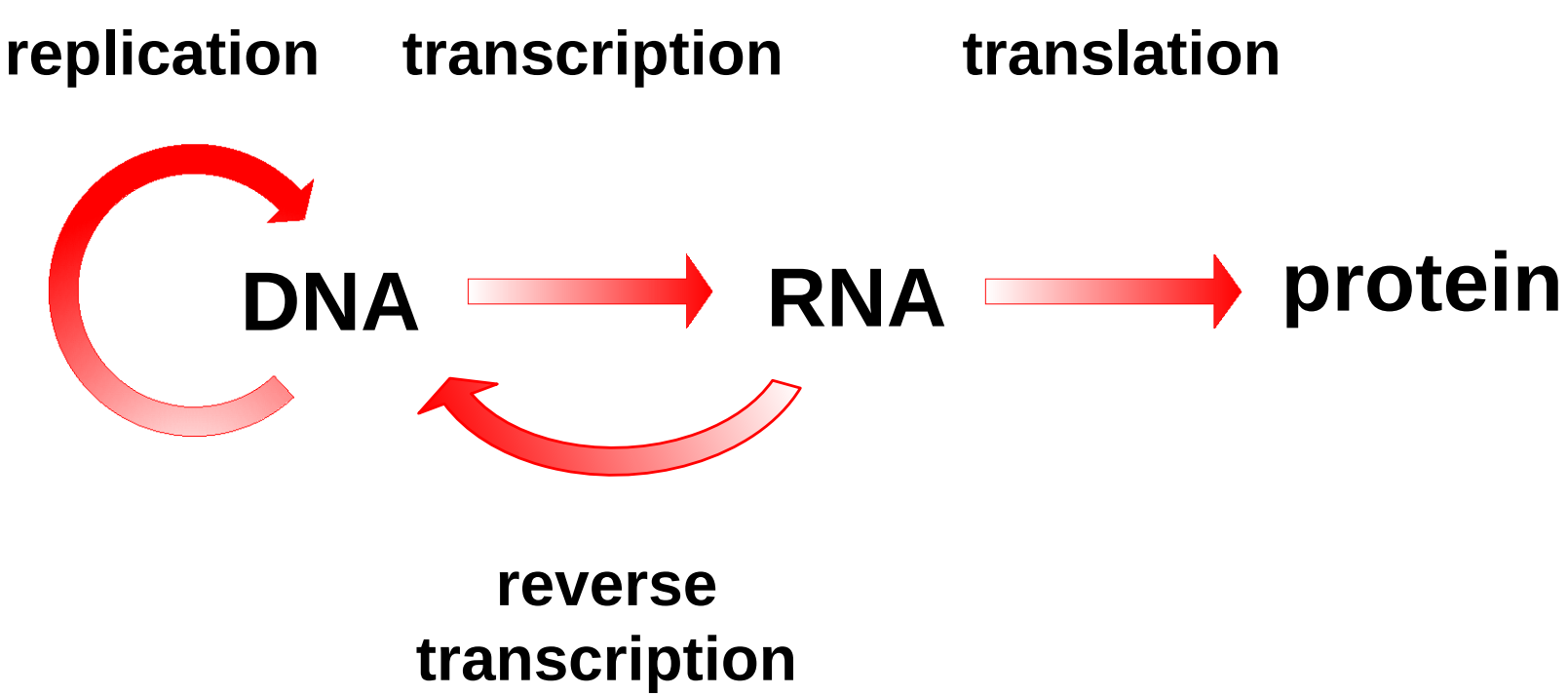
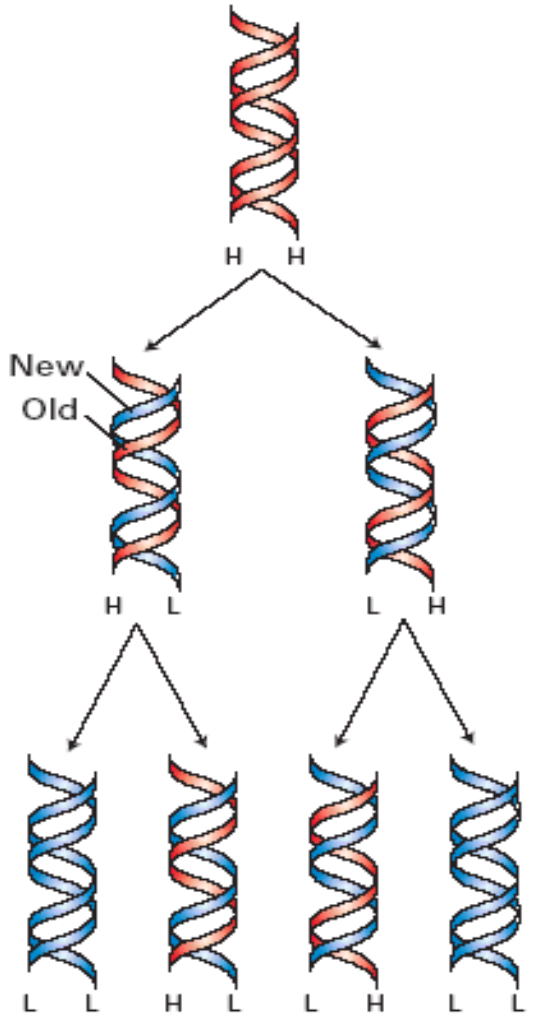
Replication

Central dogma



- **Replication: synthesis of daughter DNA from parental DNA**
- **Transcription: synthesis of RNA using DNA as the template**
- **Translation: protein synthesis using mRNA molecules as the template**
- **Reverse transcription: synthesis of DNA using RNA as the template**

DNA Replication



General Concepts of DNA Replication

DNA replication

- **A reaction in which daughter DNAs are synthesized using the parental DNAs as the template.**
- **Transferring the genetic information to the descendant generation with a high fidelity**

Daughter strand synthesis

- **Chemical formulation:**

$$(dNMP)_n + dNTP \longrightarrow (dNMP)_{n+1} + PPi$$

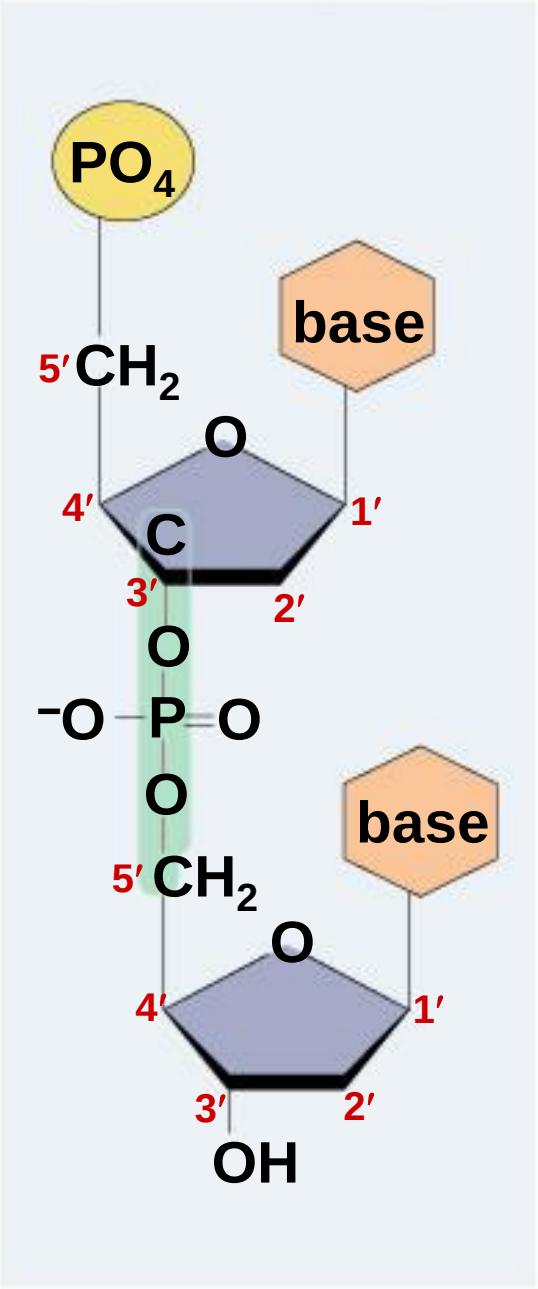
DNA strand substrate

elongated DNA strand

- **The nature of DNA replication is a series of 3′ - 5′ phosphodiester bond formation catalyzed by a group of enzymes.**

The DNA backbone

- Putting the DNA backbone together
  - refer to the 3′ and 5′ ends of the DNA



DNA replication system

Template: double stranded DNA

Substrate: dNTP

Primer: short RNA fragment with a free 3´-OH end

Enzyme: DNA-dependent DNA polymerase (DDDP),

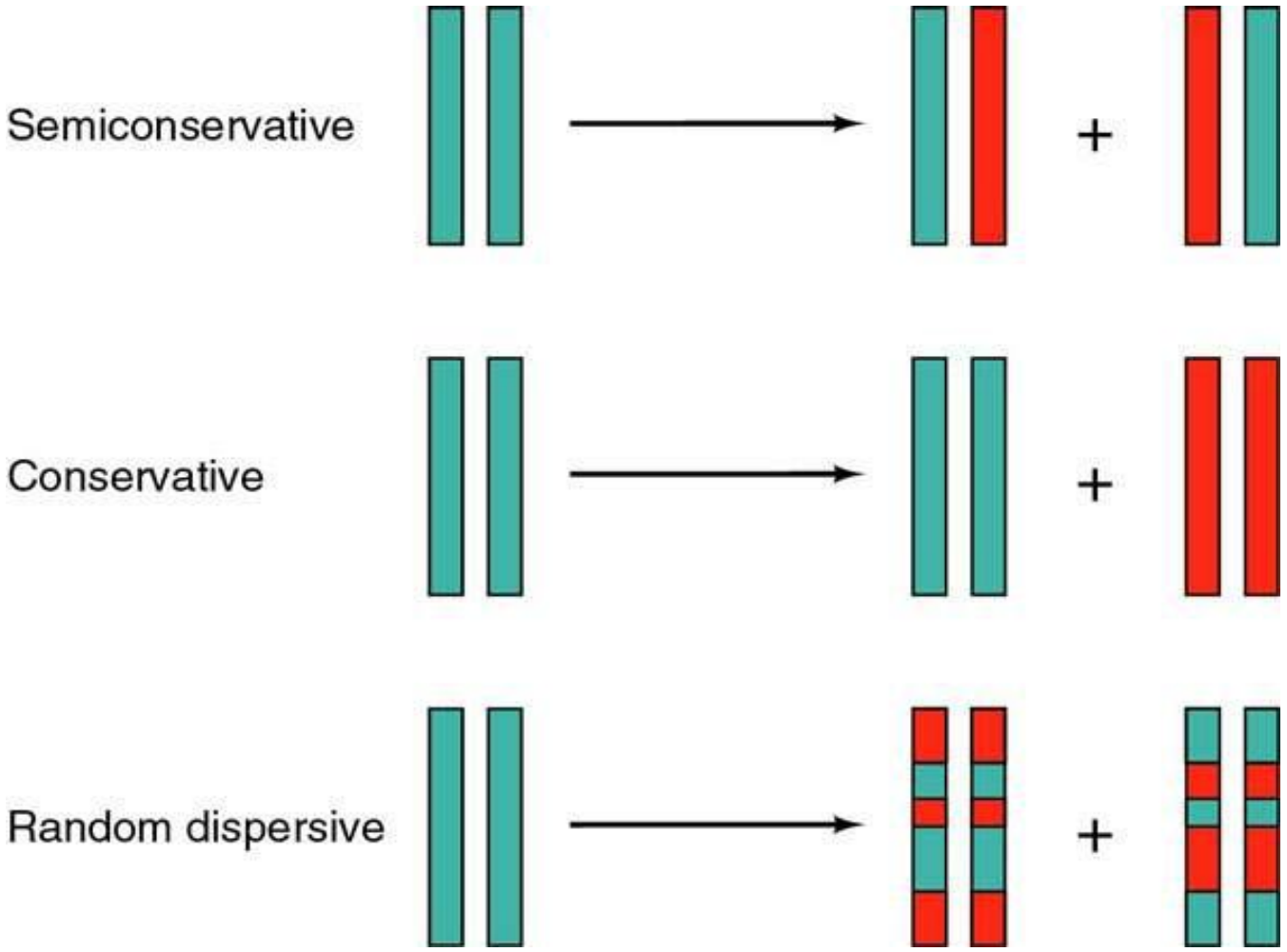
other enzymes,

protein factor

Characteristics of replication

- **Semi-conservative replication**
- **Bidirectional replication**
- **Semi-continuous replication**
- **High fidelity**

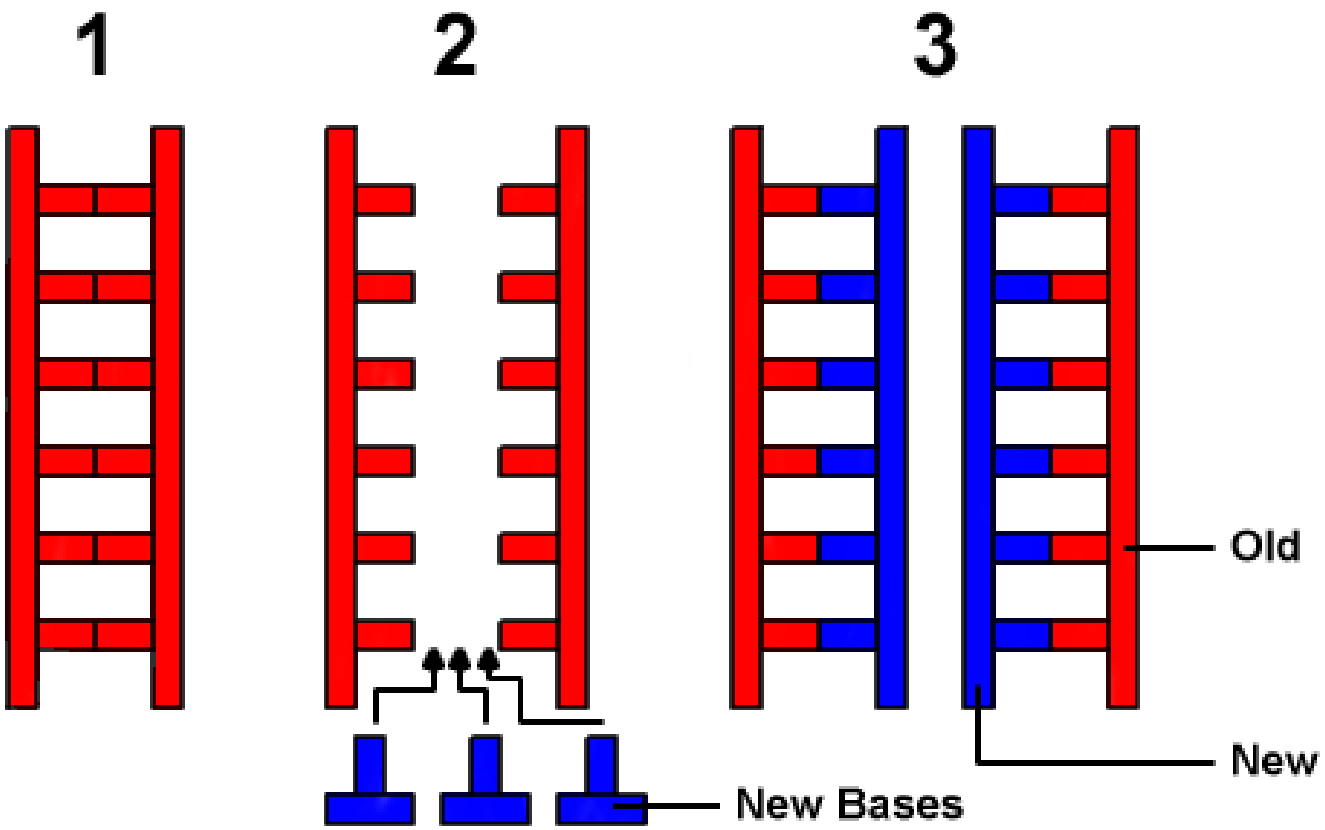
§ 1.1 Semi-Conservative Replication



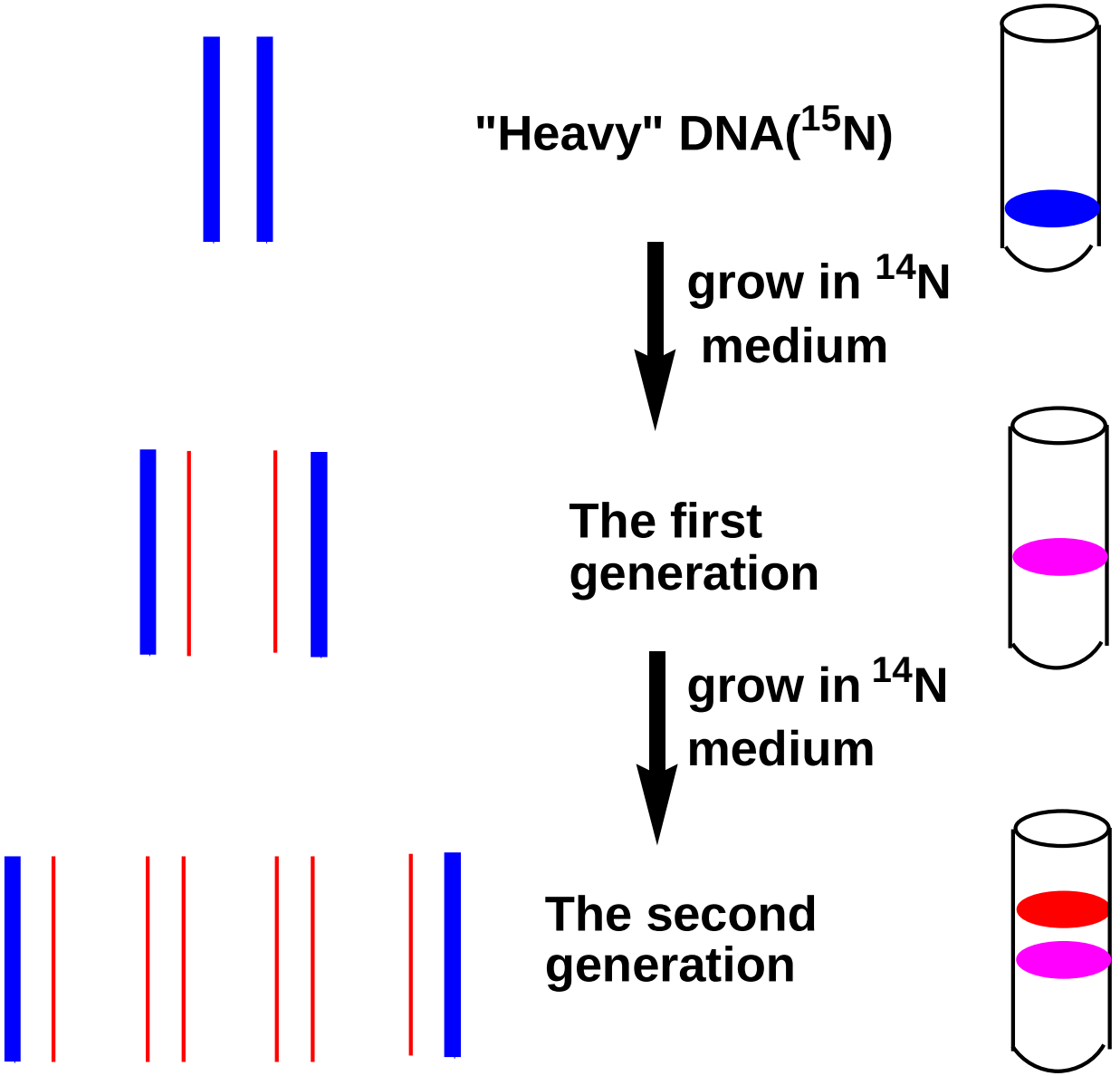
Semiconservative replication

Half of the parental DNA molecule is conserved in each new double helix, paired with a newly synthesized complementary strand. This is called semiconservative replication

Semiconservative replication



Experiment of DNA semiconservative replication



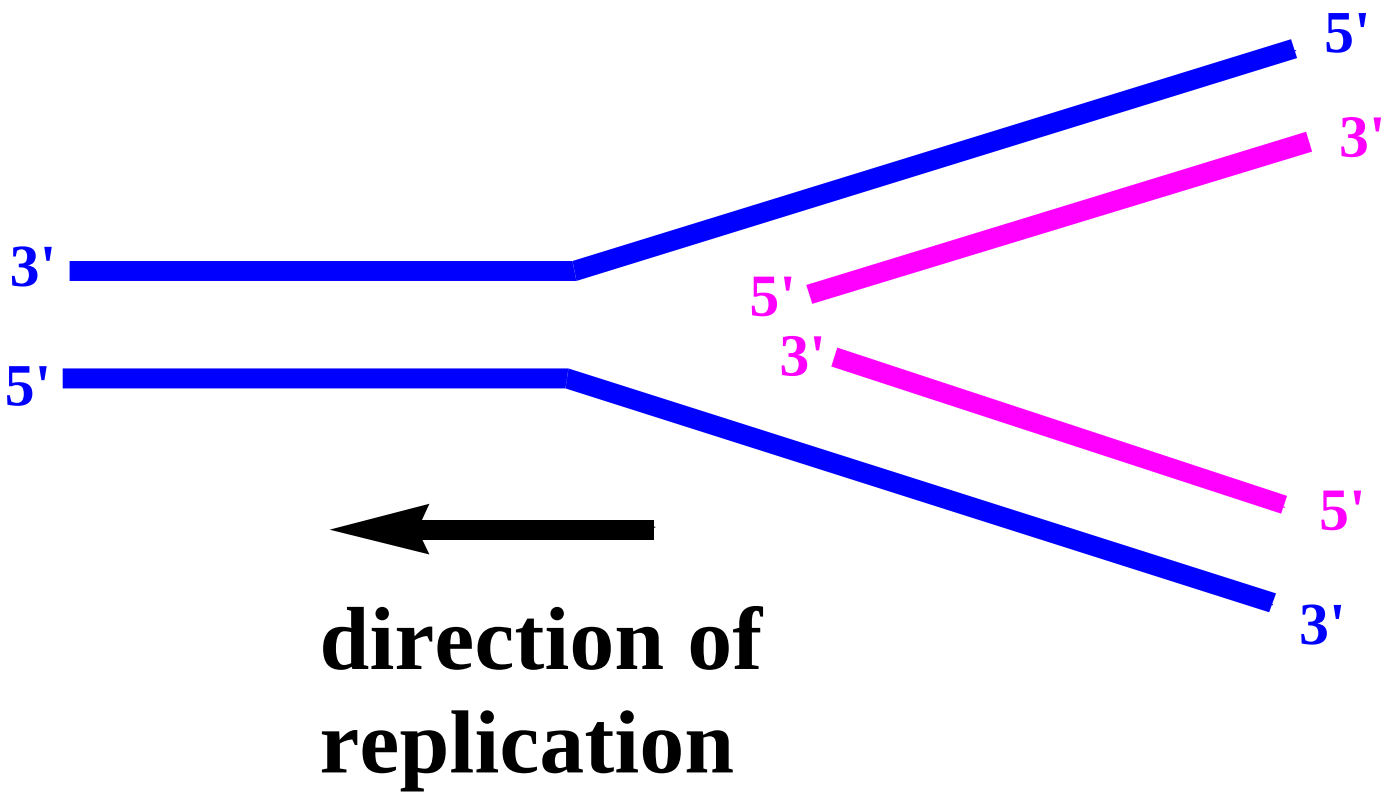
Significance

The genetic information is ensured to be transferred from one generation to the next generation with a high fidelity.

§ 1.2 Bidirectional Replication

- **Replication starts from unwinding the dsDNA at a particular point (called origin), followed by the synthesis on each strand.**
- **The parental dsDNA and two newly formed dsDNA form a Y-shape structure called replication fork.**

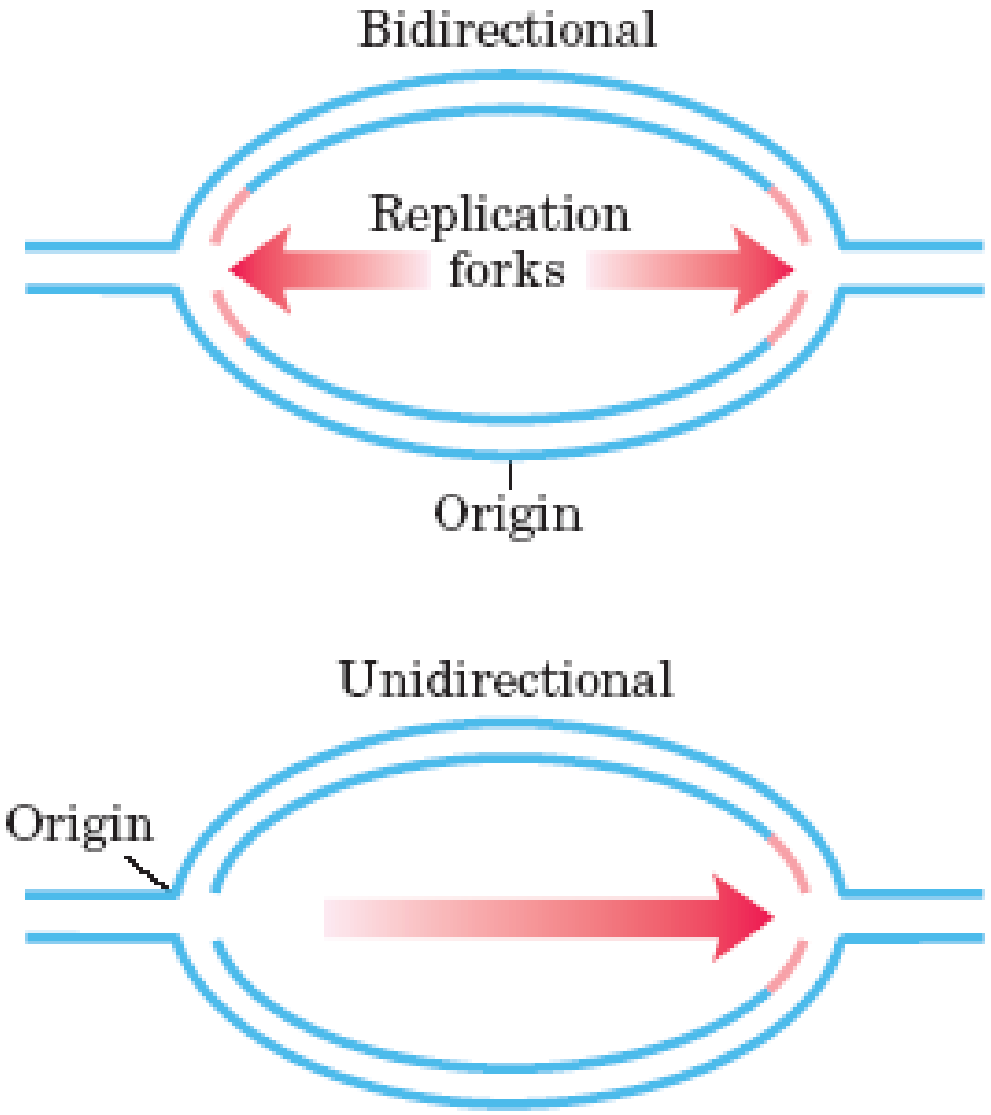
Replication fork



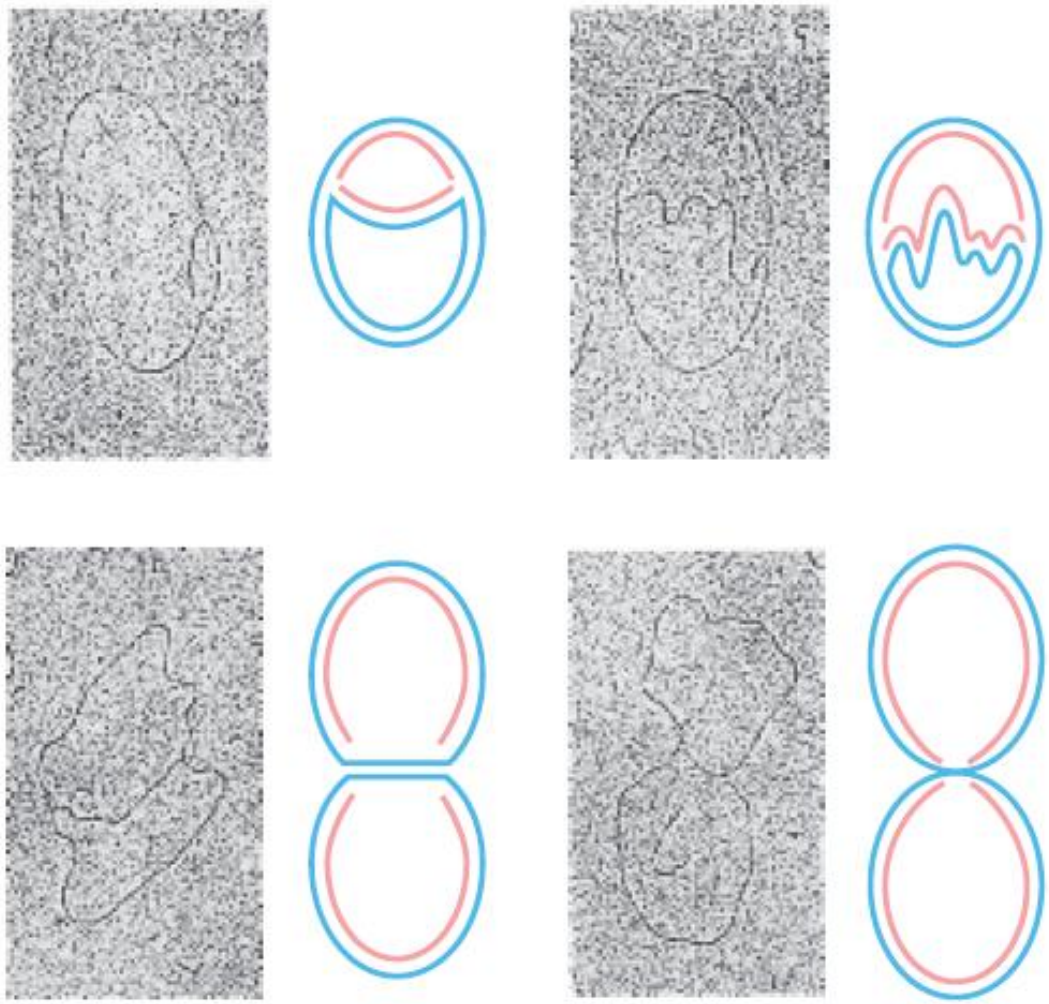
Bidirectional replication

- **Once the dsDNA is opened at the origin, two replication forks are formed spontaneously.**
- **These two replication forks move in opposite directions as the syntheses continue.**

Bidirectional replication



Replication of prokaryotes

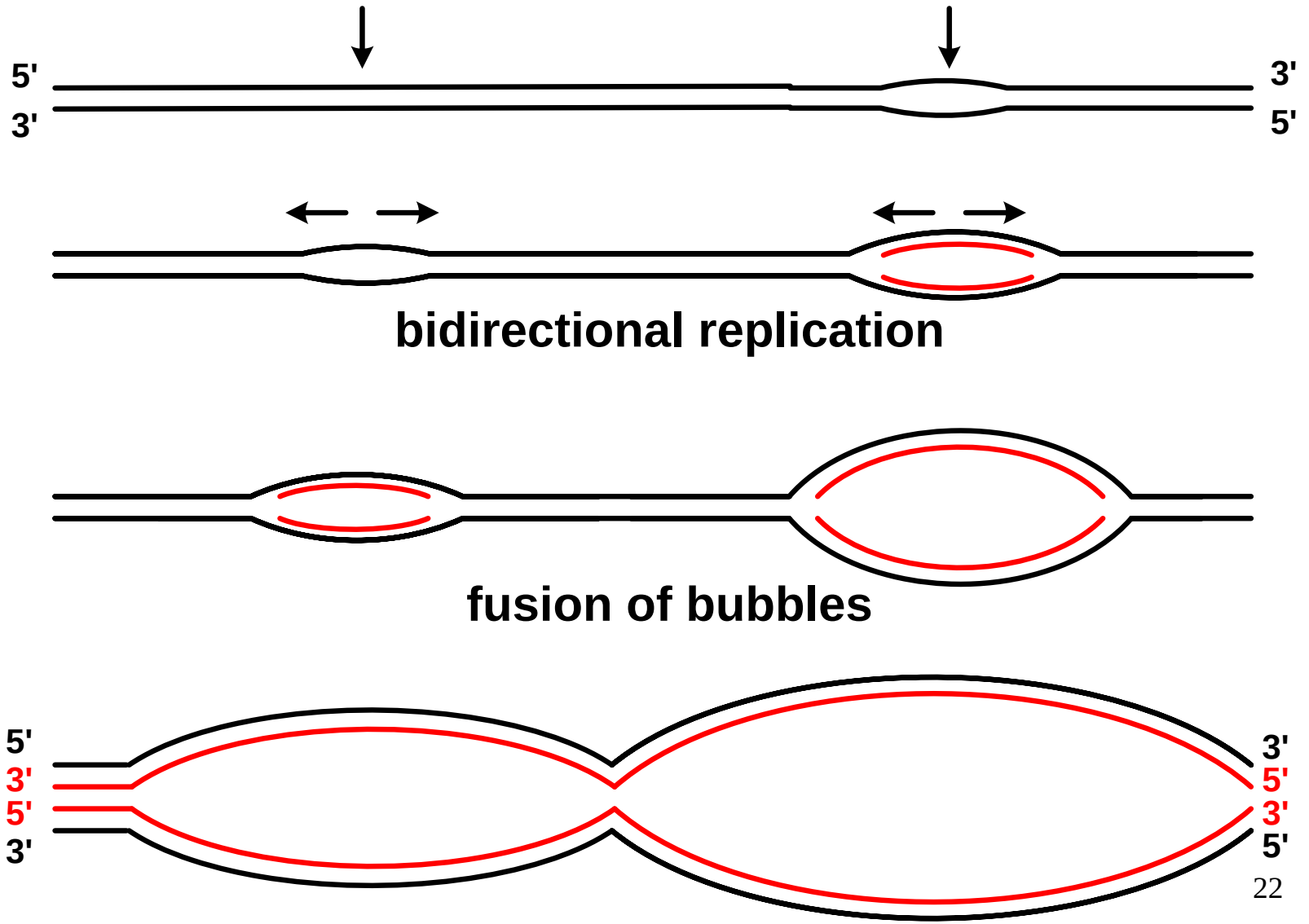


The replication process starts from the origin, and proceeds in two opposite directions. It is named θ replication.

Replication of eukaryotes

- **Chromosomes of eukaryotes have multiple origins.**
- **The space between two adjacent origins is called the replicon, a functional unit of replication.**

origins of DNA replication (every ~150 kb)

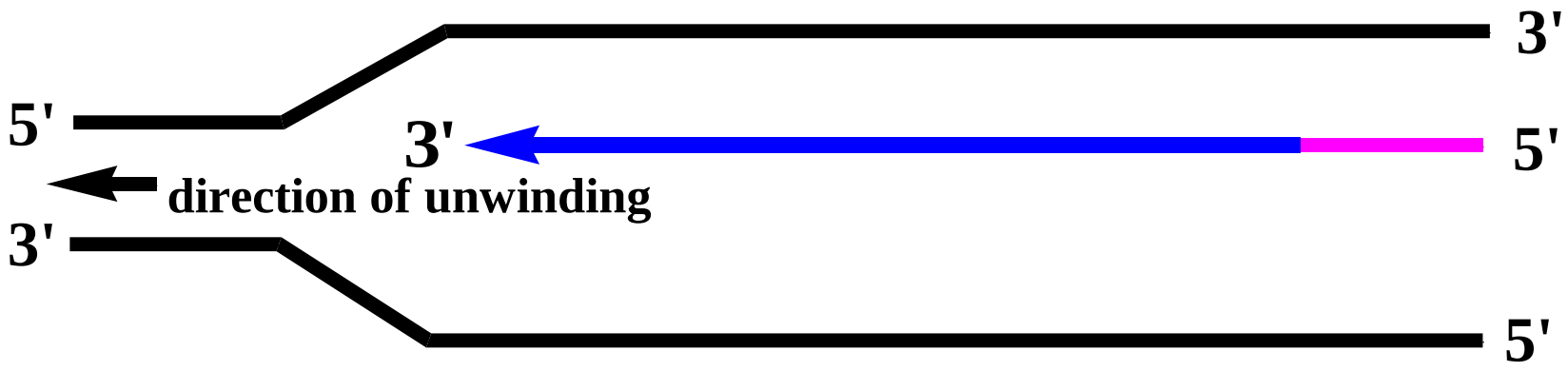


§ 1.3 Semi-continuous Replication

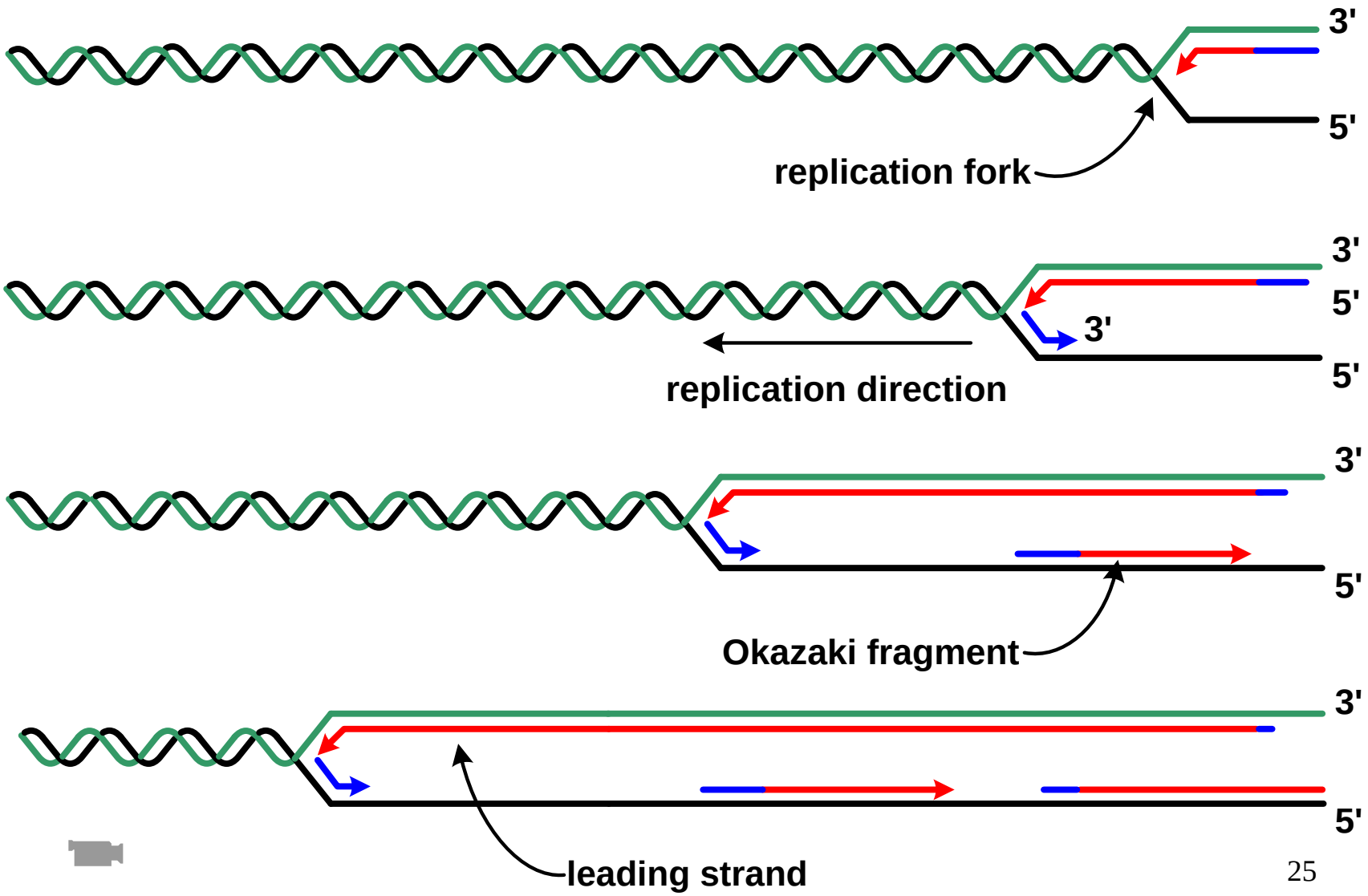
The daughter strands on two template strands are synthesized differently since the replication process obeys the principle that DNA is synthesized from the 5′ end to the 3′ end.

Leading strand

On the template having the 3´- end, the daughter strand is synthesized continuously in the 5’-3’ direction. This strand is referred to as the leading strand.



Semi-continuous replication



Okazaki fragments

- **Many DNA fragments are synthesized sequentially on the DNA template strand having the 5´- end. These DNA fragments are called Okazaki fragments. They are 1000 – 2000 nt long for prokaryotes and 100-150 nt long for eukaryotes.**
- **The daughter strand consisting of Okazaki fragments is called the lagging strand.**

Semi-continuous replication

Continuous synthesis of the leading strand and discontinuous synthesis of the lagging strand represent a unique feature of DNA replication. It is referred to as the semi-continuous replication.

Section 2

Enzymology of DNA Replication

Enzymes and protein factors

protein	Size	function
Dna A protein	50,000	recognize origin
Dna B protein	300,000	open dsDNA
Dna C protein	29,000	assist Dna B binding
DNA pol		Elongate the DNA strands
Dna G protein	60,000	synthesize RNA primer
SSB	75,600	single-strand binding
DNA topoisomerase	400,000	release supercoil constraint

§ 2.1 DNA Polymerase

DNA-pol of prokaryotes

- **The first DNA-dependent DNA polymerase (short for DNA-pol I) was discovered in 1958 by Arthur Kornberg who received Nobel Prize in physiology or medicine in 1959.**

Arthur Kornberg

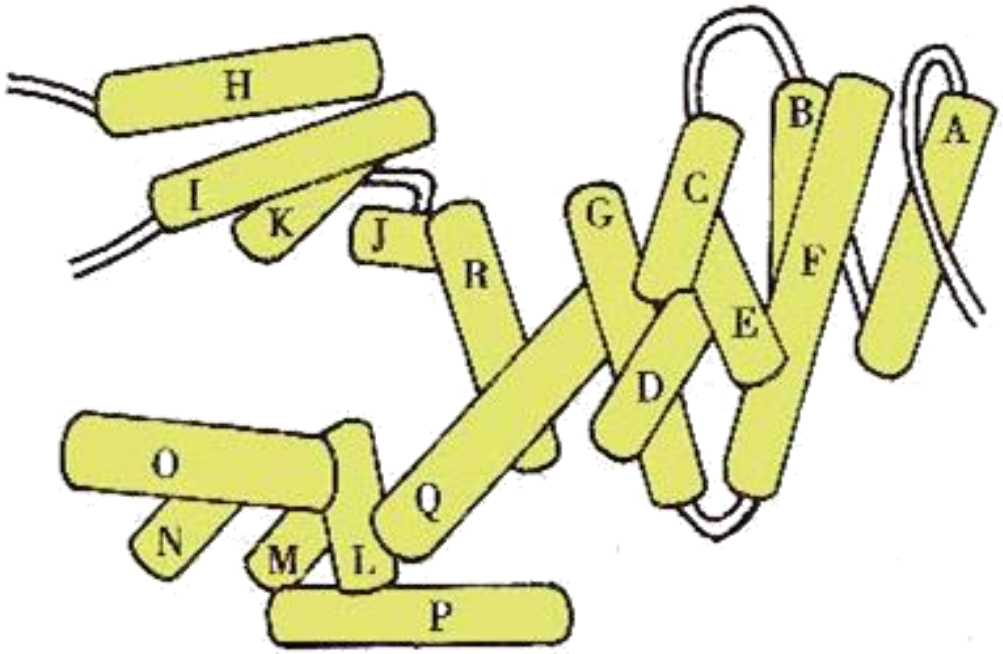
- **Later, DNA-pol II and DNA-pol III were identified in experiments using mutated *E.coli* cell line.**
- **All of them possess the following biological activity.**

  1. 5′→3′ polymerizing

  2. exonuclease

DNA-pol I

- **Mainly responsible for proofreading and filling the gaps, repairing DNA damage**



DNA-pol II

- **Temporary functional when DNA-pol I and DNA-pol III are not functional**
- **Still capable for doing synthesis on the damaged template**
- **Participating in DNA repairing**

DNA-pol III

- **A heterodimer enzyme composed of ten different subunits**
- **Having the highest polymerization activity (10^5 nt/min)**
- **The true enzyme responsible for the elongation process**

§ 2.2 Primase

- **Also called DnaG**
- **Primase is able to synthesize primers using free NTPs as the substrate and the ssDNA as the template.**
- **Primers are short RNA fragments of a several decades of nucleotides long.**

§ 2.3 Helicase

- **Also referred to as DnaB.**
- **It opens the double strand DNA with consuming ATP.**
- **The opening process with the assistance of DnaA and DnaC**

§ 2.4 SSB protein

- **Stand for single strand DNA binding protein**
- **SSB protein maintains the DNA template in the single strand form in order to**
  - **prevent the dsDNA formation;**
  - **protect the vulnerable ssDNA from nucleases.**

§ 2.5 Topoisomerase

- **Opening the dsDNA will create supercoil ahead of replication forks.**
- **The supercoil constraint needs to be released by topoisomerases.**

Topoisomerase I (topo I)

- **Also called ω-protein in prokaryotes.**
- **It cuts a phospho-diester bond on one DNA strand, rotates the broken DNA freely around the other strand to relax the constraint, and reseals the cut.**

Topoisomerase II (topo II)

- **It is named gyrase in prokaryotes.**
- **It cuts phosphoester bonds on both strands of dsDNA, releases the supercoil constraint, and reforms the phosphoester bonds.**
- **It can change dsDNA into the negative supercoil state with consumption of ATP.**

§ 2.6 DNA Ligase

- **Connect two adjacent ssDNA strands by joining the 3′-OH of one DNA strand to the 5′-P of another DNA strand.**
- **Sealing the nick in the process of replication, repairing, recombination, and splicing.**

§ 2.7 Replication Fidelity

- **Replication based on the principle of base pairing is crucial to the high accuracy of the genetic information transfer.**
- **Enzymes use two mechanisms to ensure the replication fidelity.**
  - **Proofreading and real-time correction**
  - **Base selection**

Proofreading and correction

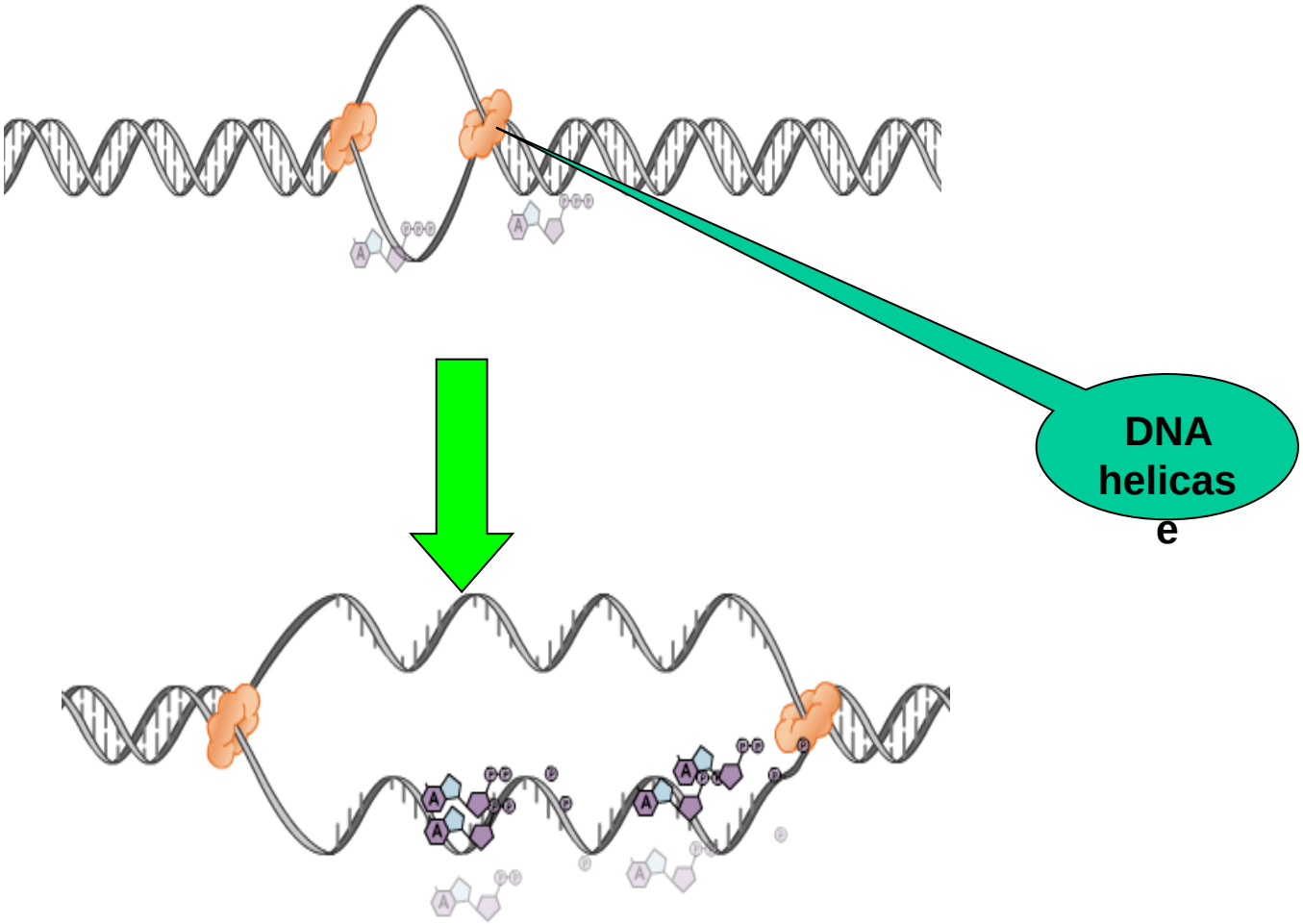
- **DNA-pol I has the function to correct the mismatched nucleotides.**
- **It identifies the mismatched nucleotide, removes it using the 3´-5´ exo-nuclease activity, add a correct base, and continues the replication.**

Section 3

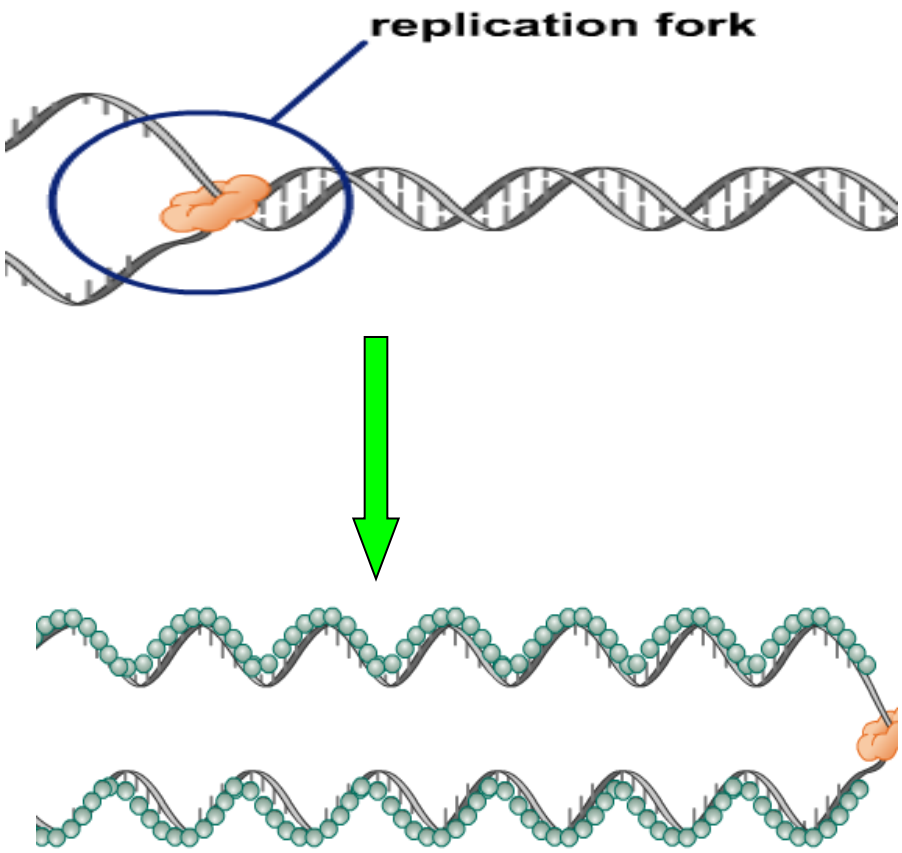
DNA Replication Process

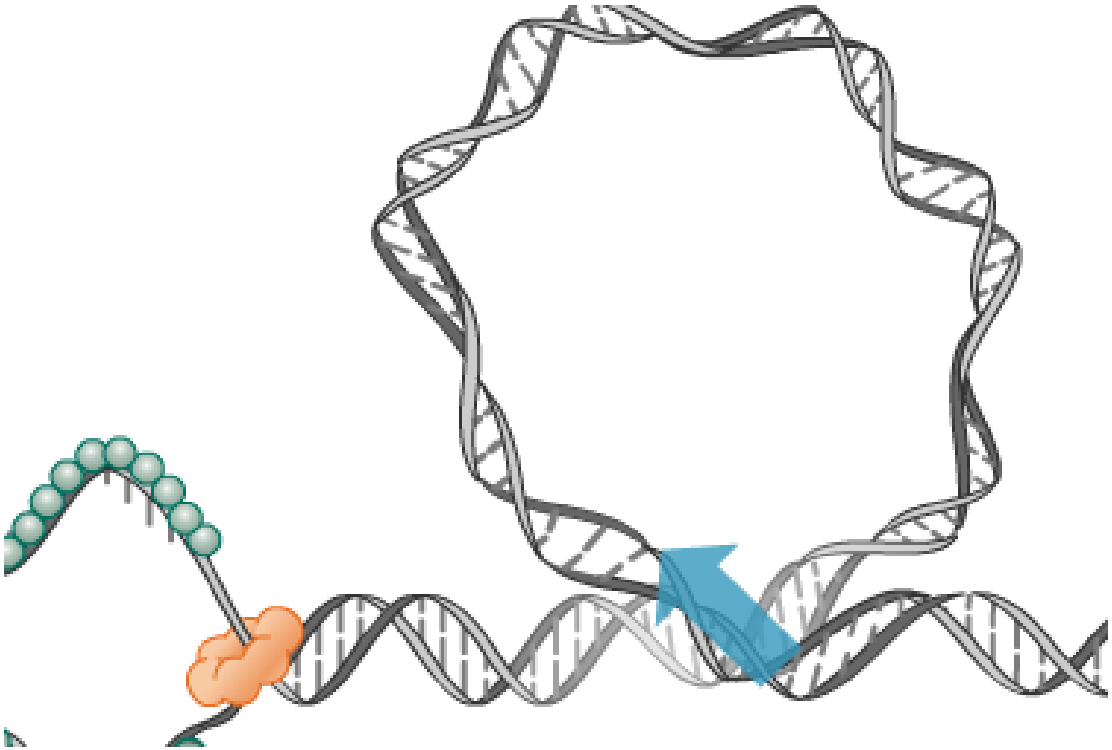
Replication in details

DNA helicases unwind the double helix

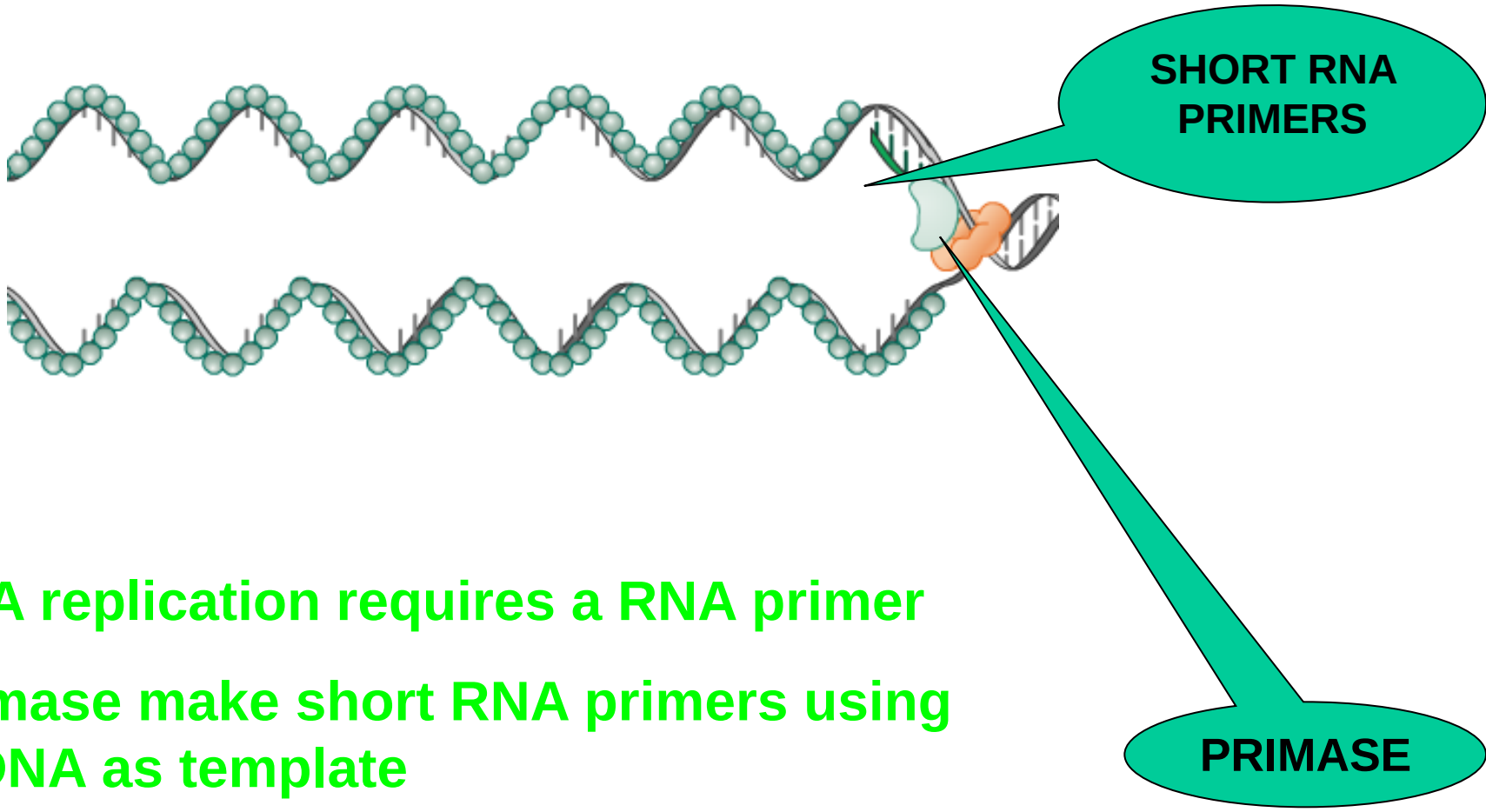


The formation of replication fork and SSBs bind to stabilise ssDNA





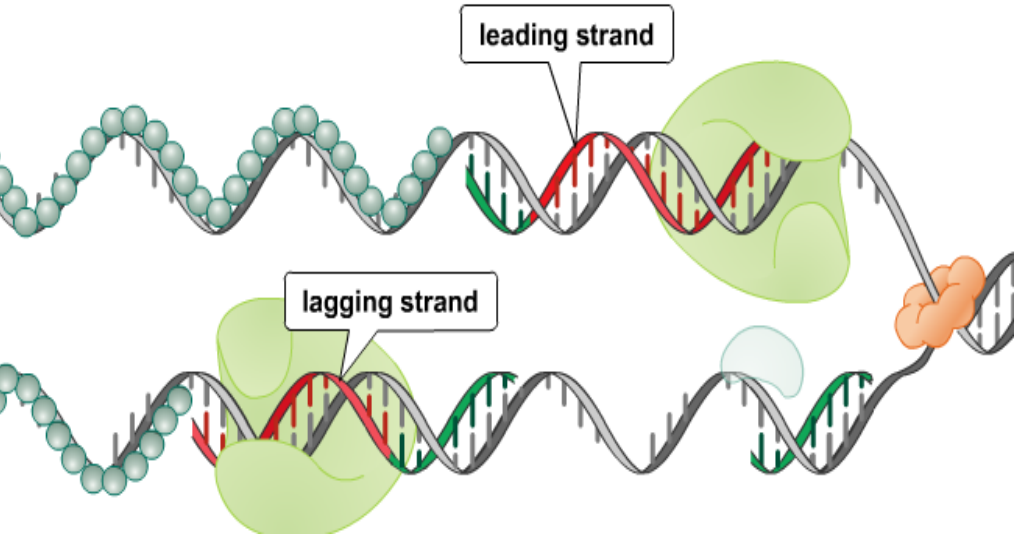
Topoisomerases remove positive supercoils .

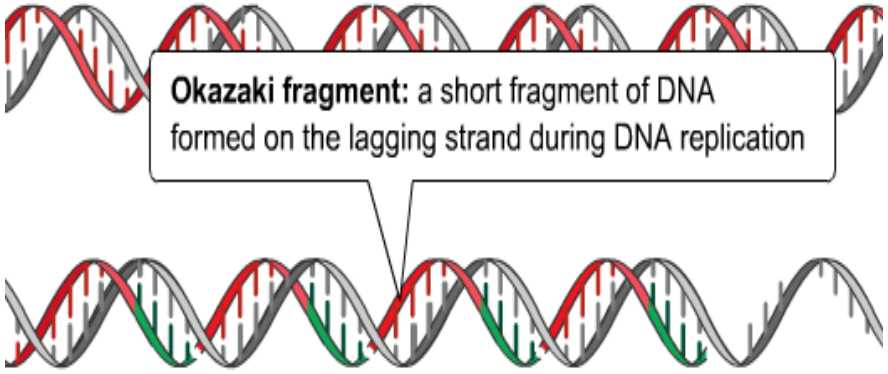


DNA replication requires a RNA primer

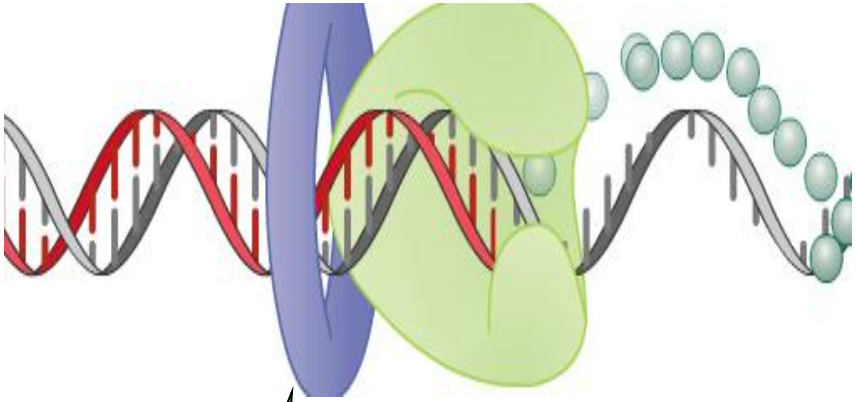
Primase make short RNA primers using ssDNA as template

DNA primase is activated by interacting with the DNA helicase





DNA polymerases catalyzes DNA synthesis

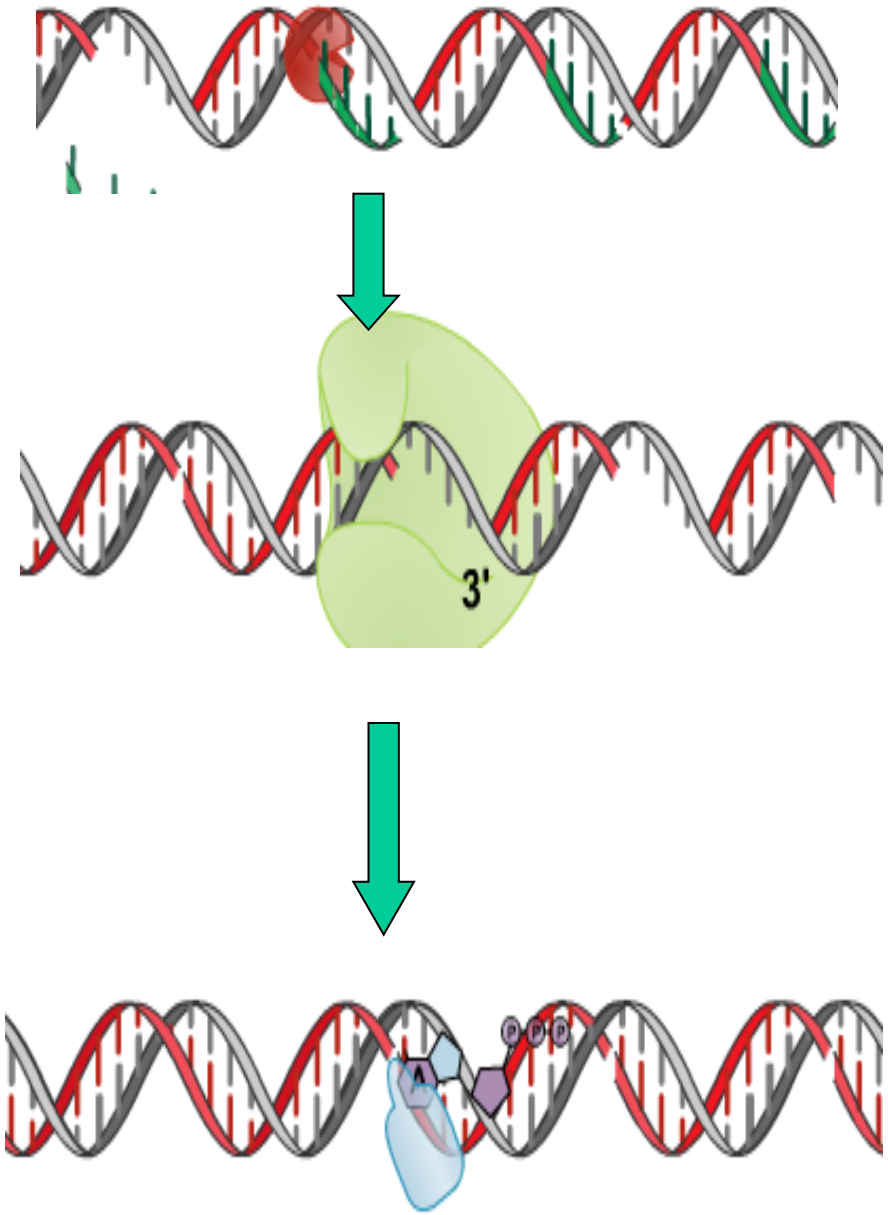


SLIDING DNA CLAMPS

Sliding DNA clamps increases

processivity

Processivity : the ability of an enzyme to catalyze many reactions before releasing its substrate



RNAse degrades RNA base paired with DNA

Removal of RNA primers leaves gaps

DNA polymerase fill the gaps

DNA ligase repairs the remaining nicks

Sequential actions

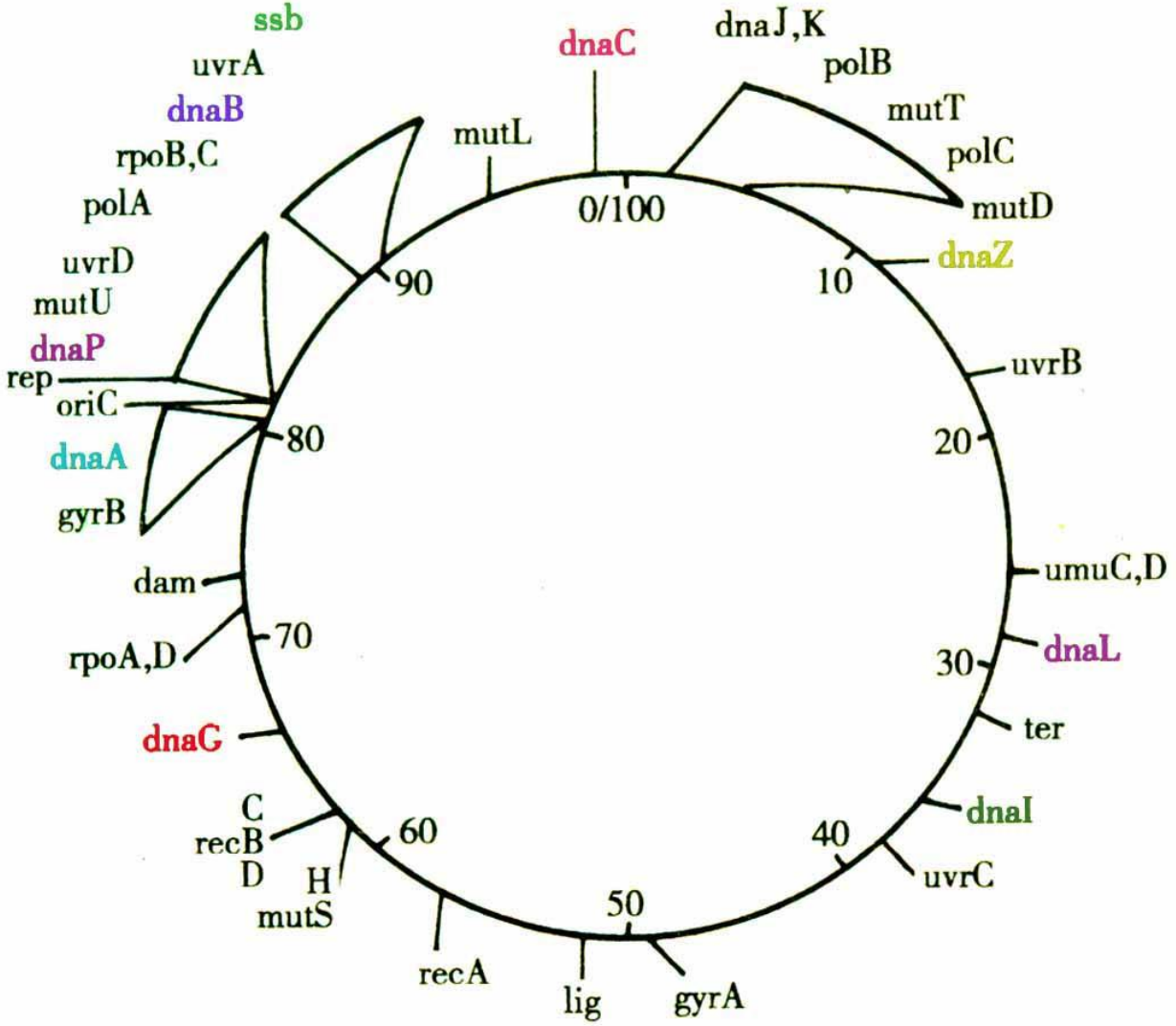
- **Initiation:** recognize the starting point, separate dsDNA, primer synthesis, …
- **Elongation:** add dNTPs to the existing strand, form phosphoester bonds, correct the mismatch bases, extending the DNA strand, …
- **Termination:** stop the replication

§ 3.1 Replication of prokaryotes

a. Initiation

- **The replication starts at a particular point called origin.**
- **The origin of *E. coli*, ori C, is at the location of 82.**
- **The structure of the origin is 248 bp long and AT-rich.**

Genome of *E. coli*



Formation of replication fork

- **DnaA recognizes ori C.**
- **DnaB and DnaC join the DNA-DnaA complex, open the local AT-rich region, and move on the template downstream further to separate enough space.**
- **DnaA is replaced gradually.**
- **SSB protein binds the complex to stabilize ssDNA.**

Primer synthesis

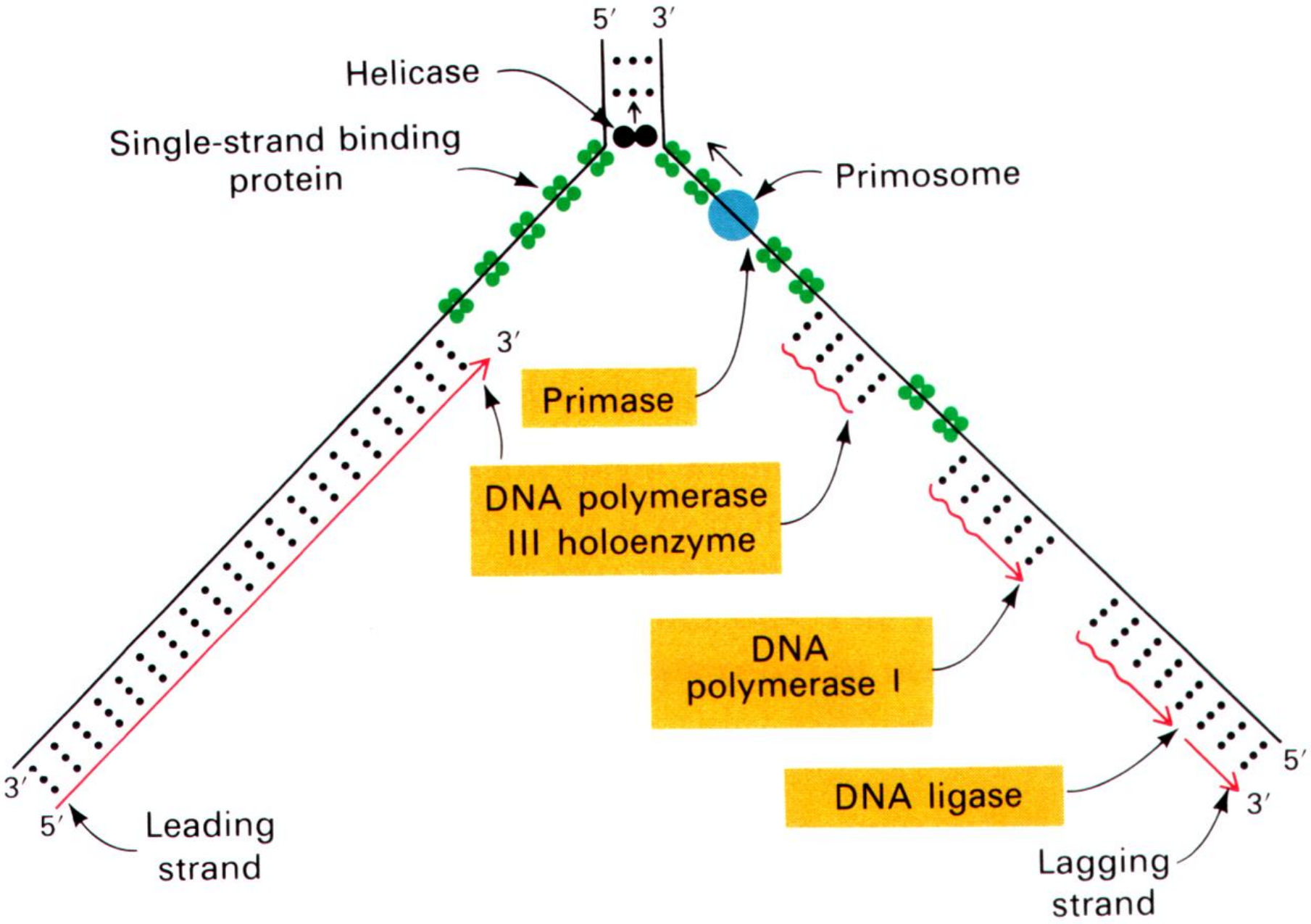
- **Primase joins and forms a complex called primosome.**
- **Primase starts the synthesis of primers on the ssDNA template using NTP as the substrates in the 5′- 3′ direction at the expense of ATP.**
- **The short RNA fragments provide free 3′-OH groups for DNA elongation.**

Releasing supercoil constraint

- **The supercoil constraints are generated ahead of the replication forks.**
- **Topoisomerase binds to the dsDNA region just before the replication forks to release the supercoil constraint.**
- **The negatively supercoiled DNA serves as a better template than the positively supercoiled DNA.**

b. Elongation

- **dNTPs are continuously connected to the primer or the nascent DNA chain by DNA-pol III.**
- **The core enzymes catalyze the synthesis of leading and lagging strands, respectively.**
- **The nature of the chain elongation is the series formation of the phosphodiester bonds.**


5′
3′
Helicase
Single-strand binding protein
Primosome
3′
Primase
DNA polymerase III holoenzyme
DNA polymerase I
DNA ligase
3′
5′
5′
3′
Leading strand
Lagging strand

c. Termination

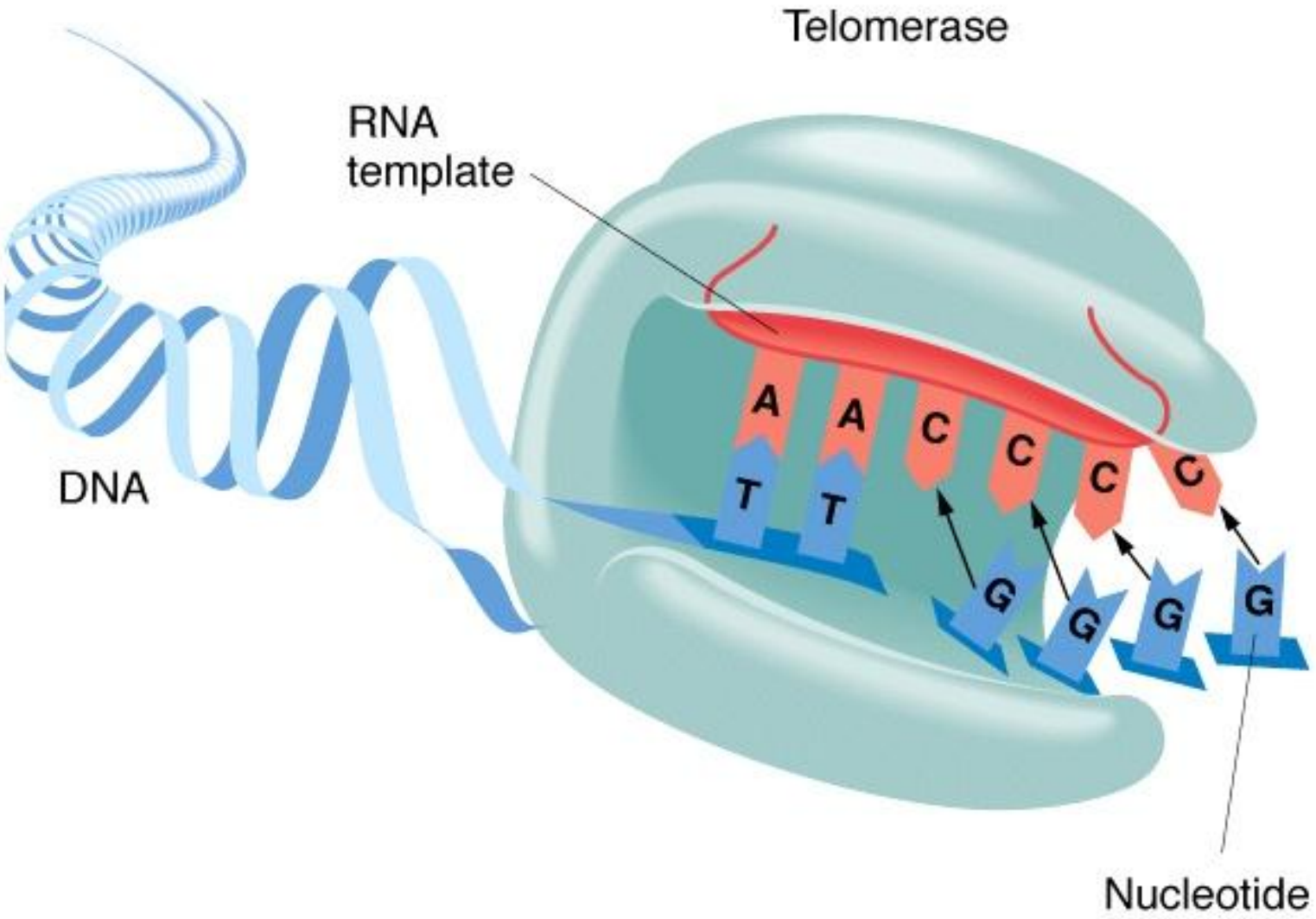
- **The replication of *E. coli* is bidirectional from one origin, and the two replication forks must meet at one point called ter at 32.**
- **All the primers will be removed, and all the fragments will be connected by DNA-pol I and ligase.**

Telomere

- **The terminal structure of eukaryotic DNA of chromosomes is called telomere.**
- **Telomere is composed of terminal DNA sequence and protein.**
- **The sequence of typical telomeres is rich in T and G.**
- **The telomere structure is crucial to keep the termini of chromosomes in the cell from becoming entangled and sticking to each other.**

Telomerase

- **The eukaryotic cells use telomerase to maintain the integrity of DNA telomere.**
- **The telomerase is composed of**
  - **telomerase RNA**
  - **telomerase association protein**
  - **telomerase reverse transcriptase**
- **It is able to synthesize DNA using RNA as the template.**


Telomerase
RNA
template
DNA
A
A
C
C
C
C
T
T
G
G
G
G
Nucleotide

Significance of Telomerase

- **Telomerase may play important roles is cancer cell biology and in cell aging.**