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Programme: M.Sc., Biochemistry

Course Title: Molecular Biology

Course Code:BC202CR

Unit 2

Replication

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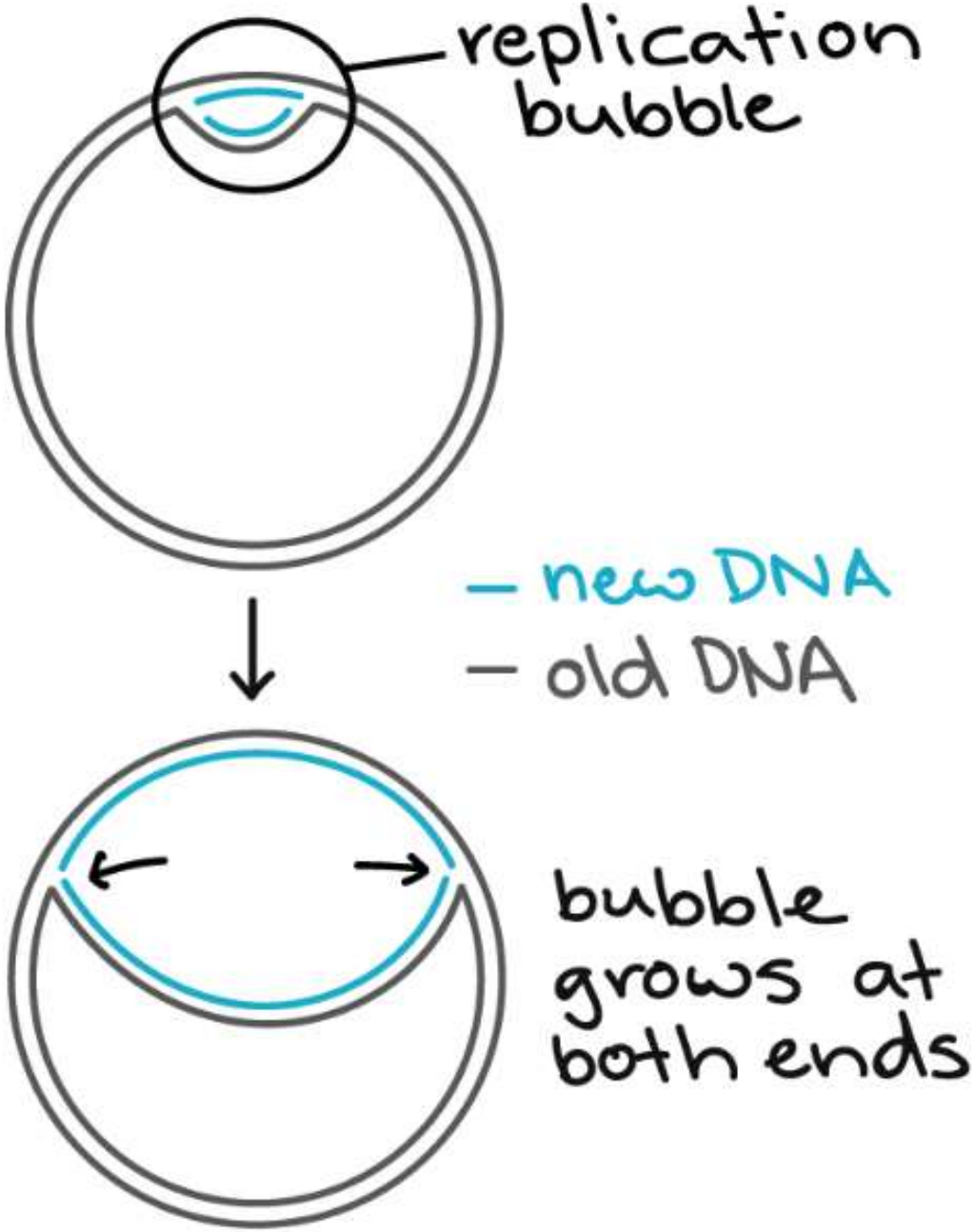
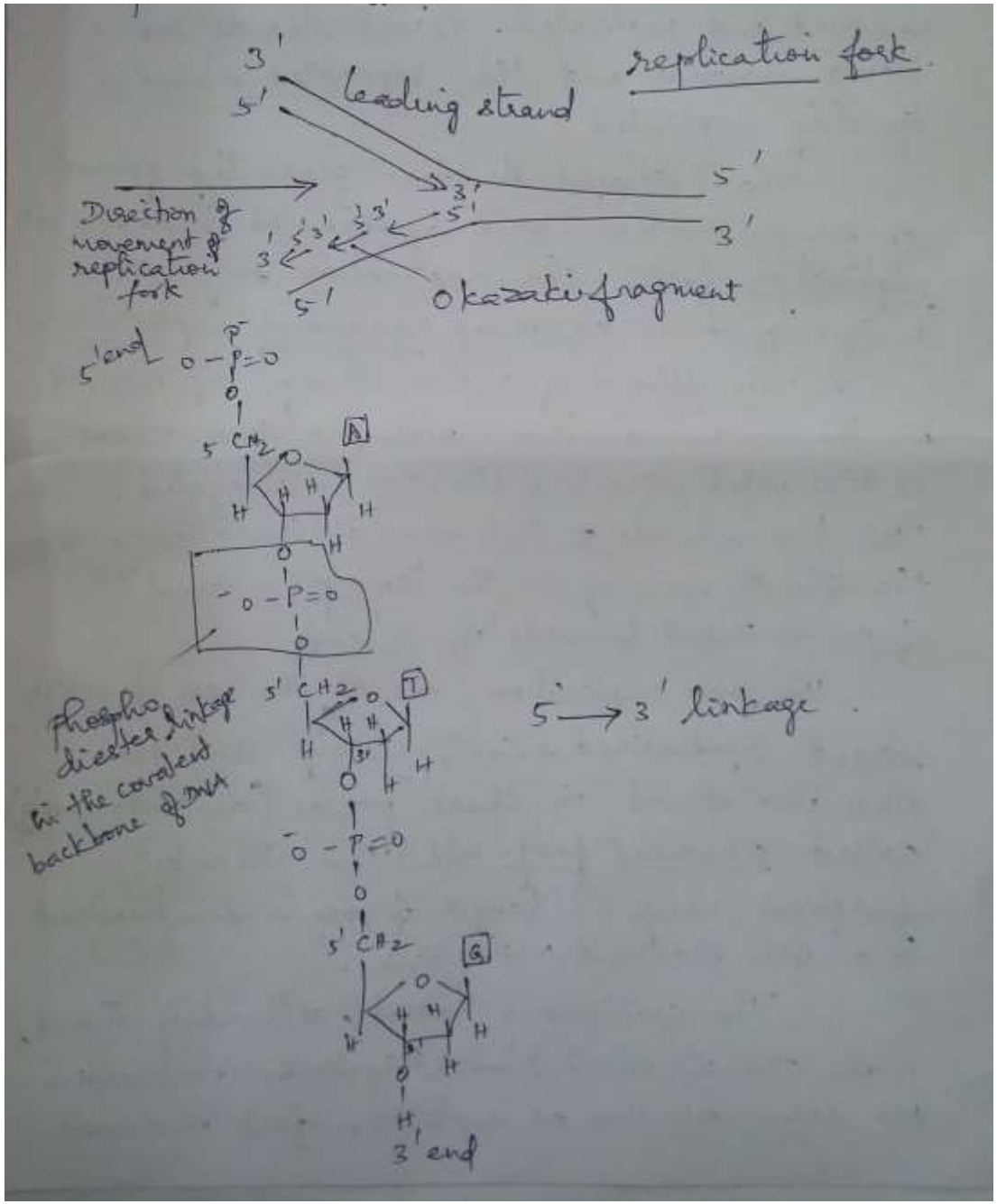
# DNA replication

- It is a highly coordinated process. In which the parent strands are simultaneously unwound and replicated.
- Parent strand is being unwound and the separated strands quickly replicated.
- The Y shaped structure generally found at the point where DNA synthesized is known as replication fork.
- The replication originate at a unique point is known as origin of replication.


replication bubble
– new DNA
– old DNA
bubble grows at both ends

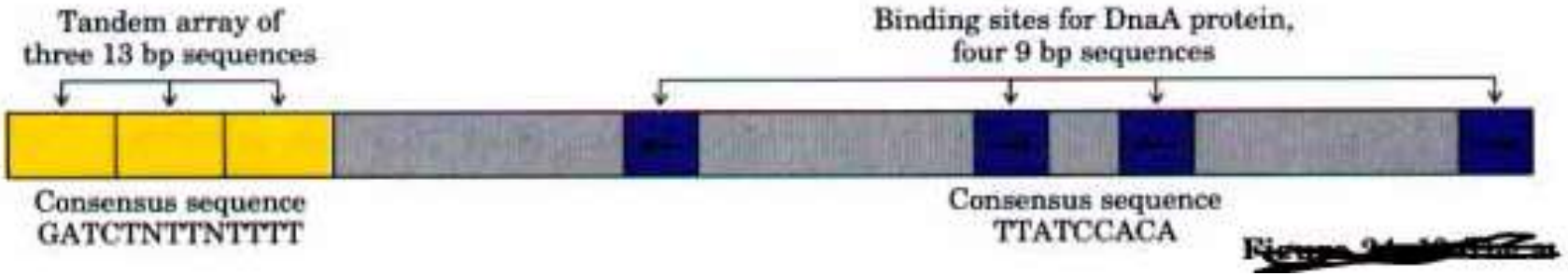
- A new strand of DNA is always synthesized in the 5’→3’ direction with the free 3’-OH as the point at which the DNA is elongated.
- The two strands of DNA are antiparellel, the strand serving as template is read from its 3’ end towards its 5’end.
- In DNA replication, one of the new daughter strand synthesized continuously and other DNA strand in short pieces (discontinuously) called Okazaki fragments.

- The Okazaki fragments range in length from few hundred to few thousand nucleotides.
- The continuous strand, or leading strand, is the one in which 5’ → 3’ synthesis proceeds in the same direction as the replication fork movement.
- The discontinuous strand or lagging strand is the one in which 5’ → 3’ synthesis proceeds in the direction opposite to the direction of fork movement.


replication fork
3'
5'
leading strand
5'
3'
3'
5'
Direction of movement of replication fork
3'
5'
3'
5'
Okazaki fragment
5' end
A
T
G
3' end
Phospho diester linkage in the covalent backbone of DNA
5' → 3' linkage

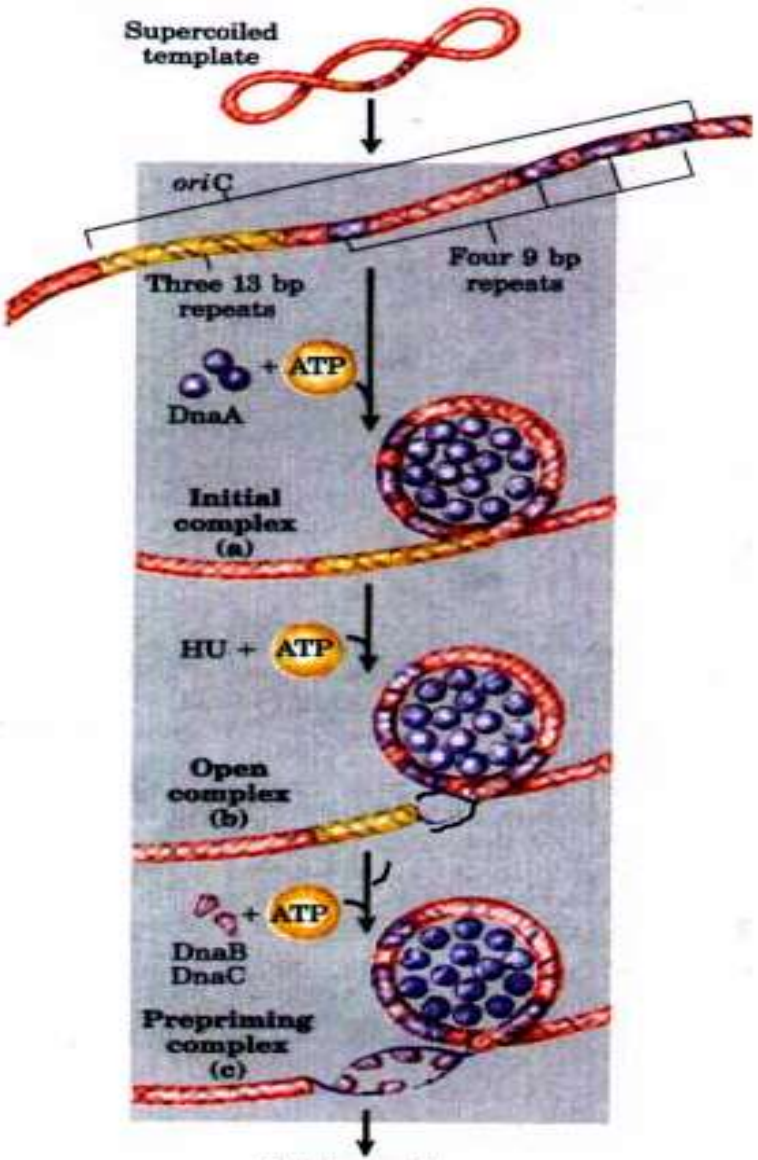
- In E.coli, replication occurs bidirectionally. Replication in E.coli chromosome proceeds in 3 stages.
- 1. Initiation , 2. Elongation  3. Termination
- **Initiation**
- The E.coli replication origin, ori C consists of 245 bp. They are highly conserved. It consists of two series of short repeats.Three repeats of 13bp sequence and 4 repeats of 9bp sequence.

# Arrangement of sequences in the E.coli replication



- 9 different proteins or enzymes are involved in the initiation phase of replication.
- They open the DNA helix at the origin of replication and establish a prepriming complex for subsequent reactions.
- 1. A single complex of four to five DnaA protein molecules binds to the four 9bp repeats in the origin.

**[redacted]** A model for initiation of replication at the *E. coli* origin, *oriC*. **(a)** About 20 DnaA protein molecules, each with a bound ATP, bind at the four 9 base pair repeats. The DNA is wrapped around this complex. **(b)** The three 13 base pair repeats are then denatured sequentially to give



- 2. Then recognizes and denatures the DNA in the region of three 13bp repeats which are rich in A=T pairs. It requires ATP and bacterial Histone like protein HU.
- 3. The DnaC protein then loads the DnaB protein on to the unwound region. Two ring shaped hexamers of DnaB, one loaded onto each DNA strand act as helicases unwinding the DNA bidirectionally and creating two potential replication forks.

- 4. Many molecules of single stranded DNA binding protein (SSB) bind cooperatively to single stranded DNA, stabilizing the separated strands and prevents renaturation.
- 5. Gyrase relieves the topological stress produced by the DnaB helicase.
- 6. The timing of replication initiation is affected by DNA methylation and interactions with the bacterial plasma membrane.
- The Ori C DNA is methylated by the DAM methylase (DAM =DNA Adenine methylation).

- Immediately after replication, the DNA is hemimethylated , the parent strands have methylated Ori C but the new strands do not. At the time of release from the plasma membrane Ori C must be fully methylated before it can bind Dna A protein.

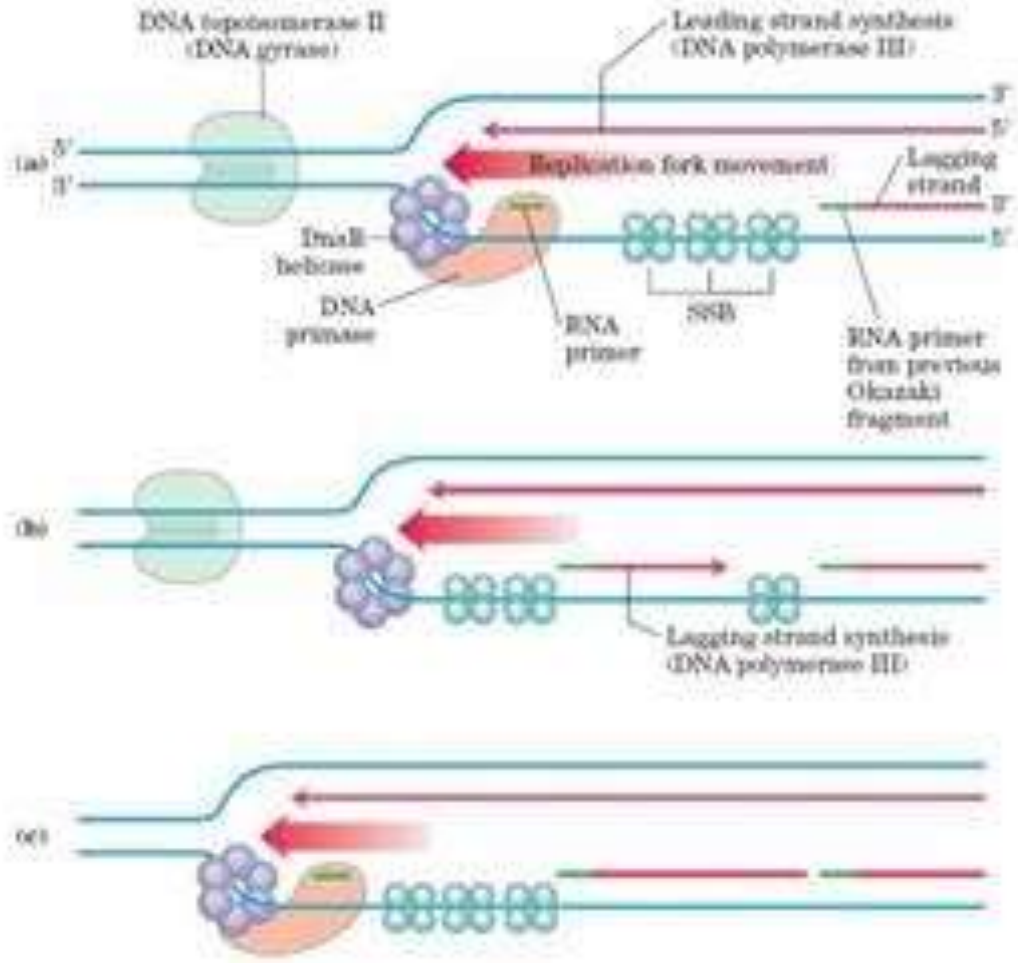
# Elongation

- It involves leading and lagging strand synthesis. Several enzymes are involved in the synthesis of both strands.
- Leading strand synthesis occurs continuously and lagging strand with Okazaki fragment (discontinuously).
- 1. Synthesis begins with primer (Short RNA – 10 – 60 nucleotides) by the enzyme primase (Dna G protein) at the replication origin.

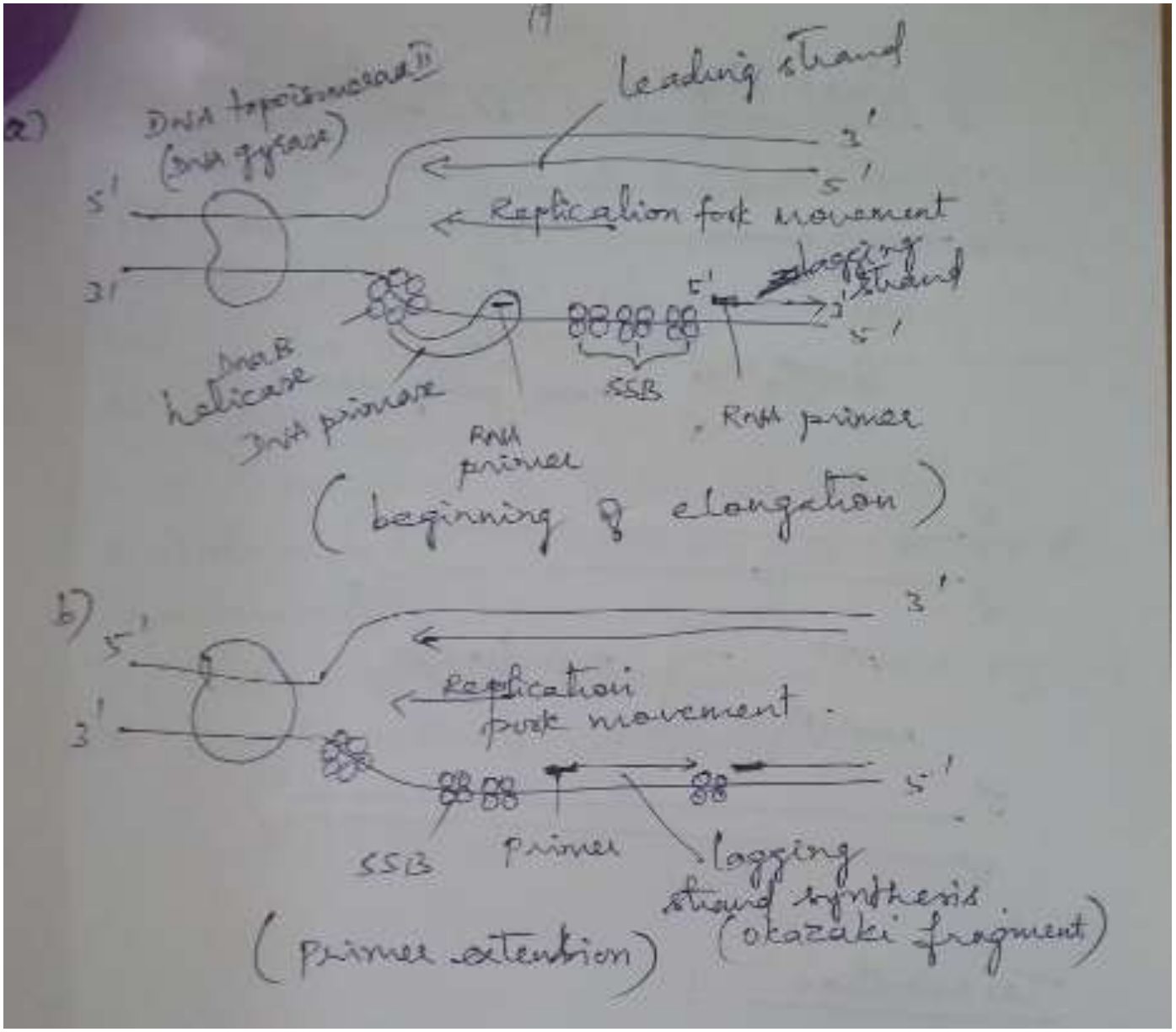
- 2. Deoxyribo nucleotides are added to the primer by DNA polymerase III.
- Leading and lagging strand synthesis should occur coordinately by a single DNA polymerase III dimer, which is accomplished by looping the DNA of the lagging strand.
- The Dna B helicase and Dna G primase constitutes a functional unit within the replication complex called primosome.

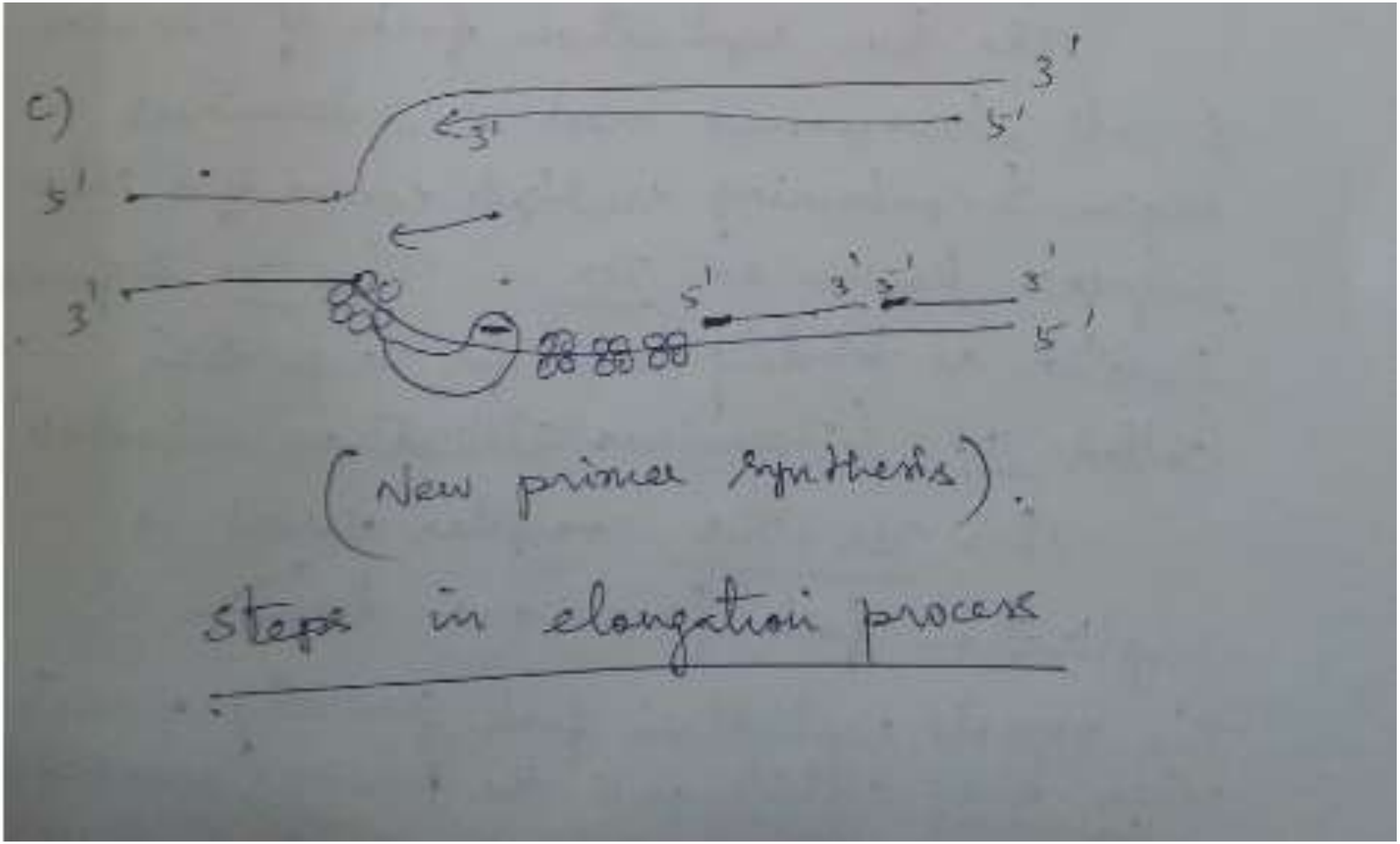
- The Dna G primase occasionally associated with helicase and synthesizes a short RNA primer.
- A new β sliding clamp is then positioned at the primer by the clamp loading complex of DNA polymerase III.
- When the synthesis of an Okazaki fragment has been completed, replication halts and the core subunit of DNA polymerase III dissociate from their β sliding clamp and associates with the new clamp leads to the synthesis of new Okazaki fragment.

- Once an Okazaki fragment has been completed its RNA primer is removed and replaced with DNA by DNA polymerase I.
- The nick is sealed by DNA ligase.



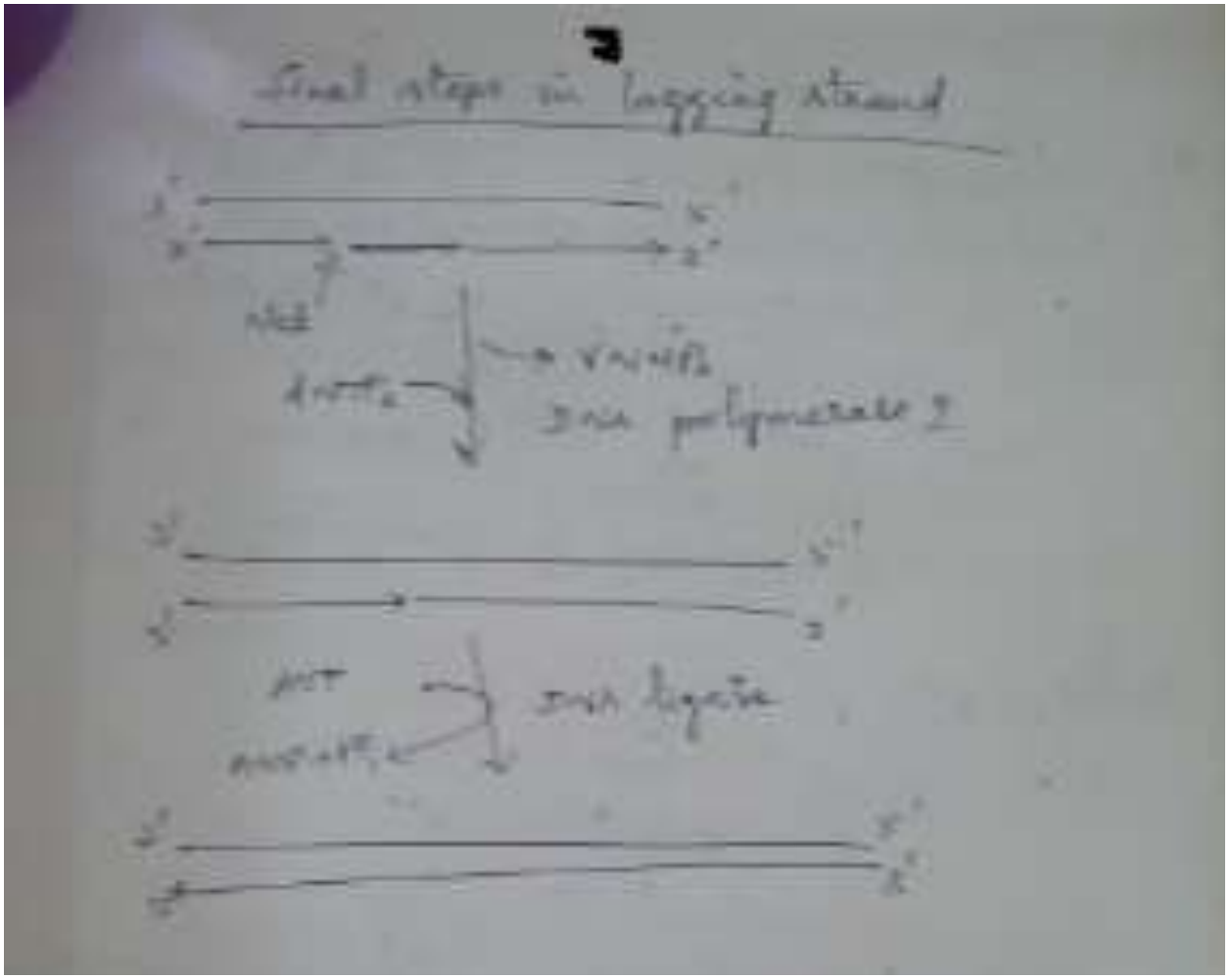

**Synthesis of Okazaki fragments.** (a) At intervals, primase synthesizes an RNA primer for a new Okazaki fragment. Note that if we consider the two template strands as lying side by side, lagging strand synthesis formally proceeds in the opposite direction from fork movement. (b) Each primer is extended by DNA polymerase III. (c) DNA synthesis continues until the fragment extends as far as the primer of the previously added Okazaki fragment. A new primer is synthesized near the replication fork to begin the process again.

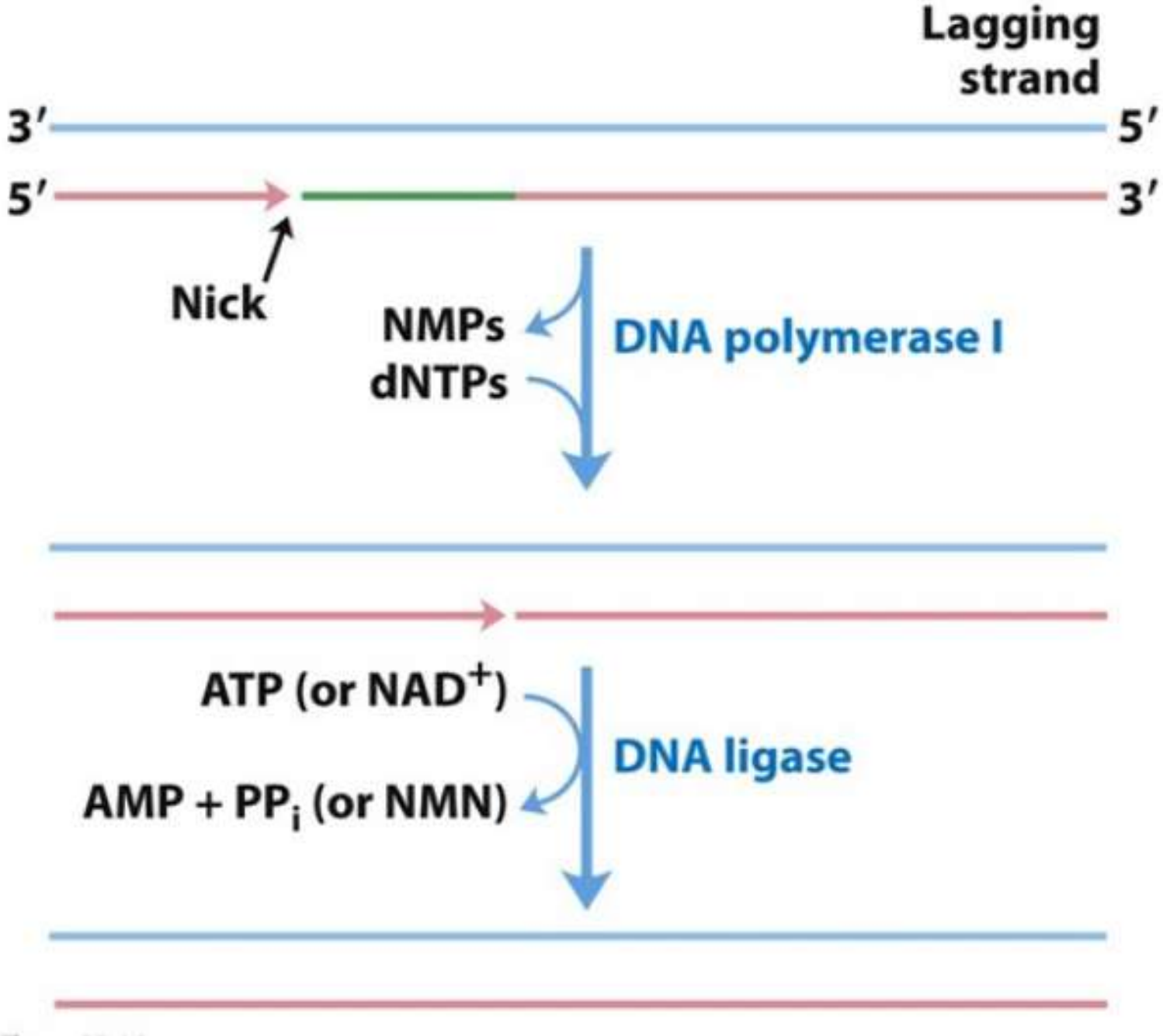

a)
DNA topoisomerase II
(DNA gyrase)
leading strand
3'
5'
5'
3'
Replication fork movement
lagging strand
5'
3'
5'
DnaB
helicase
DNA primase
RNA
primer
SSB
RNA primer
RNA primer
( beginning & elongation )
b)
5'
3'
3'
5'
Replication
fork movement
5'
SSB
primer
lagging
strand synthesis
(okazaki fragment)
( primer extention )




(New primer synthesis).

Steps in elongation process


Final steps in logging strand
DNA polymerase I
DNA ligase

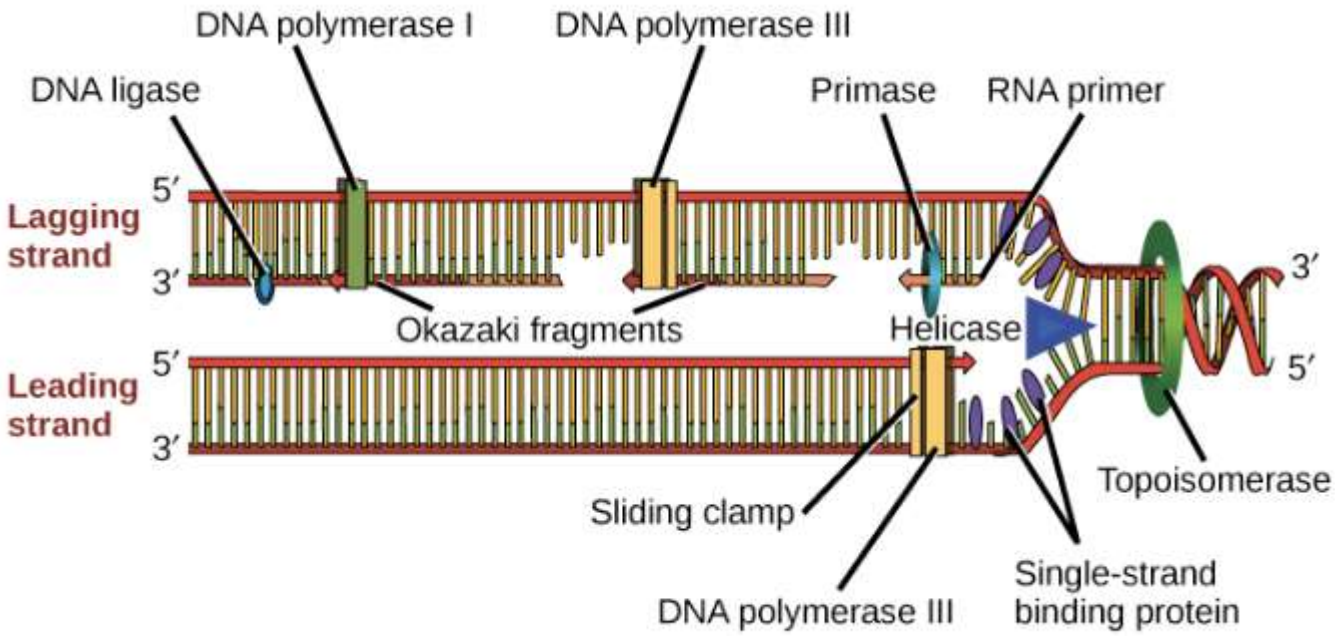

Lagging strand
3′
5′
5′
3′
Nick
NMPs
dNTPs
DNA polymerase I
ATP (or NAD+)
AMP + PPi (or NMN)
DNA ligase

# Termination

- The two replication forks of circular E.coli chromosomes meet at a terminus region containing multiple copies of a 20bp sequence known as **Ter**.
- This Ter sequence function as binding sites for a protein called **Tus** (Terminus utilization substance).
- This **Ter – Tus** complex arrest a replication fork from one direction.

- The opposite replication fork generally arrested when they collide with the previous arrested fork.
- The final few hundred base pairs of DNA between these large proteins complexes are then replicated. Completing two interlinked circular chromosomes.
- DNA circles linked in this way are known as **Catenanes.**

- Separation of catenated circles in E.coli requires topoisomerase IV ( a type II topoisomerase).
- The separated chromosomes then segregate into daughter cells at cell division.


DNA polymerase I
DNA polymerase III
DNA ligase
Primase
RNA primer
5′
Lagging strand
3′
Okazaki fragments
Helicase
3′
5′
5′
Leading strand
3′
Topoisomerase
Sliding clamp
Single-strand binding protein
DNA polymerase III

- **Helicase** opens up the DNA at the replication fork.
- **Single-strand binding proteins** coat the DNA around the replication fork to prevent rewinding of the DNA.
- **Topoisomerase** works at the region ahead of the replication fork to prevent supercoiling.
- **Primase** synthesizes RNA primers complementary to the DNA strand.
- **DNA polymerase III** extends the primers, adding on to the 3' end, to make the bulk of the new DNA.
- RNA primers are removed and replaced with DNA by **DNA polymerase I**.
- The gaps between DNA fragments are sealed by **DNA ligase**.