CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.2: DNA REPLICATION

Learning Outcomes

You should be able to:

(b) describe the process of DNA replication and how the end replication problem arises

Use the knowledge gained in this section in new situations or to solve related problems.

References

Reece, J B, Urry, L A, Cain, M L, Wasserman, S A, Minorsky, P V and Jackson, R B (2017) **Campbell Biology (11th Edition)** (Pearson Higher Education) ISBN 0321739752

I. Introduction

The structure of DNA was proposed by Watson and Crick in 1953. With an understanding of DNA structure, experimental evidence supported the proposal that DNA replicates in a semi-conservative manner.

During S phase of interphase, DNA content is doubled to form two sister chromatids joined at the centromere, in preparation of subsequent nuclear division.

II. Evidence of Semi-Conservative DNA replication

Matthew Meselson and Frank Stahl obtained evidence for the **semi-conservative replication** of DNA and published their results in 1958.

The **Meselson and Stahl experiment** was as follows:

- Cultures of *Escherichia coli* (*E. coli*) bacteria were grown with the presence of 'heavy' isotope of nitrogen, ^{15}N, instead of the ordinary 'light' isotope of nitrogen, ^{14}N.
- After many generations, the bacteria culture all contained the heavy form, ^{15}N.
- Some bacteria from the culture were treated to release their DNA into a solution. The solution with DNA was then centrifuged at high speed.
- The result was a single band of DNA, a heavy band that contained the ^{15}N. This is known as the parental generation.

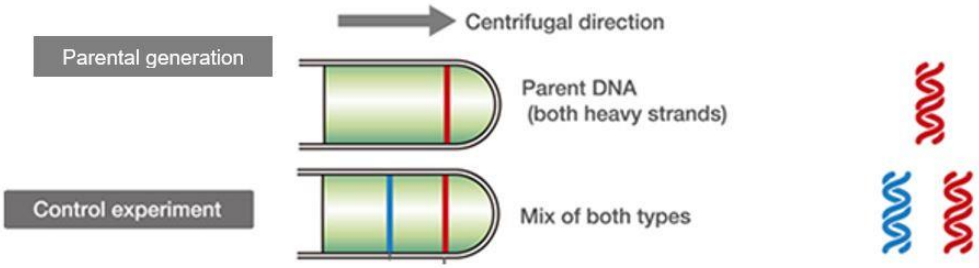


Fig. 2 Parental generation compared to control experiment with both ^{14}N and ^{15}N

- The remaining bacteria were placed in a ^{14}N culture medium and allowed to grow for 20 minutes, which is the generation time for *E. coli* grown in optimal conditions.
- A sample of bacteria was then taken, processed, and centrifuged to produce the first generation (generation 1).
- This same process was continued so that the second and third generations were obtained, each being processed and centrifuged.

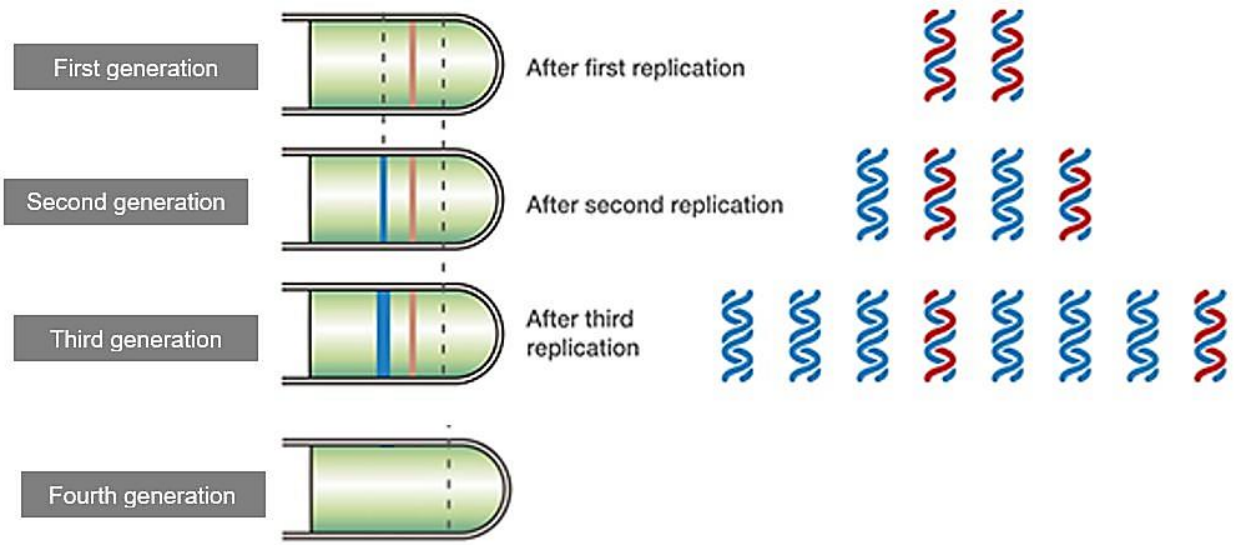


Fig. 3 Results of second and third generations

The **Meselson and Stahl experimental results** were as follows:

- In the first generation, the DNA band occurred as an intermediate band in the middle of positions of ^{14}N and ^{15}N bands. This indicates that the DNA molecules (double helix) comprises of one ^{15}N strand and one ^{14}N strand.

  This shows that two ^{15}N parental strands of DNA separated to act as a template for the replication of a new ^{14}N daughter strand.

  After replication, the parental ^{15}N strand rewinds with a newly synthesised ^{14}N strand to form a new $^{14}N^{15}N$ daughter DNA molecule.

- In the second generation, DNA molecules from the first generation separated and each act as a template for the synthesis of a new ^{14}N daughter strand.

  After replication, one ^{15}N parental strand rewinds with newly synthesised ^{14}N daughter strand to form a new daughter DNA molecule; while the other ^{14}N parental strand rewinds with another newly synthesised ^{14}N daughter strand to form a new daughter DNA molecule.

  Thus, one daughter DNA molecule consists of $^{14}N^{15}N$ forming an intermediate band while the $^{14}N^{14}N$ daughter molecule forms a light band.

- This proceeds on with the third generation resulting in a larger proportion, thus thicker, light band ($^{14}N^{14}N$)

- Thus, the experimental evidence supports semi-conservative mode of replication.

Checkpoint 1

Illustrate the positions and thickness of the DNA bands in the fourth generation of *E. coli* cultured in ^{14}N medium in Fig. 3.

III. Process of Semi-conservative Replication

(i) Initiation

- **Helicase** binds at the **origin of replication** (oriR). Helicase hydrolysed ATP to release energy to **unwind and unzip** the DNA double helix into two single strands by breaking the hydrogen bonds between the complementary base pairs.

- This results in the formation of a **replication bubble** with 2 **replication forks**. Helicase continues to unwinds and separates the DNA **bidirectionally** away from the oriR.

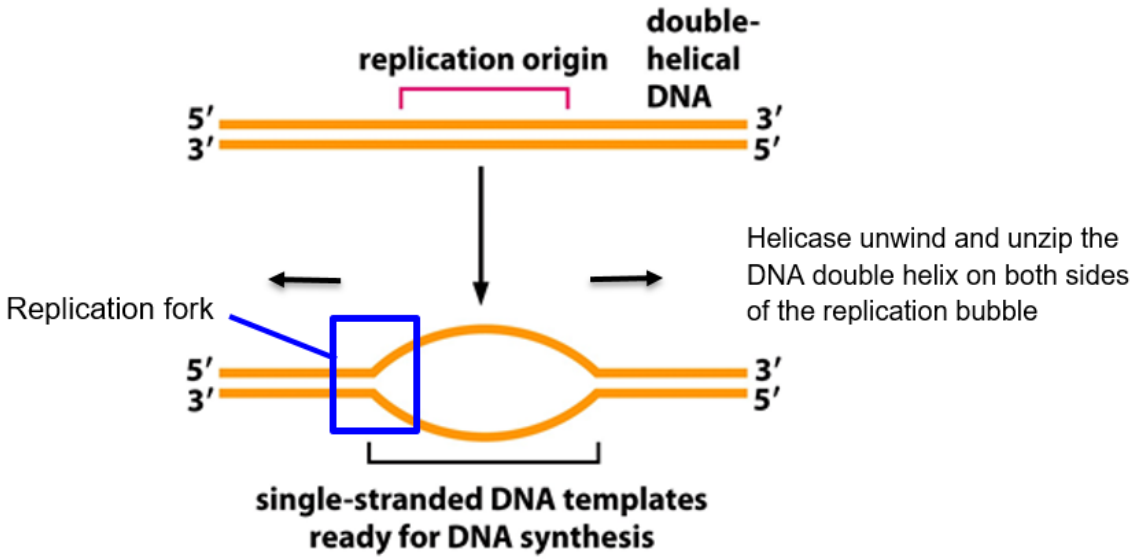


Fig. 4 Formation of replication bubble

- **Single strand binding proteins** (SSBPs) bind to single-stranded regions of DNA near the replication fork to keep the strands apart to prevent the replication bubble from rewinding.

- As replication continues, the unwinding of the double helix causes tighter twisting (supercoiling) in the DNA regions that are yet to unwind and unzip.

- **Topoisomerase / DNA gyrase** helps relieve the supercoiling by breaking phosphodiester bonds in the sugar-phosphate backbone to allow the helix to untwist in the opposite direction before sealing the backbone.

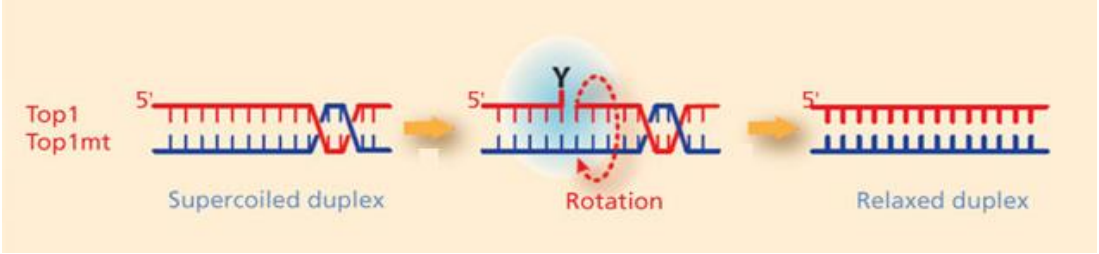


Fig. 5 Role of topoisomerase

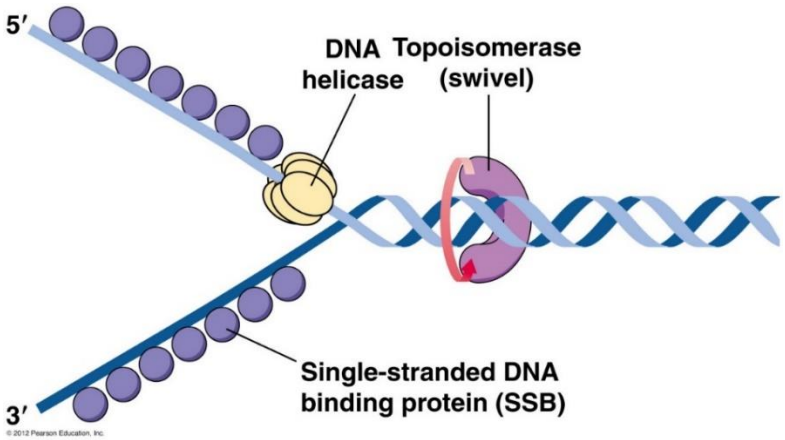


Fig. 6 Helicase, single-strand binding proteins and topoisomerase at a replication fork

(ii) Elongation

- **Primase** binds to the separated DNA strands and synthesizes a short stretch of complementary RNA called a **primer**, using the DNA strand as a template, in the direction of 5' → 3'.

- It brings in ribonucleoside triphosphates (rNTPs) **complementary to the DNA template** and catalyses phosphodiester bonds between adjacent ribonucleotides.

- The RNA primer provides a **free 3'OH end** for **DNA polymerase** to recognise and bind.

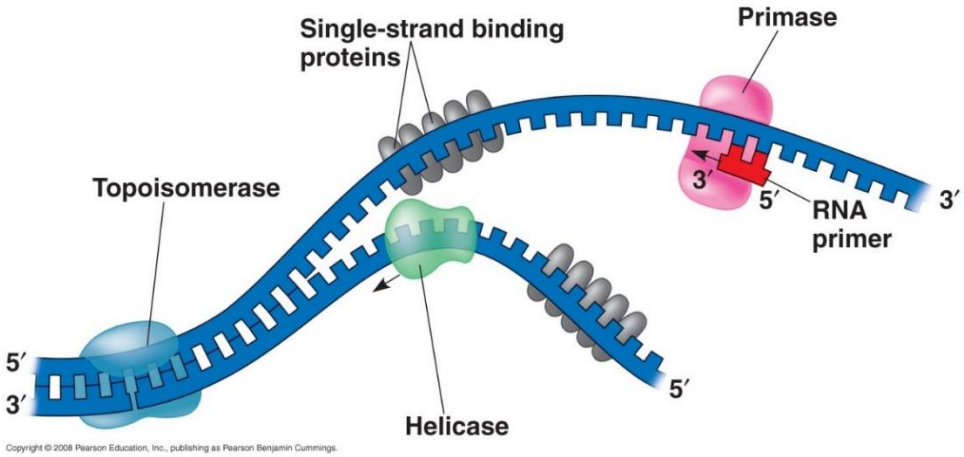


Fig. 7 Role of primase

FYI	The main DNA polymerase enzyme responsible for replication in prokaryotes is DNA polymerase III whereas in eukaryote, it is DNA polymerase δ.

- **DNA polymerase** brings in **free deoxyribonucleoside triphosphates (dNTPs)** that are complementary to the parent DNA template.

- DNA polymerase hydrolyses phosphate bonds in the 5' phosphate groups of incoming dNTPs to release energy for the synthesis of the daughter strand.

- DNA polymerase adds the resultant **deoxyribonucleotides** to the free 3'-OH end of the (RNA) primer and catalyses the formation of phosphodiester bonds between adjacent nucleotides.

- Hence, the **synthesis** of the complementary daughter strand occurs in the **5' → 3' direction** and the parental strand is **read** in the direction of **3' → 5'**.

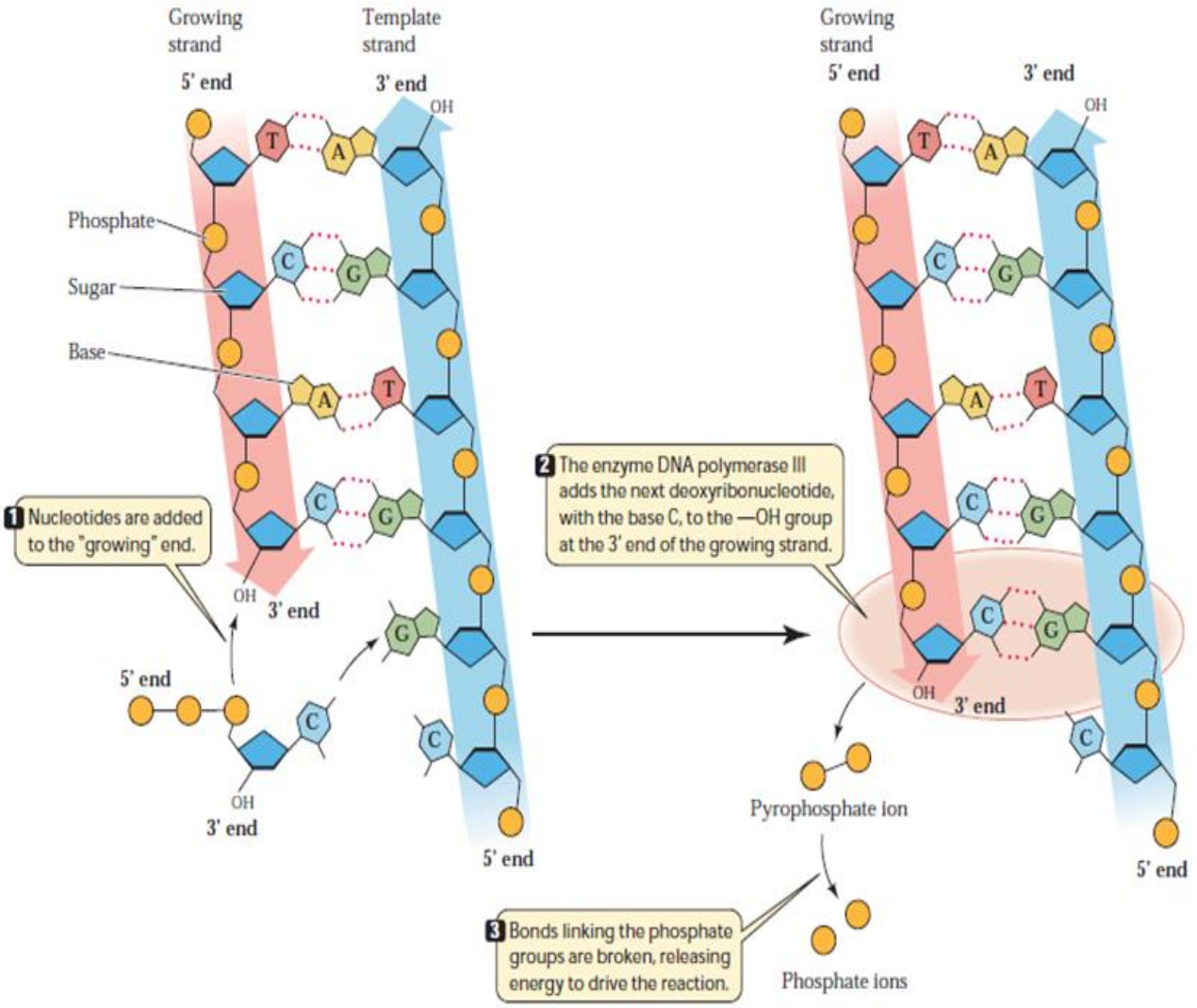


Fig. 8 Elongation at 3' OH end of the growing daughter strand during DNA replication in a bacterial (prokaryote) cell.

- Daughter strands that are synthesized **continuously towards the replication fork** in the direction of **5' → 3'** using only **one RNA primer** are called **leading strands.**

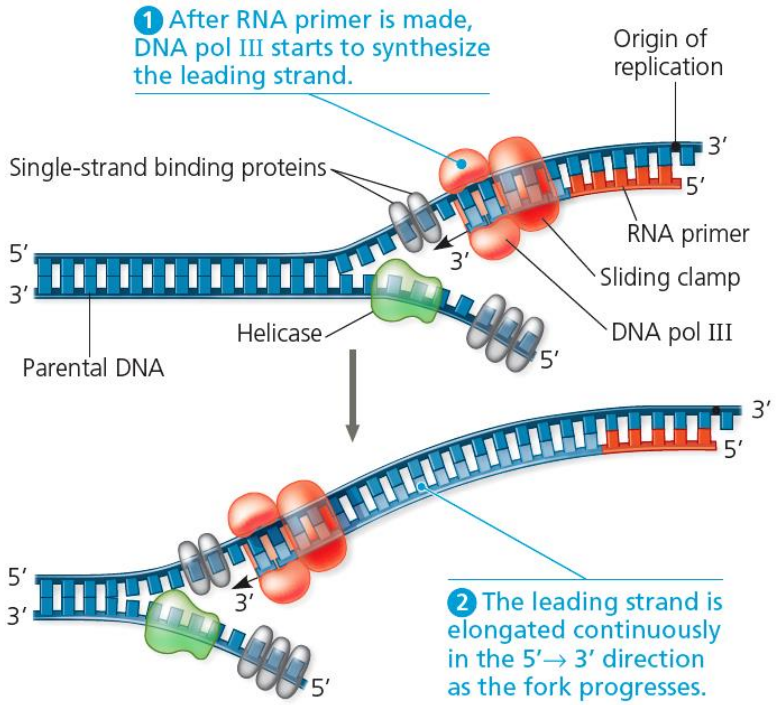
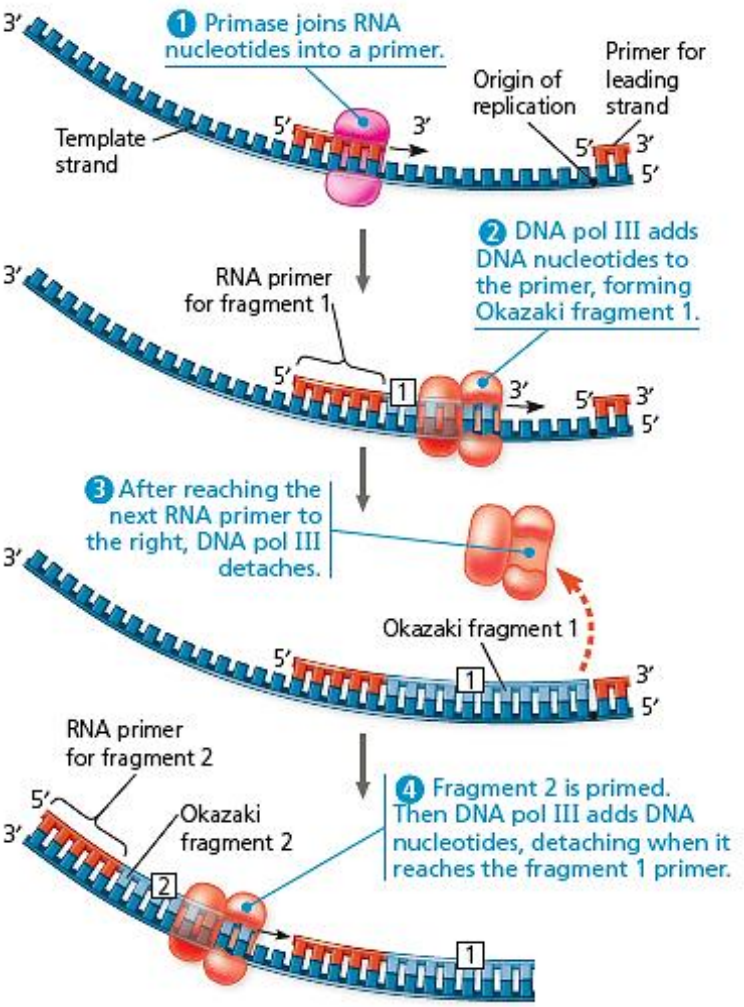


Fig. 9 Leading strand synthesis in prokaryotes

- Daughter strands that are synthesized **discontinuously away from the replication fork** in the direction of **5' → 3'** using **multiple RNA primers** are called **lagging strands**.

- This is because the replication fork opens behind the 5' end of the lagging strand, thus a new primer needs to be synthesized each time a new polynucleotide strand is synthesized.

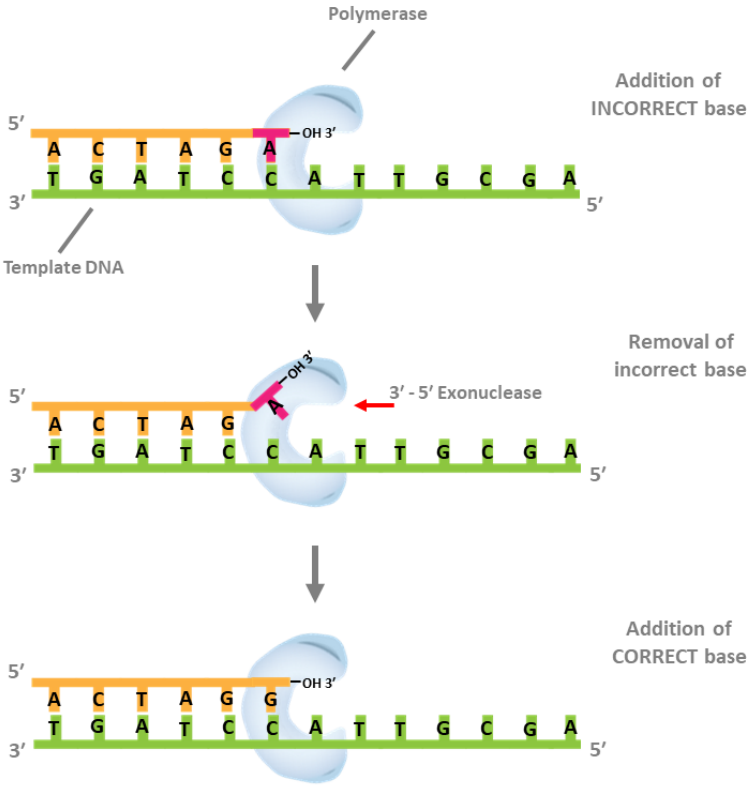
- The lagging strand is thus synthesized discontinuous in the form of **Okazaki fragments**.



(Right) Fig. 10 Lagging strand synthesis in prokaryotes

The daughter strands are synthesized in such a way because:

- DNA polymerase cannot initiation the synthesis of a new DNA polynucleotide strand,
- DNA polymerase can only elongate existing polynucleotide strand at the free 3'-OH end, thus it can only synthesize polynucleotide strand in the 5' ➝ 3' direction and,
- pairing of DNA strands are in anti-parallel manner.



- As DNA polymerase moves along the template, part of the enzyme **proofreads** the previous region. This is to check if proper base pairing has taken place between the bases.
- If an incorrect deoxyribonucleotide was added, it would be removed by the 3' → 5' exonuclease activity of DNA polymerase and replaced with the correct deoxyribonucleotide.

(Left) Fig. 11 Proofreading by DNA polymerase

- The lagging strand is synthesized in Okazaki fragments, interspersed with RNA primers. RNA primers need to be removed and Okazaki fragments must be joined to form a continuous DNA daughter strand.

FYI	The DNA polymerase enzyme responsible for removal of primers and replacing with DNA in prokaryotes is DNA polymerase I whereas in eukaryote, it is DNA polymerase ε.

- A different **DNA polymerase** removes the RNA primer and replaces it with deoxyribonucleotides complementary to the parent strand using the preceding 3'-OH from another Okazaki fragment.
- Hence the final RNA primer at the 3'-end of the parental strand will not be replaced as there is no preceding 3'-OH.
- **DNA ligase** catalyses the formation of phosphodiester bond between the 3'-OH of one Okazaki fragment with the 5'-phosphate group of another Okazaki fragment to form a continuous strand of DNA.

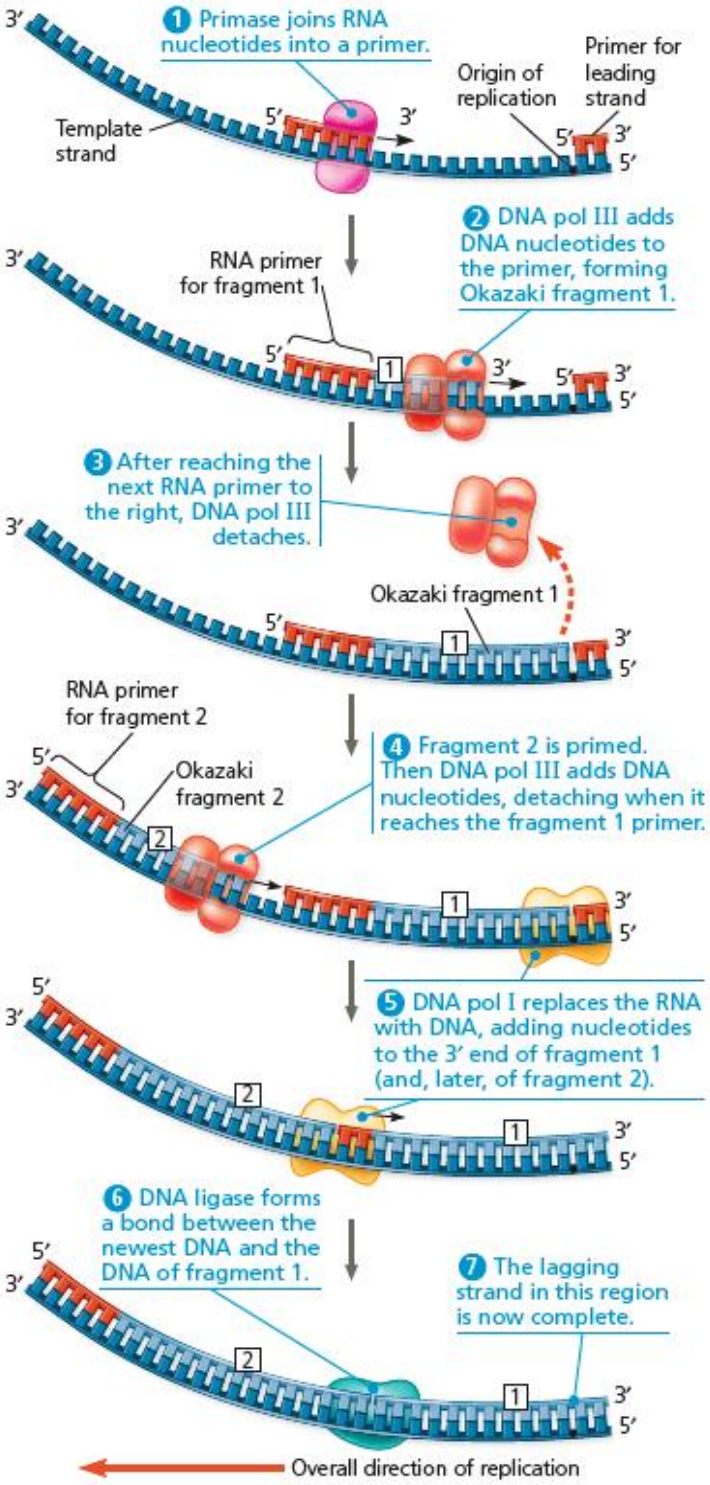


Fig. 12 DNA ligase joins Okazaki fragments in a bacterial cell.

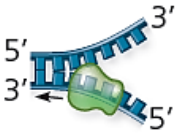
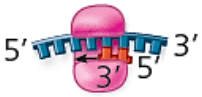
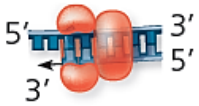
Protein	Function
Helicase 	Unwinds parental double helix at replication forks
Single-strand binding protein 	Binds to and stabilizes single-stranded DNA until it is used as a template
Topoisomerase 	Relieves overwinding strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase 	Synthesizes an RNA primer at 5′ end of leading strand and at 5′ end of each Okazaki fragment of lagging strand
DNA pol III 	Using parental DNA as a template, synthesizes new DNA strand by adding nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I 	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides added to 3′ end of adjacent fragment
DNA ligase 	Joins Okazaki fragments of lagging strand; on leading strand, joins 3′ end of DNA that replaces primer to rest of leading strand DNA

Fig. 13 Roles of proteins in prokaryote DNA replication

(iii) Termination

- Replication of a parental DNA molecule produces two new DNA molecules, each comprising of a parental strand and a newly synthesized daughter strand.

- The **daughter DNA strand at the 5'-end is shorter than the parental strand at the 3'-end.** This leaves a single-stranded stretch of parental DNA extending beyond the 5' end of the daughter strand, called the **3' overhang**.

- After each round of replication, the new daughter DNA strand is shorter than its parental template. Thus, DNA molecules gets progressively shorter with each replication. This is called the **end replication problem**.

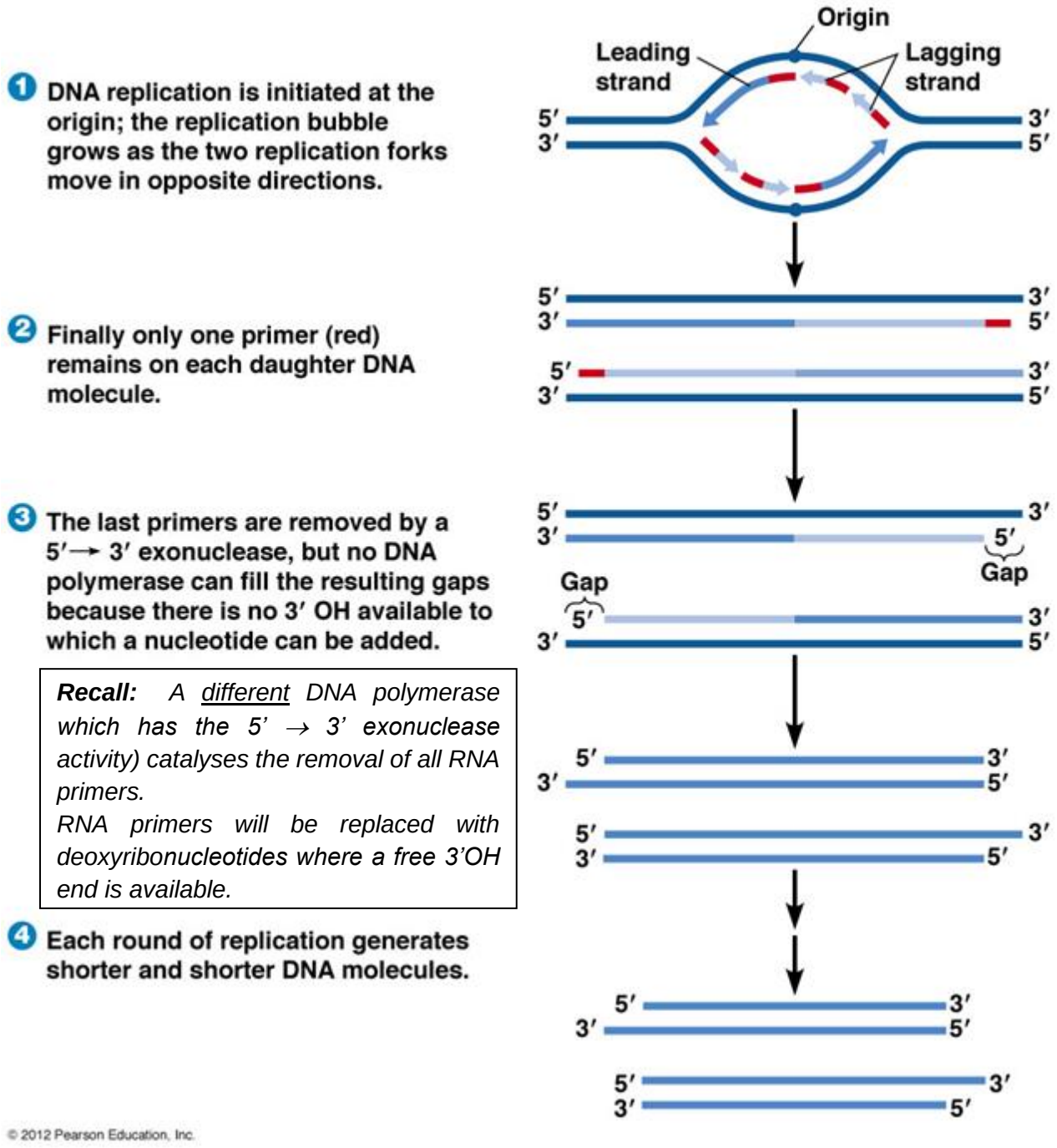


Fig. 14 End replication problem

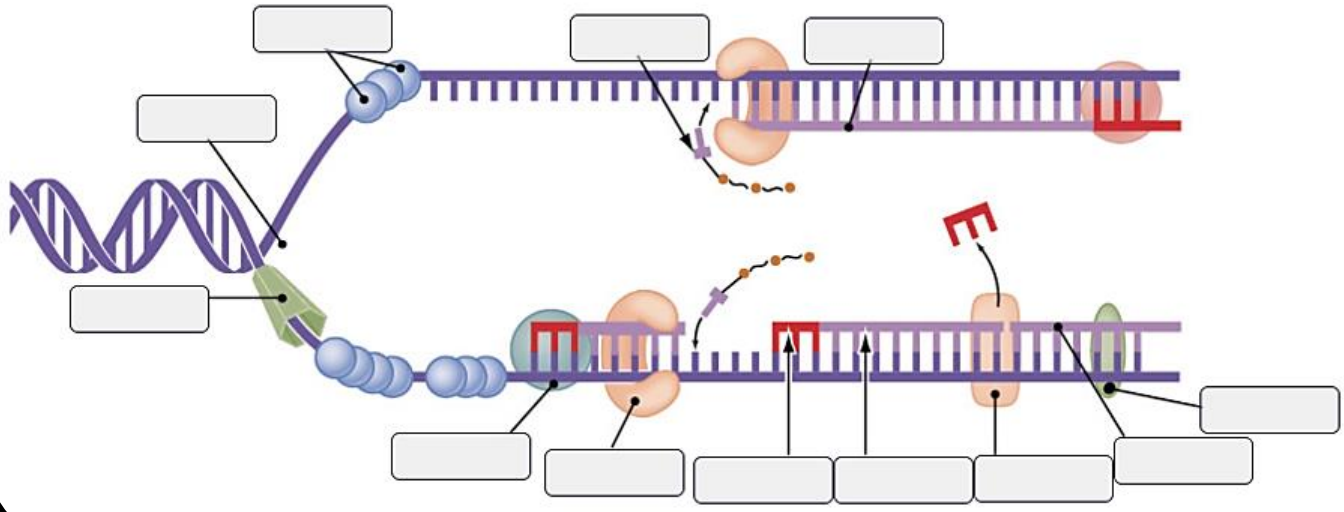
- As DNA replication occurs during every cell division, DNA molecules get shorter each time the cell divides.

- Thus, each cell can only divide a limited number of times (Hayflick Limit) as the DNA molecule will shorten to a critical length which triggers **apoptosis** (**programmed cell death**), causing the cell to die. Somatic cells experience **replicative senescence**, i.e. no longer carry out DNA replication and nuclear divisions, after undergoing a limited number of cell divisions.

Checkpoint 2

The diagram shows part of a DNA molecule undergoing replication in a prokaryote.
Label the diagram with the appropriate labels.

DNA polymerase I	DNA polymerase III	DNA ligase	Helicase
Leading strand	Lagging strand	Okazaki fragment	Primase
RNA primer	Replication fork	Single-stranded binding proteins	Nucleotide



Annex A: Models of DNA Replication

There were 3 possible models of replication that the scientists contemplated - conservative replication, semi-conservative replication and dispersive replication.

(i) Conservative model
This model of replication suggests that the parental DNA molecule acts as a template and directs synthesis of an entirely new double-stranded daughter DNA molecule.

After each round of replication, the parental DNA molecule is conserved.

(ii) Semi-conservative model
This model of replication suggests that the two parental strands separate by breaking the complementary hydrogen bonds. Each parental strand acts as a template for DNA replication.

After each round of replication, each daughter DNA molecule comprises one parental strand and one daughter strand with hydrogen bonds form between complementary base pairs.

(iii) Dispersive model
This model of replication suggests that the parental DNA strands break up into short segments and are used as templates for the synthesis of new daughter segments.

After each round of replication, each daughter DNA molecule contain a mixture of parental and newly synthesised daughter segments.

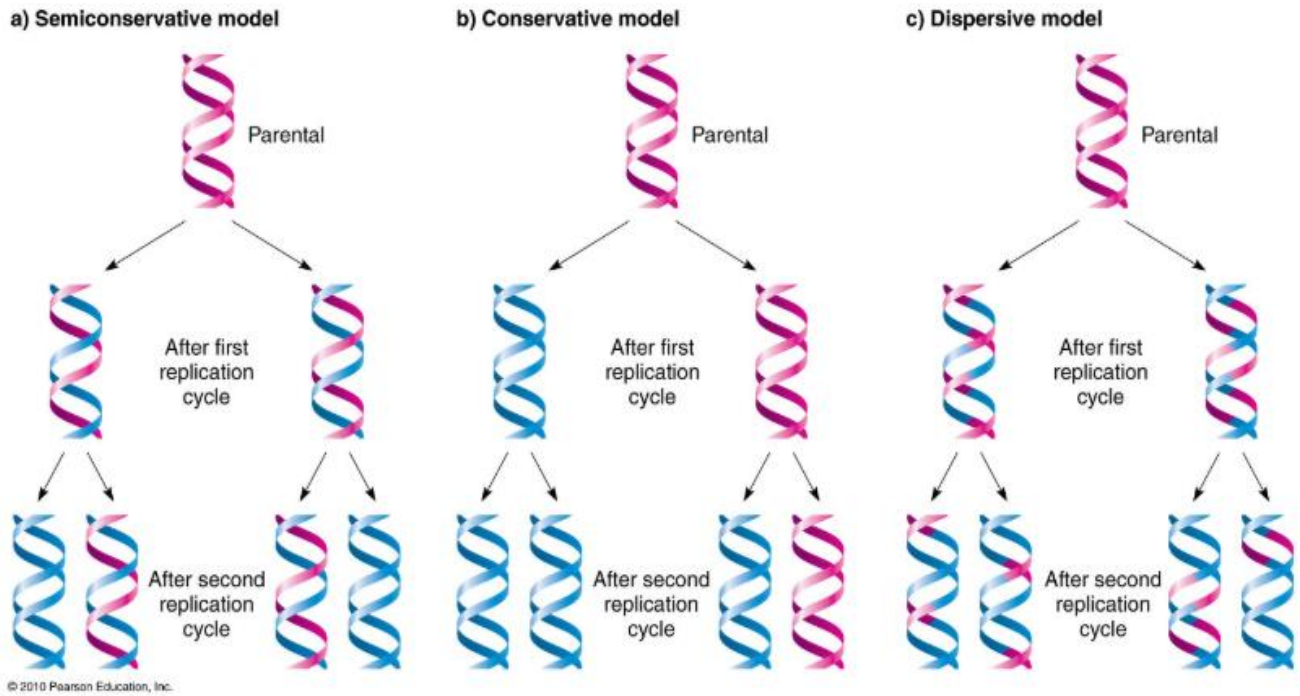
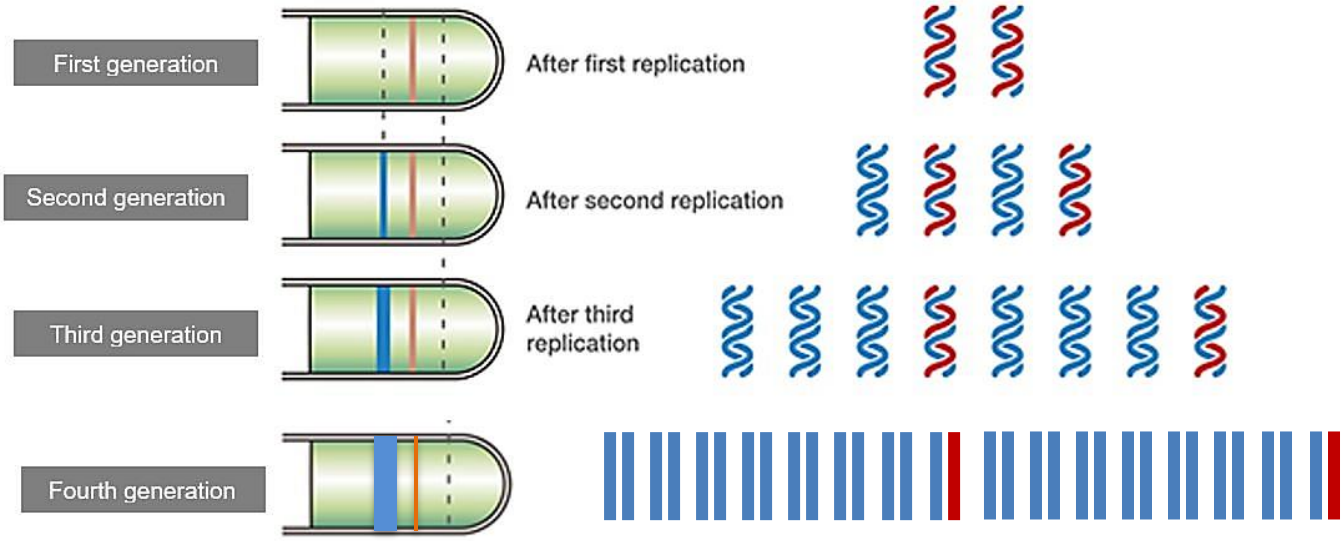


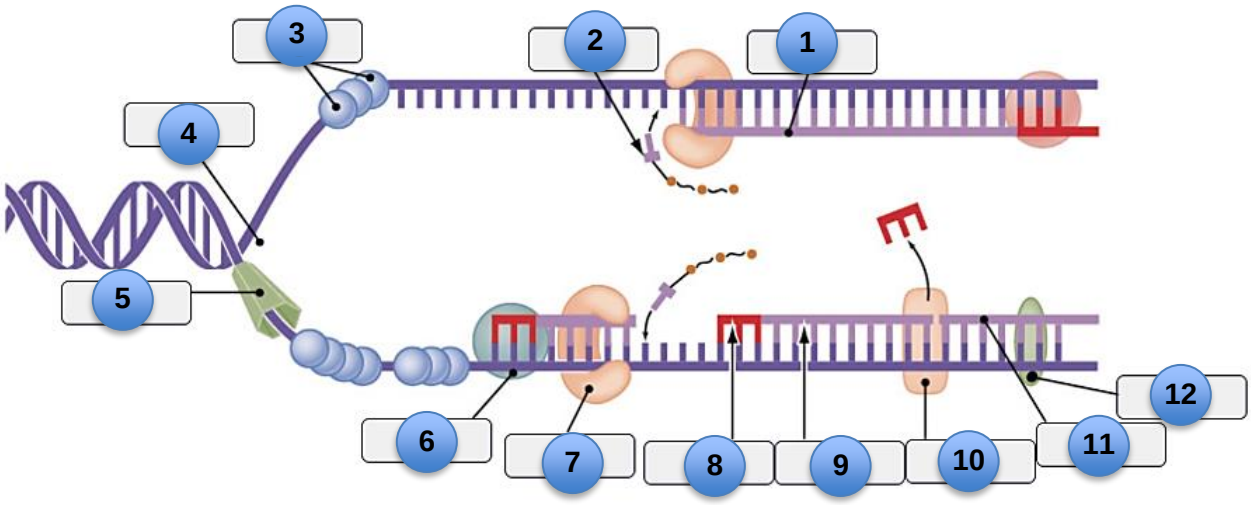
Fig. 1 Models of DNA replication

Answers

Checkpoint 1



Checkpoint 2



(10) DNA polymerase I	(7) DNA polymerase III	(12) DNA ligase	(5) Helicase
(1) Leading strand	(9) Lagging strand	(11) Okazaki fragment	(6) Primase
(8) RNA primer	(4) Replication fork	(3) Single-stranded binding proteins	(2) Nucleotide