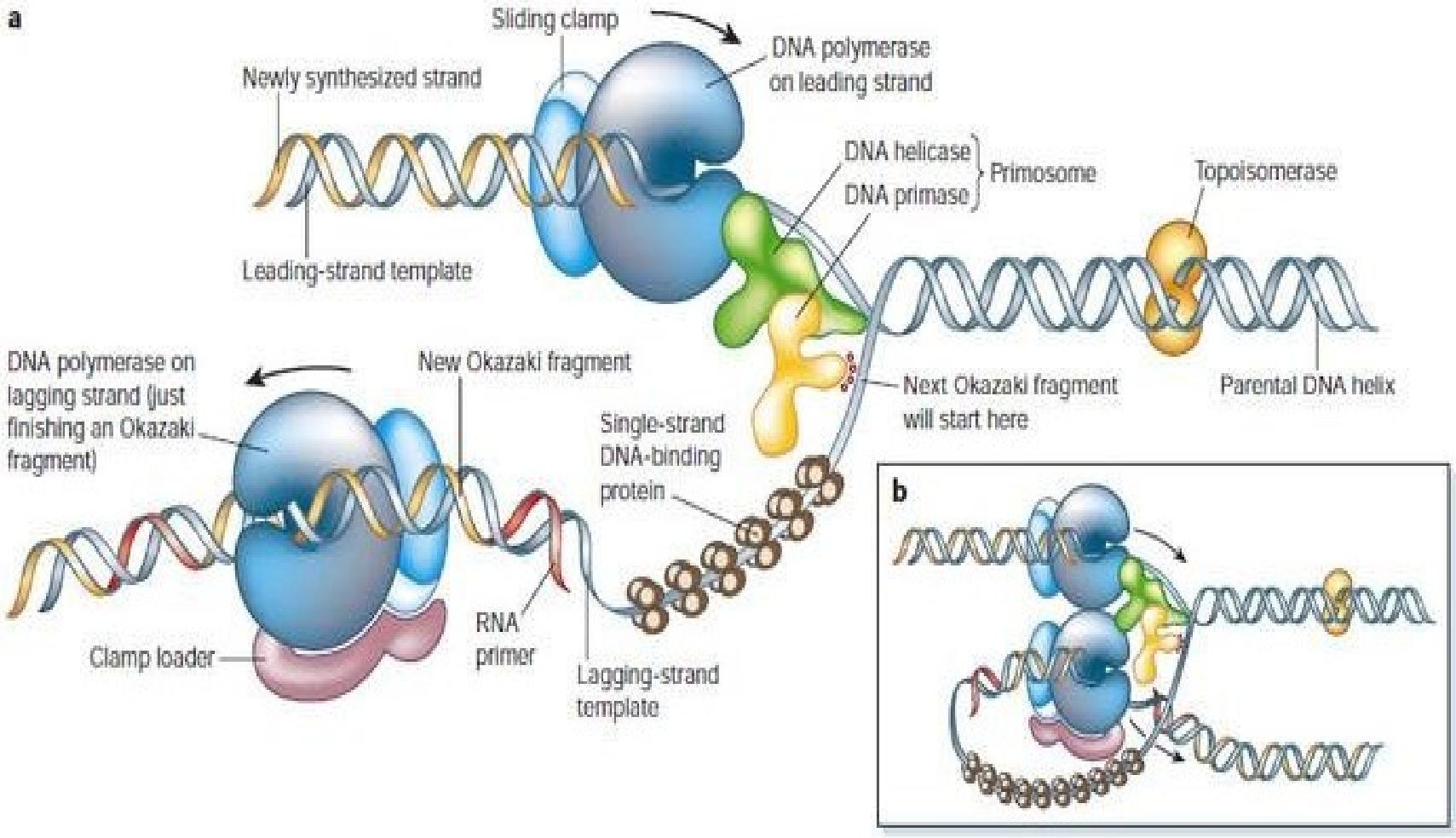

sugar-phosphate
backbone of DNA
single strand
DNA bases
3′
5′
2 nm
domain A
domain B
(A)
single-strand binding protein (SSB)



(B)

β clamp

- **As the leading strand is being synthesized, corresponding portion of** Lagging strand is looped through a clamp enabling coordinate synthesis of both strands.
- Both core complex and clamp dissociate after synthesis of **Okazaki fragments and** again associate the next Okazaki fragment .


a
Sliding clamp
DNA polymerase on leading strand
Newly synthesized strand
DNA helicase
DNA primase
Primosome
Topoisomerase
Leading-strand template
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
New Okazaki fragment
Next Okazaki fragment will start here
Parental DNA helix
Single-strand DNA-binding protein
RNA primer
Clamp loader
Lagging-strand template
b

Activities of enzymes topoisomerase type I / II and supercoils of DNA

Positive supercoils of DNA	Negative supercoils of DNA(
are formed when the DNA molecule is twisted in the same direction as the right handed helix of B-form DNA about its axis.	are formed when the DNA molecule is twisted in the opposite direction as the right handed helix of B-form DNA about its axis.
Introduced by topoisomerase I and relaxed by topoisomerase II.	Introduced by topoisomerase II and relaxed by topoisomerase I.
The amount /activities of enzymes topoisomerase type I and II are regulated to maintain appropriate degree of negative supercoiling.	

Supercoils and DNA Polymerases

- Super coils are formed as double helix separates from one side & replication proceed at the other side (twisted ropes pooled apart)
- **DNA Topoisomerase Type I** –nuclease activity –cuts single strand (to overcome problem of supercoiling) & reseal the strand by ligase activity.
- **DNA Topoisomerase Type II(**called **DNA Gyrase** in prokaryotes): cuts both strands (to overcome problem of supercoiling) & reseal the strands by Ligase activity. It introduces negative supercoils to DNA using free energy from ATP hydrolysis.
  - ❑ Cancer treatment

❖ **Camphotherin** –an inhibitor of DNA Topoisomerase Type I

❖ **Amasacrime** & **Etoposide-** inhibitors of DNA Topoisomerase TYPE II

Primase