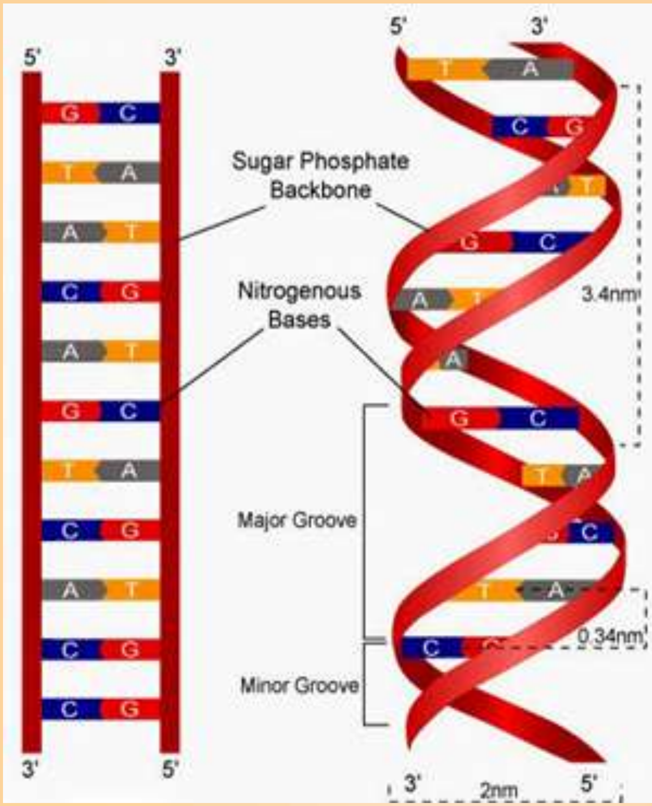
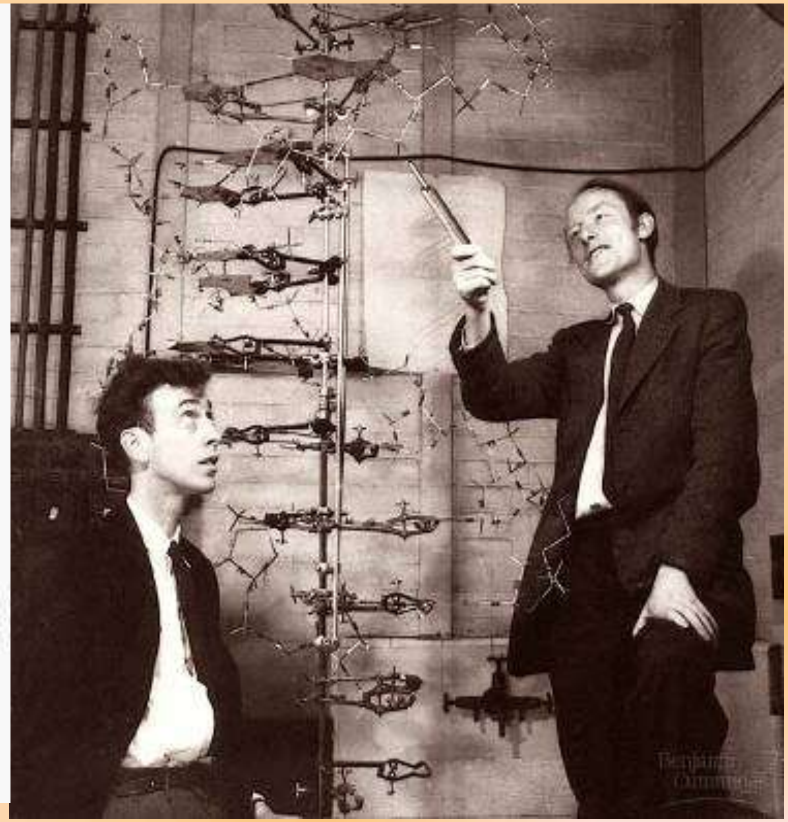
# DNA REPLICATION



***MOOHAMMAD SHAHRUKH***
***M.Sc. Medical Biochemistry***
***(Ph.D. Scholar)***

- Deoxyribonucleic acid (DNA) is a macromolecule that carries genetic information from generation to generation. It is responsible to preserve the identity of the species over millions of years. **DNA may be regarded as** a **reserve bank of genetic information or a** memory bank.

# The central dogma of life

- The **biological information flows from DNA to RNA, and from there to proteins. This is the** central dogma of life.
- **It is ultimately** the DNA that controls every function of the cell through protein synthesis.

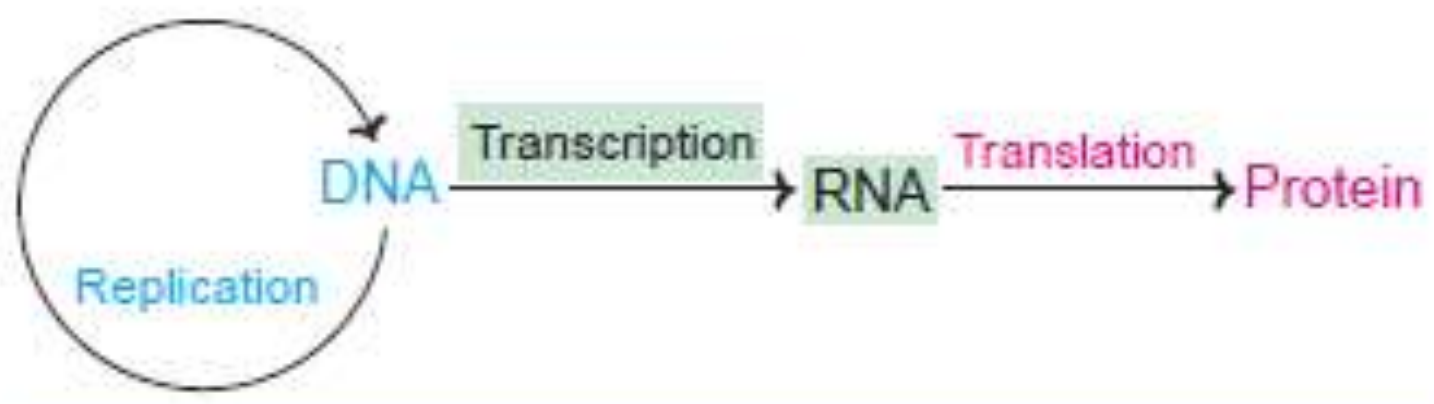



*Fig. 24.1 : The central dogma of life.*

# REPLICATION OF DNA

- DNA is the genetic material. When the cell divides, the daughter cells receive an identical copy of genetic information from the parent cell.
- Replication is a **process in which DNA copies itself to produce identical daughter molecules of DNA.**
- Synthesis of a new DNA molecule is a complex process involving a series of steps.

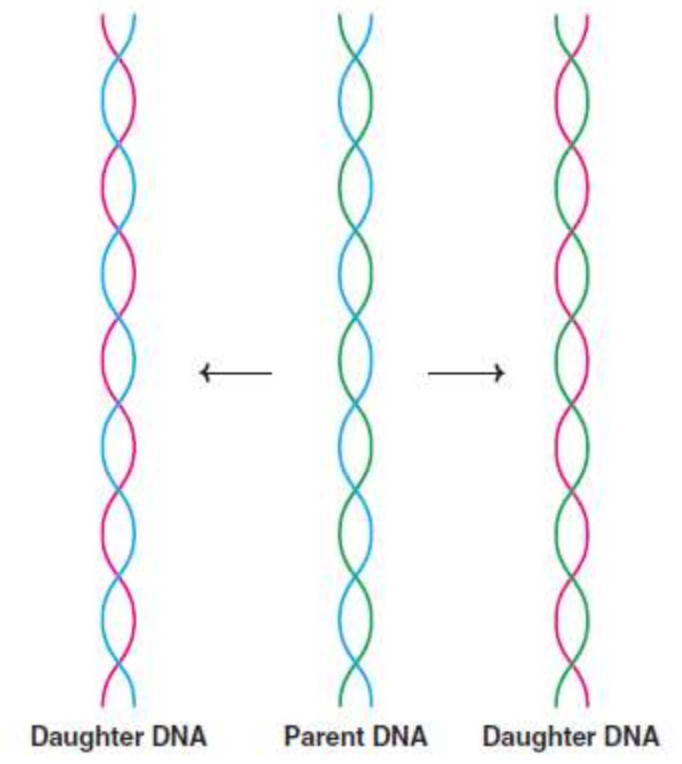



*Fig. 24.2 : DNA replication—semiconservative.*

# TYPES OF REPLICATION

Replication is of **two types:**

**1. Conservative, and**

**2. Semi-conservative.**

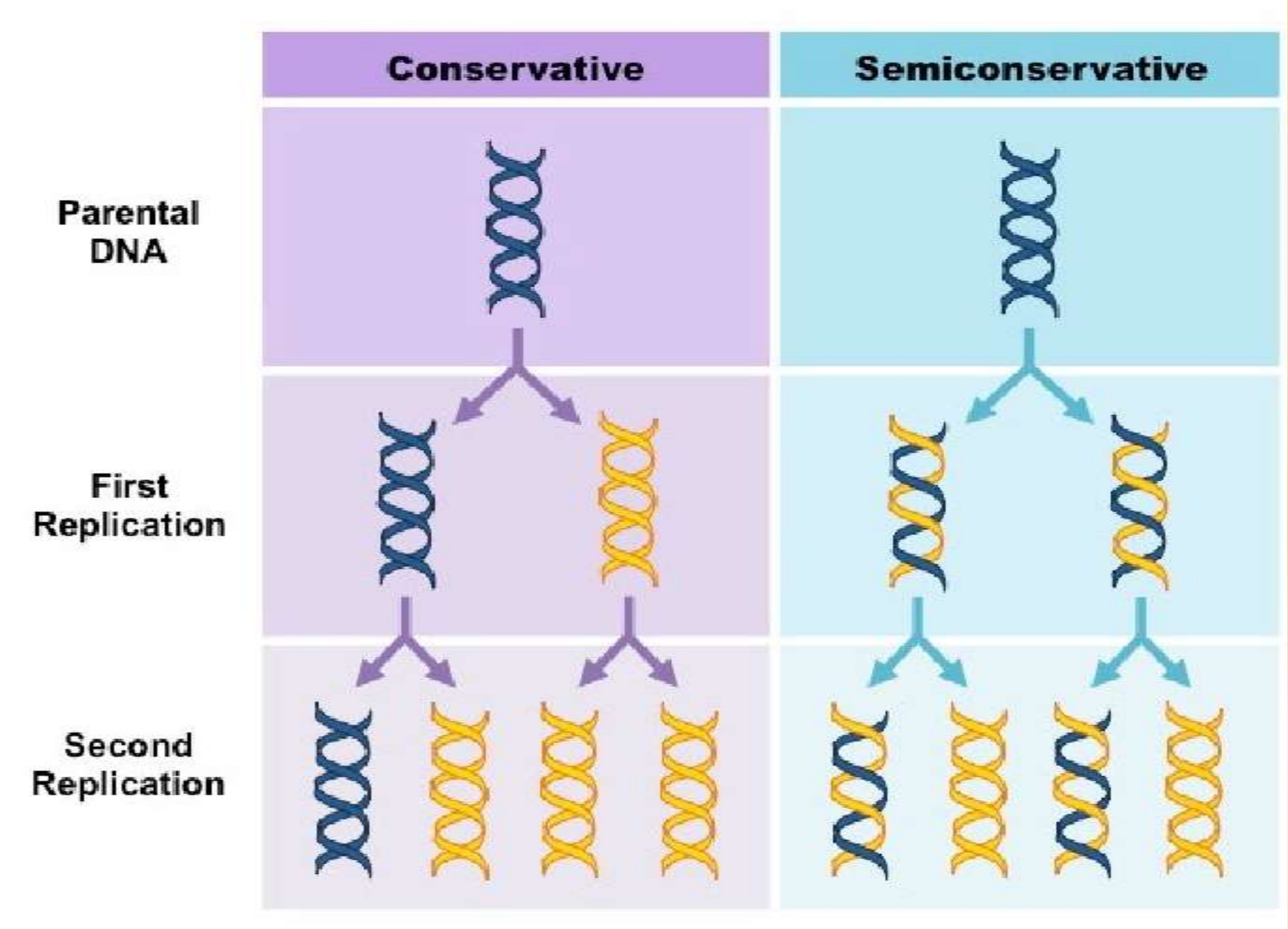
***1. Conservative replication:*** ***In conservative replication*** the parental strands never completely separate. Thus, After one round of replication, one daughter duplex contains only parental strands and the other only daughter strands.

# TYPES OF REPLICATION

2. ***Semiconservative replication:*** ***The process of unwinding*** of the double-helical daughter molecules, each of which is composed of a parental strand and a newly synthesized strand formed from the complementary strand. This is called semiconservative replication.

- **Meselson and Stahl demonstrated experimentally** that the process of replication in *E. coli was semiconservative.*

# TYPES OF REPLICATION



# OTHER TYPES OF REPLICATION

*Theta replication:* ***Most prokaryotic DNA replicates*** when it is in a circular form; such replication is called as Theta replication.

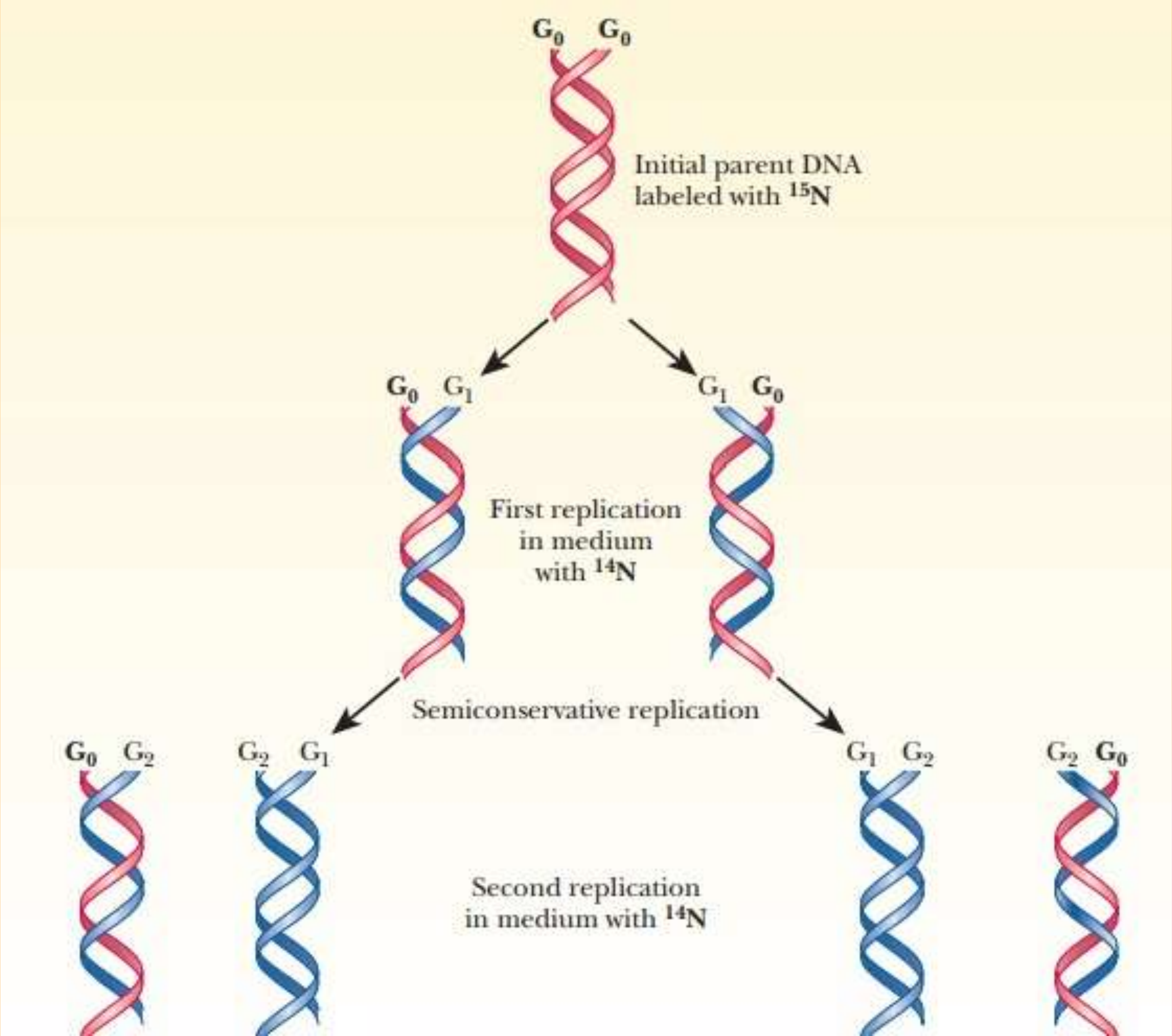
❖The process of DNA replication is complex and involves many cellular functions and several verification procedures to ensure the fidelity in replication.

❖The first important observation was made by **Arthur Kornberg** in *E. coli, an enzyme called DNA polymerase now also* called Kornberg's enzyme.

❖This enzyme has multiple catalytic sites—a complex structure and requires dATP, dCTP, dGTP and dTTP.

# REPLICATION IN PROKARYOTES

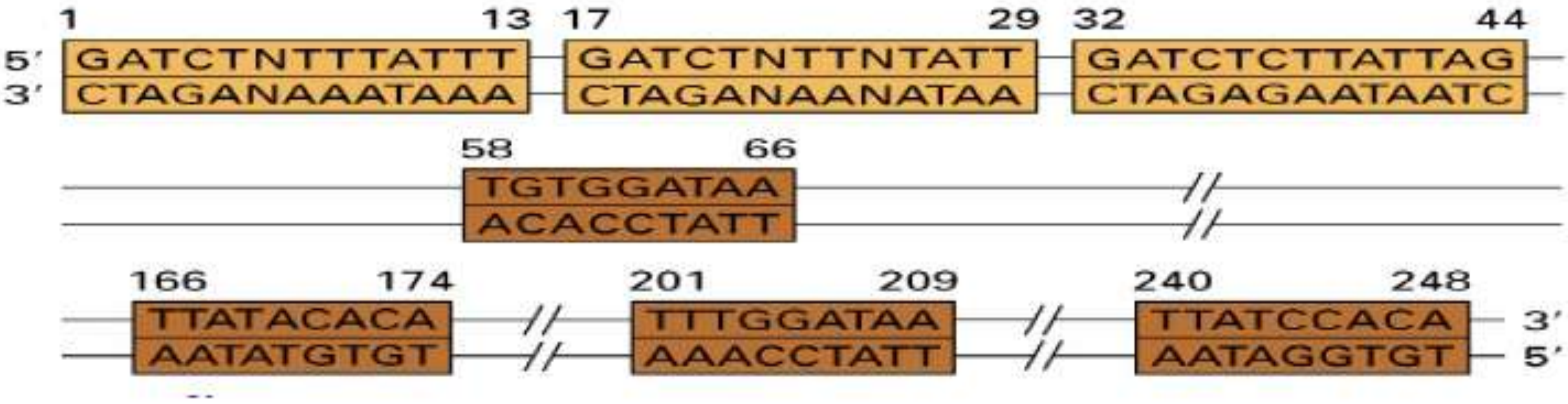
**In prokaryotes, replication is semi-conservative.**



# REPLICATION IN PROKARYOTES

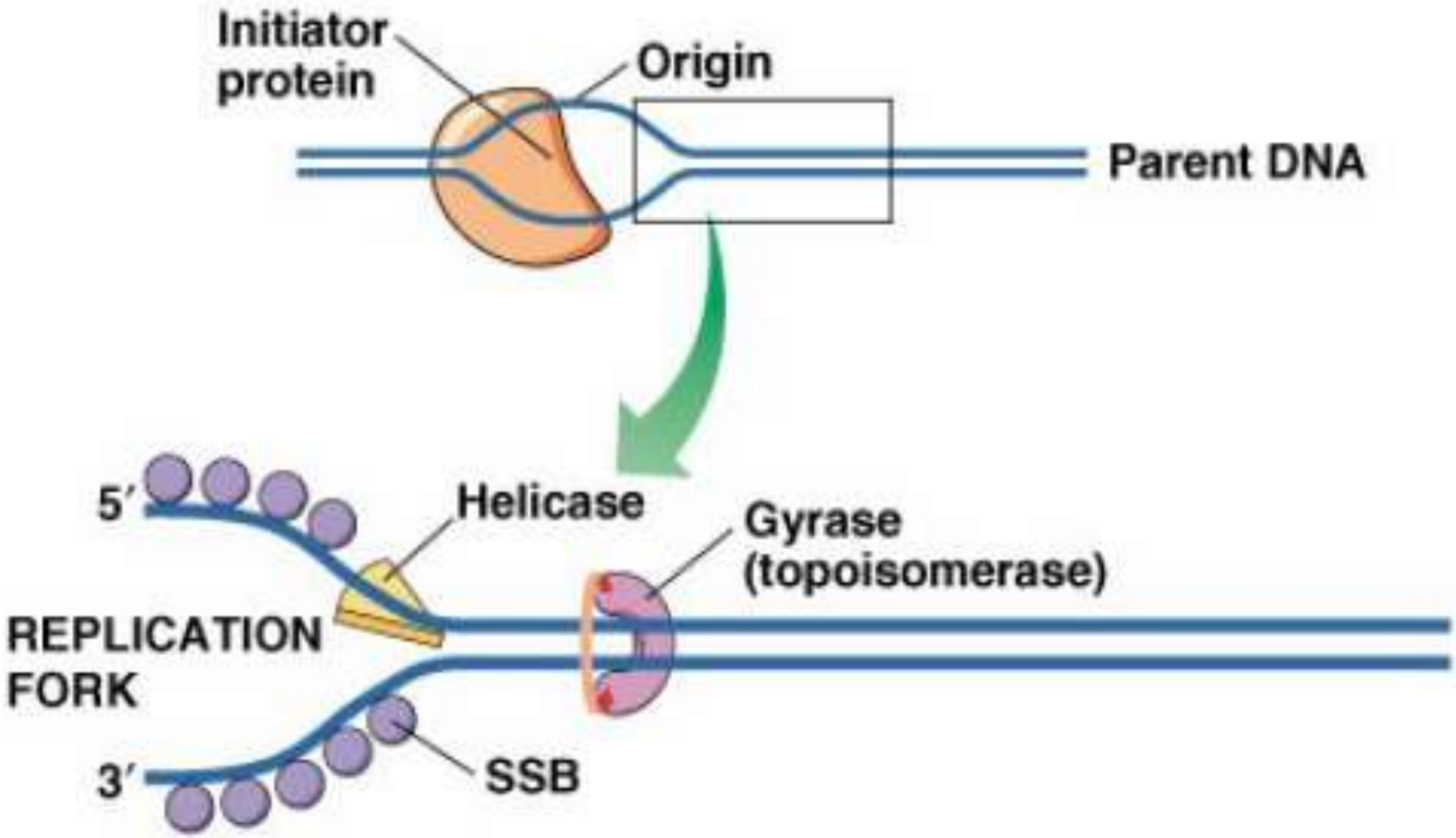
## Origin of Replication:

- The initiation of DNA synthesis occurs at a site called **origin of replication.**
- Strands are separated to allow replication machinery contact with the DNA
  - Many A-T base pairs because easier to break 2 H-bonds that 3 H-bonds



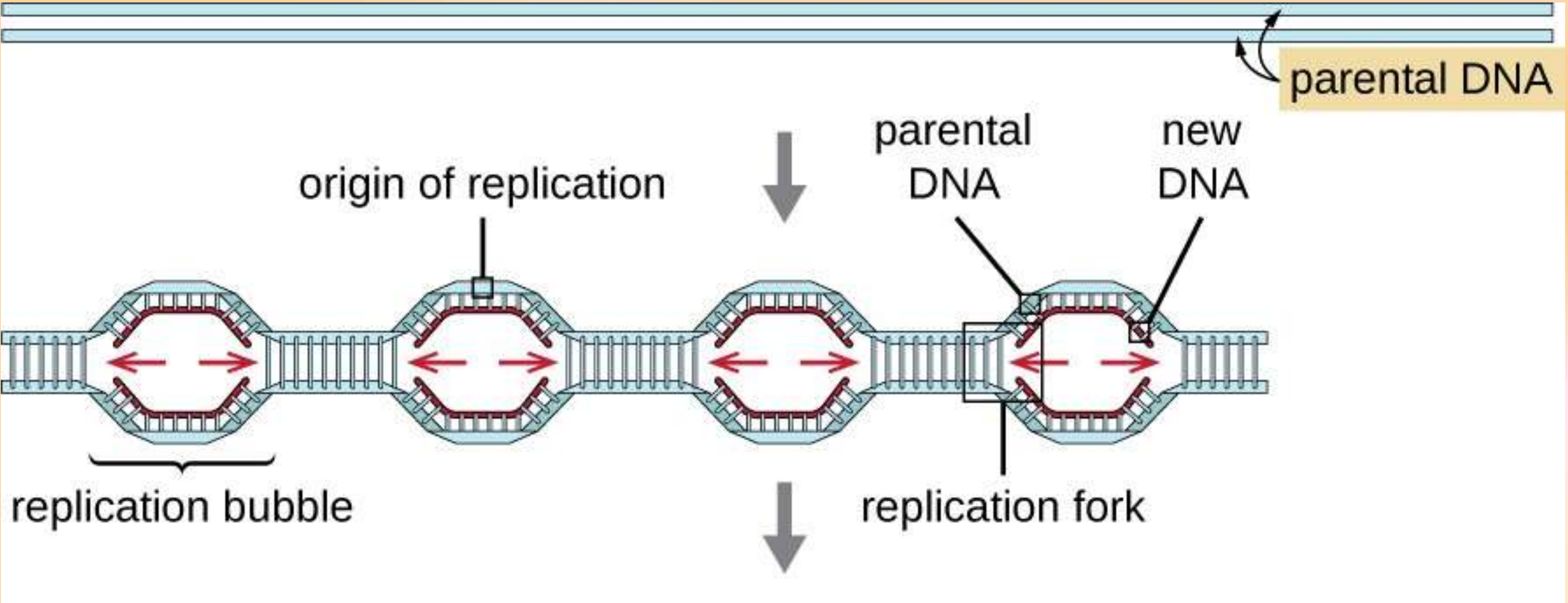
# Initiation

- Initiator protein (DnaA) binds to the origin and separates strands



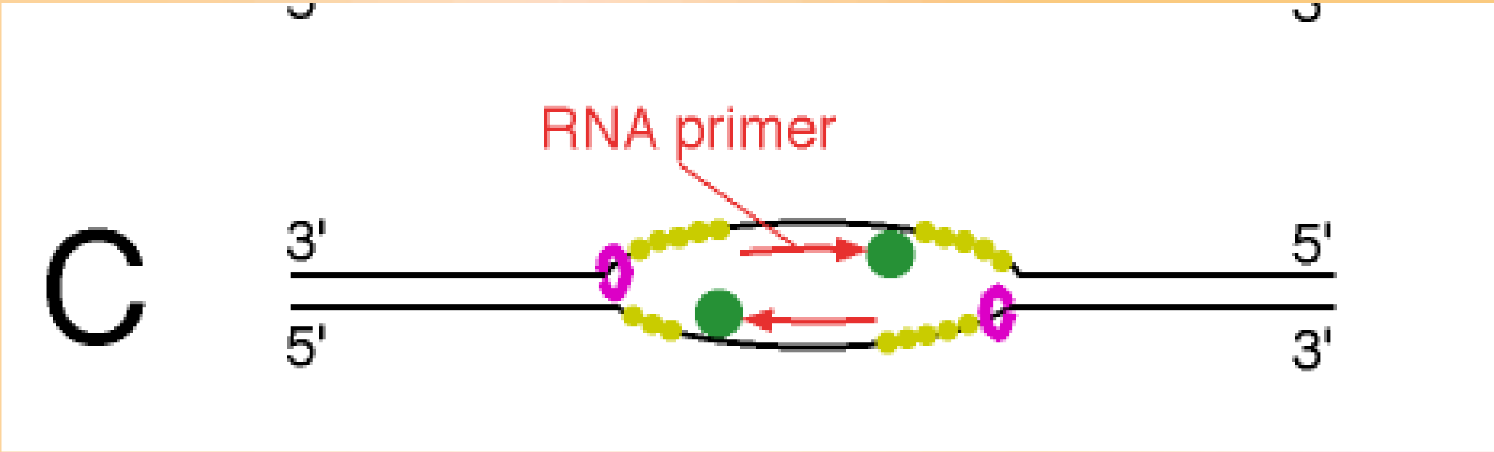
# Replication bubbles

- The two complementary strands of DNA separate at the site of replication to form a bubble.
- Multiple replication bubbles are formed in eukaryotic DNA molecules, which is essential for a rapid replication process



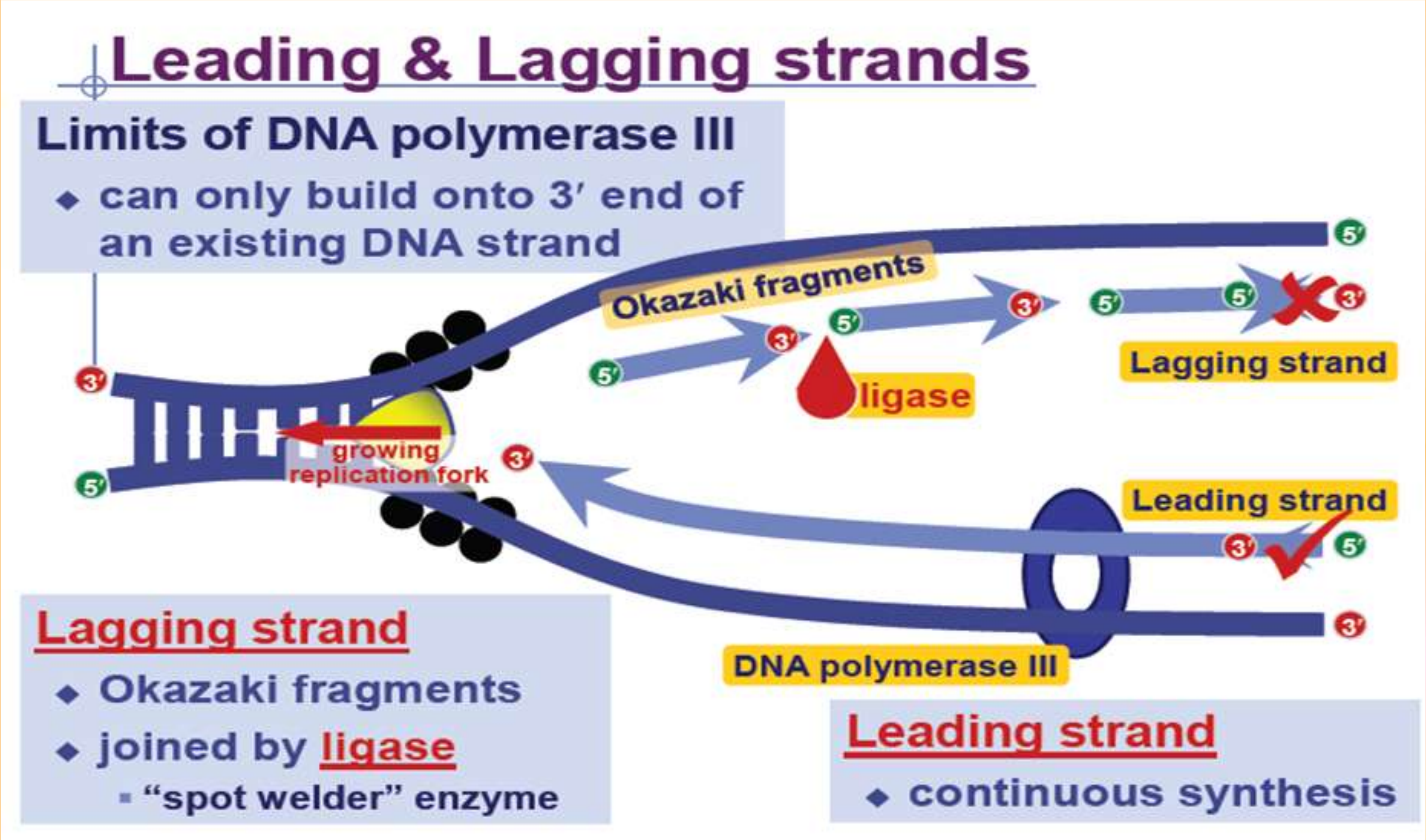
# RNA primer

- A short fragment of RNA (about 5-50 nucleotides, variable with species) is required as a primer.
- The enzyme **primase (a specific RNA** polymerase) in association with single-stranded binding proteins forms a complex called **primosome, and produces RNA primers.**



# DNA synthesis is semidiscontinuous and bidirectional

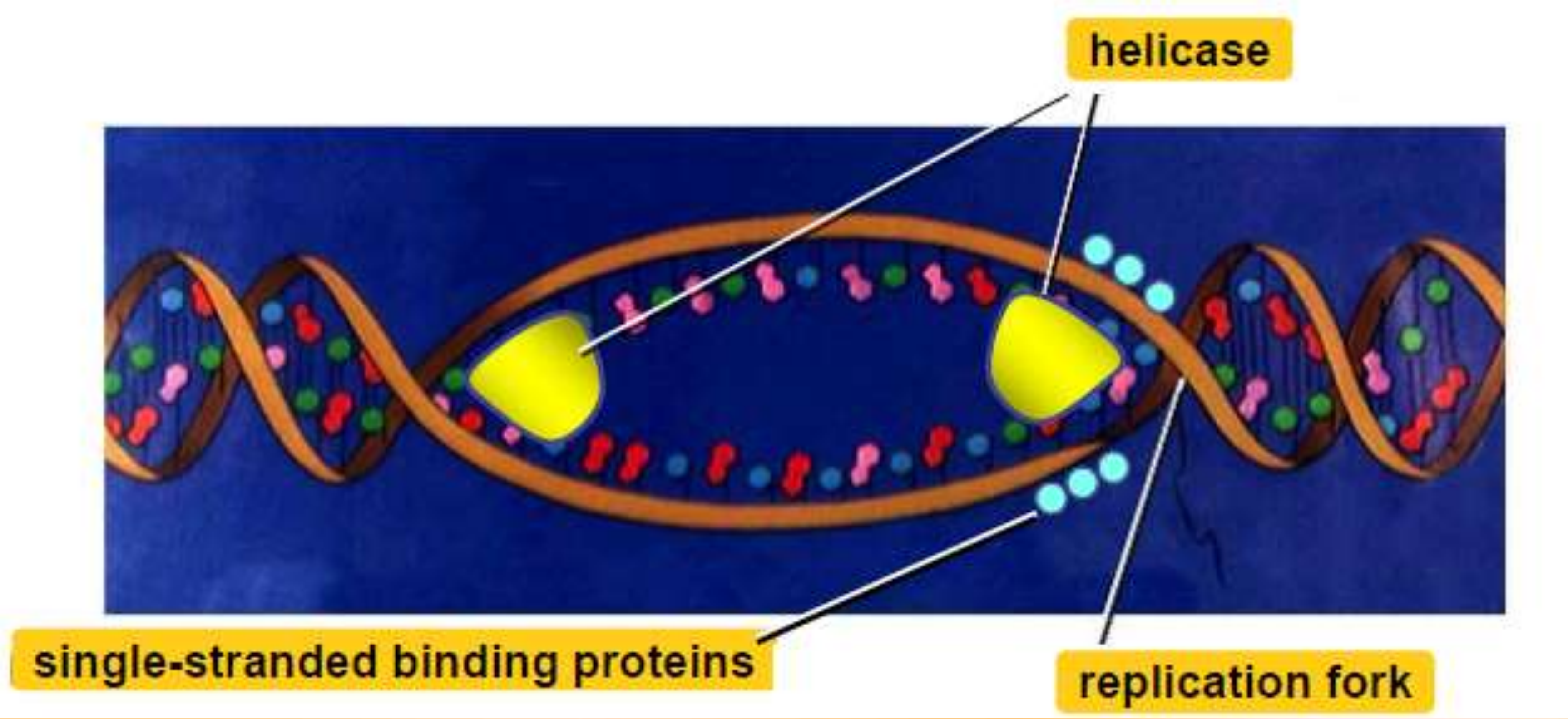
- The replication of DNA occurs in 5’ to 3’ direction, simultaneously, on both the strands of DNA.



# Replication fork and DNA synthesis

- The separation of the two strands of parent DNA results in the formation of a replication fork.

1. **DNA helicases :** Helicases move along the DNA helix and separate the strands. Their function is comparable with a **zip opener. Helicases are** dependent on ATP for energy supply.



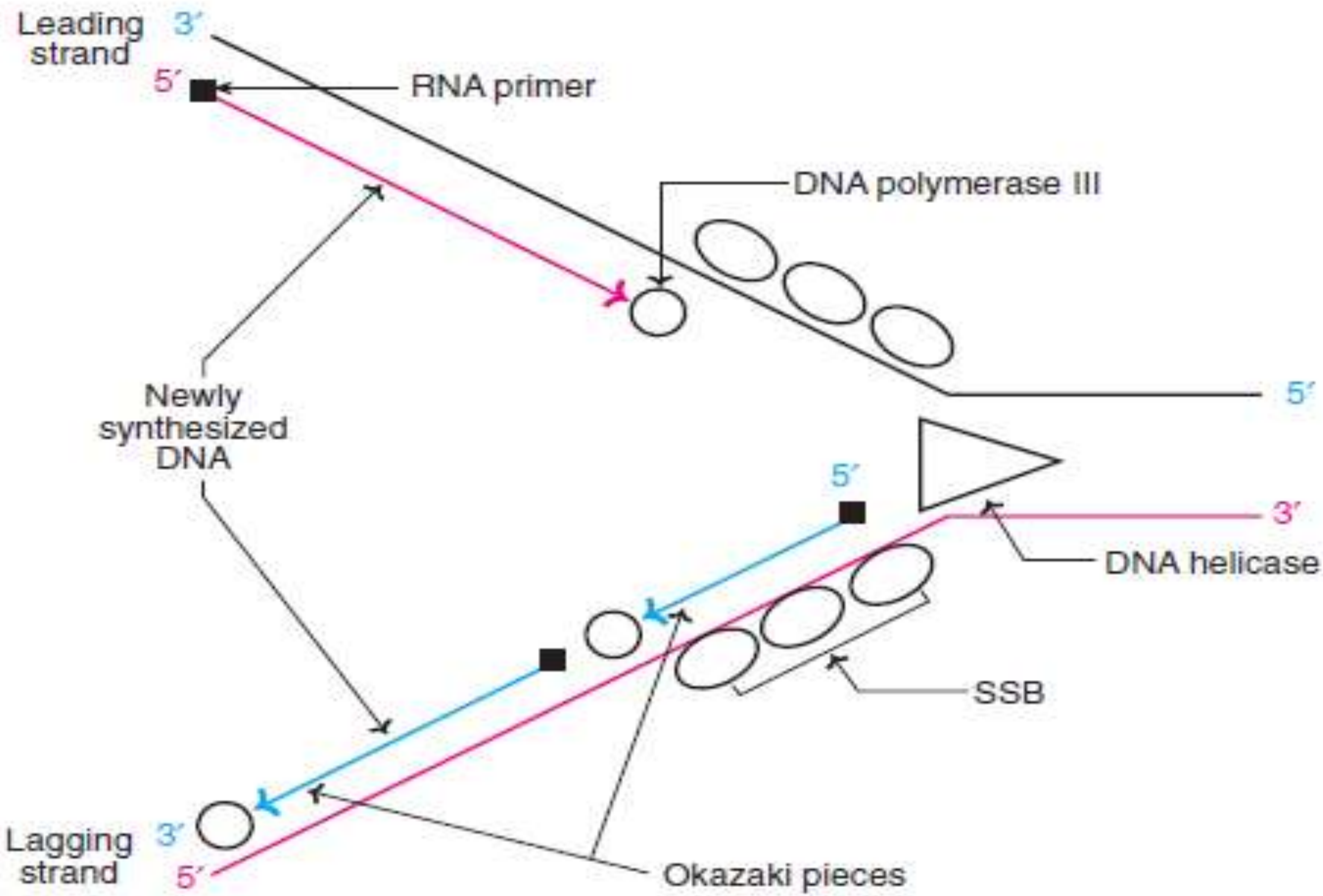
# Replication fork and DNA synthesis

**2. Single-stranded DNA binding (SSB) proteins :** These are also known as DNA helix-destabilizing proteins. SSB proteins bind only to single-stranded DNA (separated by helicases), keep the two strands separate and provide the template for new DNA synthesis.

**3. DNA polymerase III:** The synthesis of a new DNA strand, catalysed by DNA polymerase III, occurs in 5'→3' direction. This is antiparallel to the parent template DNA strand. The presence of all the four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP and dTTP) is an essential prerequisite for replication to take place.

# Replication fork and DNA synthesis

- **Okazaki pieces : The small fragments of the discontinuously synthesized DNA are called** Okazaki pieces. These are produced on the lagging strand of the parent DNA. Okazaki pieces are later joined to form a continuous strand of DNA. DNA polymerase I and DNA ligase are responsible for this process.




**Replication fork**

# Replication fork and DNA synthesis

- **Proof-reading function of DNA polymerase III:** DNA polymerase III also has a **proof-reading activity. It checks the** incoming nucleotides and allows only the correctly matched bases (i.e. complementary bases) to be added to the growing DNA strand.

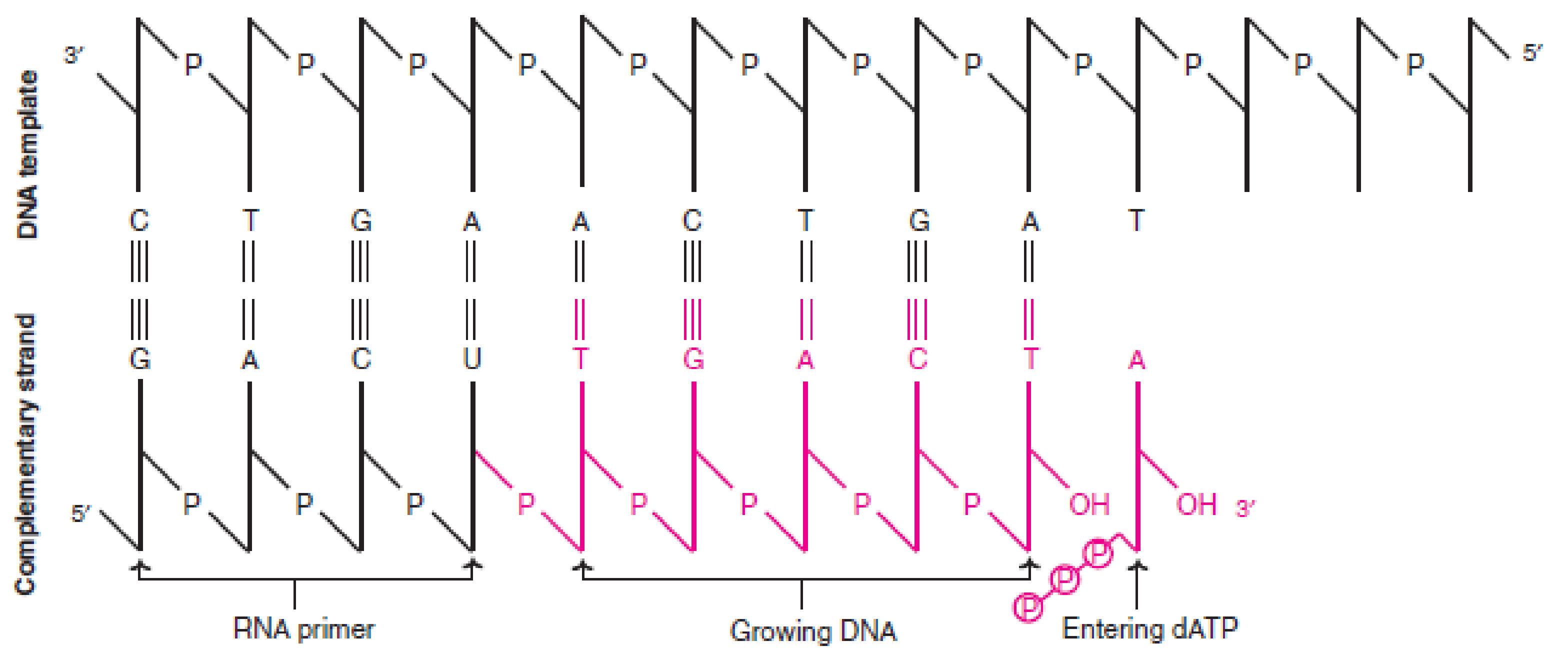



*Fig. 24.5 : DNA replication with a growing complementary strand.*

# Replication fork and DNA synthesis

- **Replacement of RNA primer by DNA:** DNA polymerase I comes into picture. It removes the RNA primer and takes its position. DNA polymerase I catalyses the synthesis (5’→3’ direction) of a fragment of DNA that replaces RNA primer

The enzyme **DNA ligase** **catalyses the** formation of a phosphodiester linkage betweenthe DNA synthesized by DNA polymerase III and the small fragments of DNA produced by DNA polymerase I. This process—nick sealing-requires energy, provided by the breakdown of ATP to AMP and PPi.

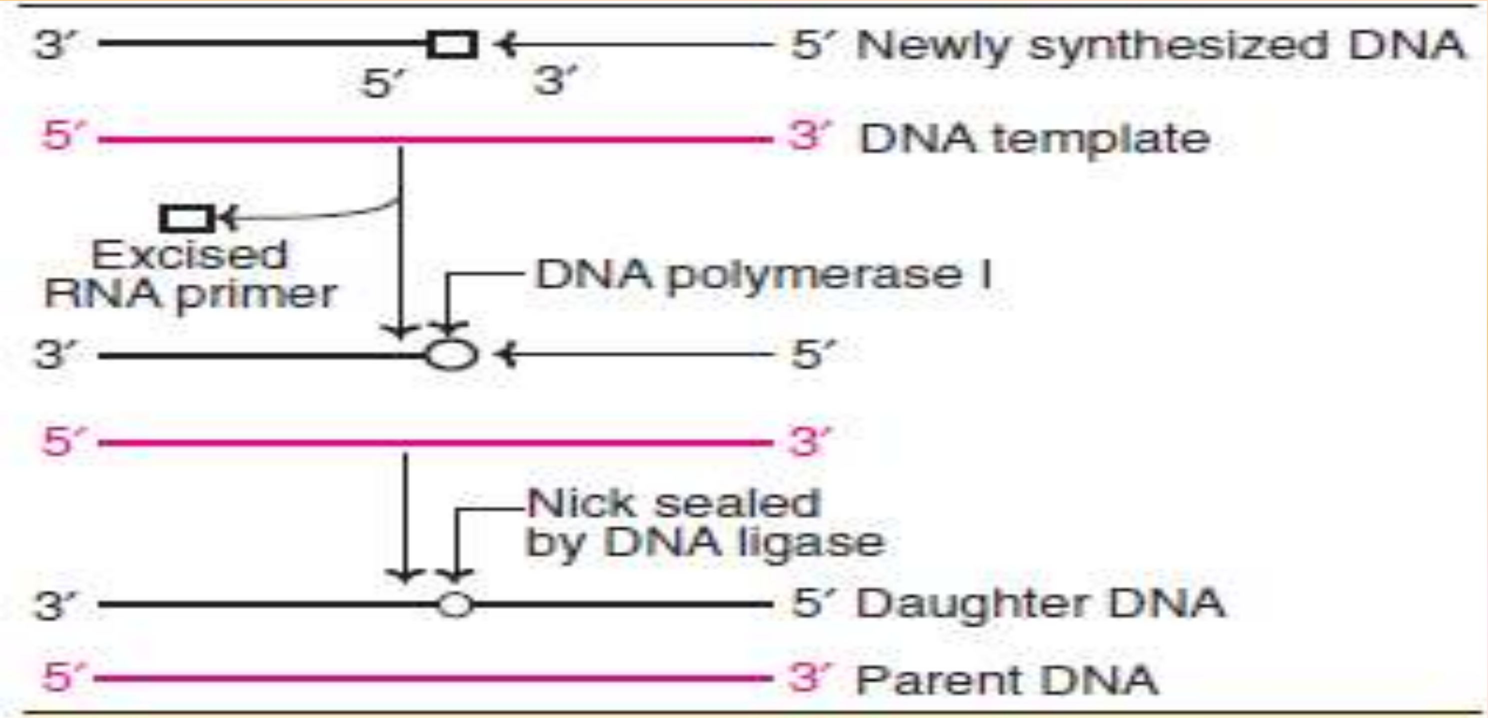
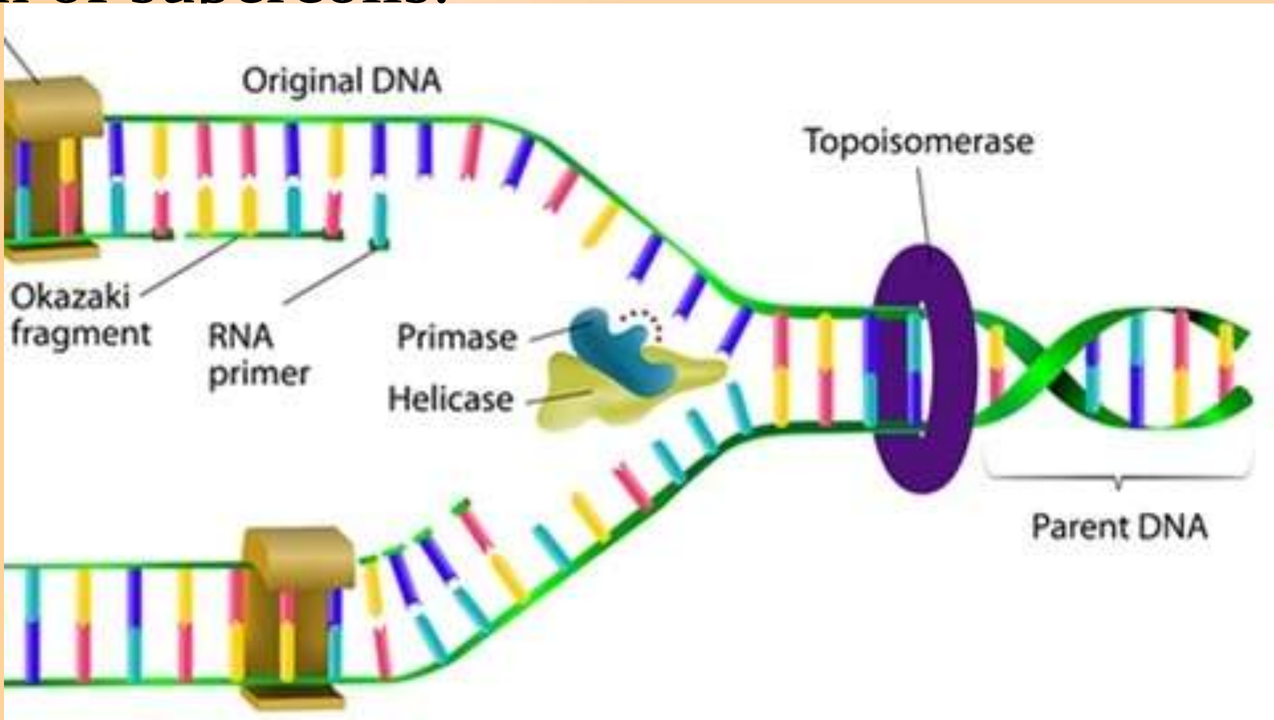



***Fig. 24.6 :** Overview of the action of DNA polymerase I and DNA ligase.*

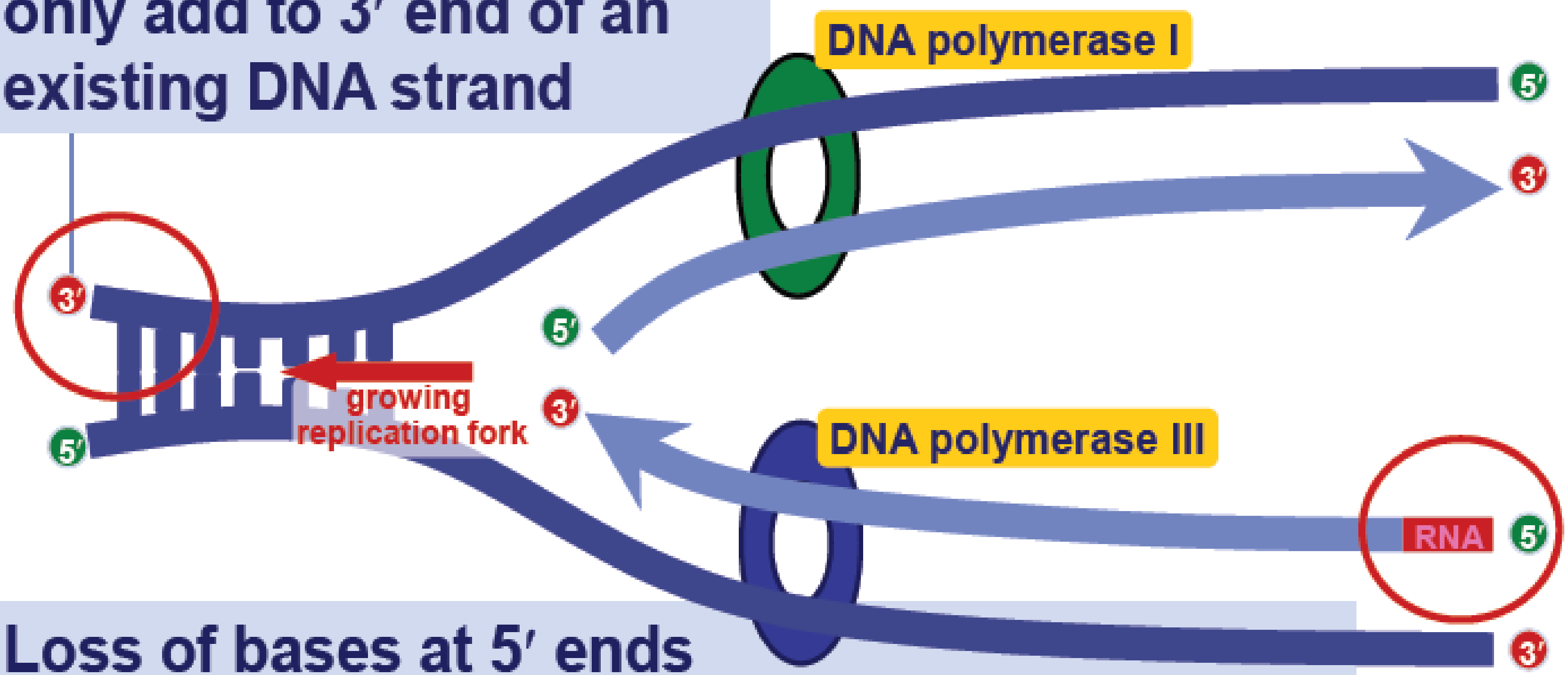
# DNA Topoisomerases:

- Type I DNA topoisomerase cuts the single DNA strand (nuclease activity) to overcome the problem of supercoils and then reseals the strand (ligase activity).
- Type II DNA topoisomerase (also known as DNA gyrase) cuts both strands and reseals them to overcome the problem of supercoils.



# Chromosome shortning:

All DNA polymerases can only add to 3′ end of an existing DNA strand



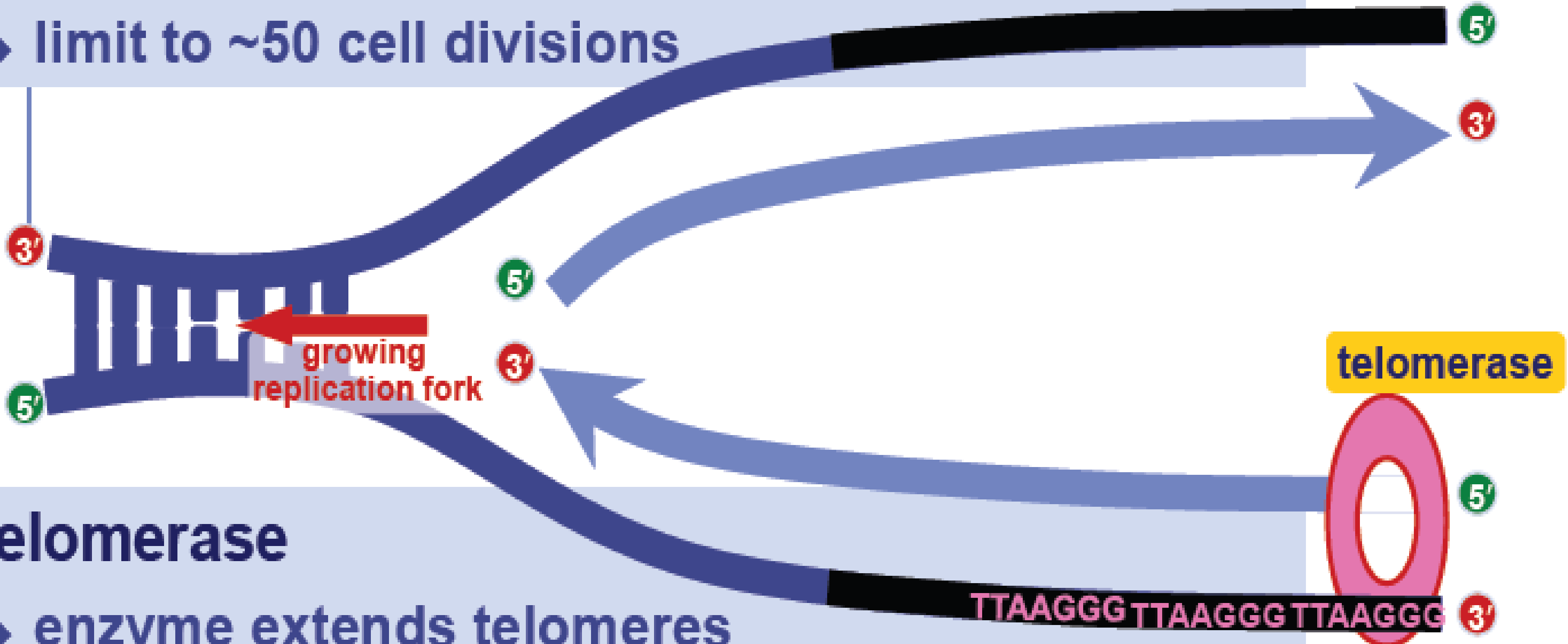

Loss of bases at 5′ ends in every replication

- chromosomes get shorter with each replication
- limit to number of cell divisions?

# Telomeres

**Repeating, non-coding sequences at the end of chromosomes = protective cap**

- limit to ~50 cell divisions




## Telomerase

- enzyme extends telomeres
- can add DNA bases at 5′ end
- different level of activity in different cells
  - high in stem cells & cancers -- Why?

# Summary of prokaryotes replication

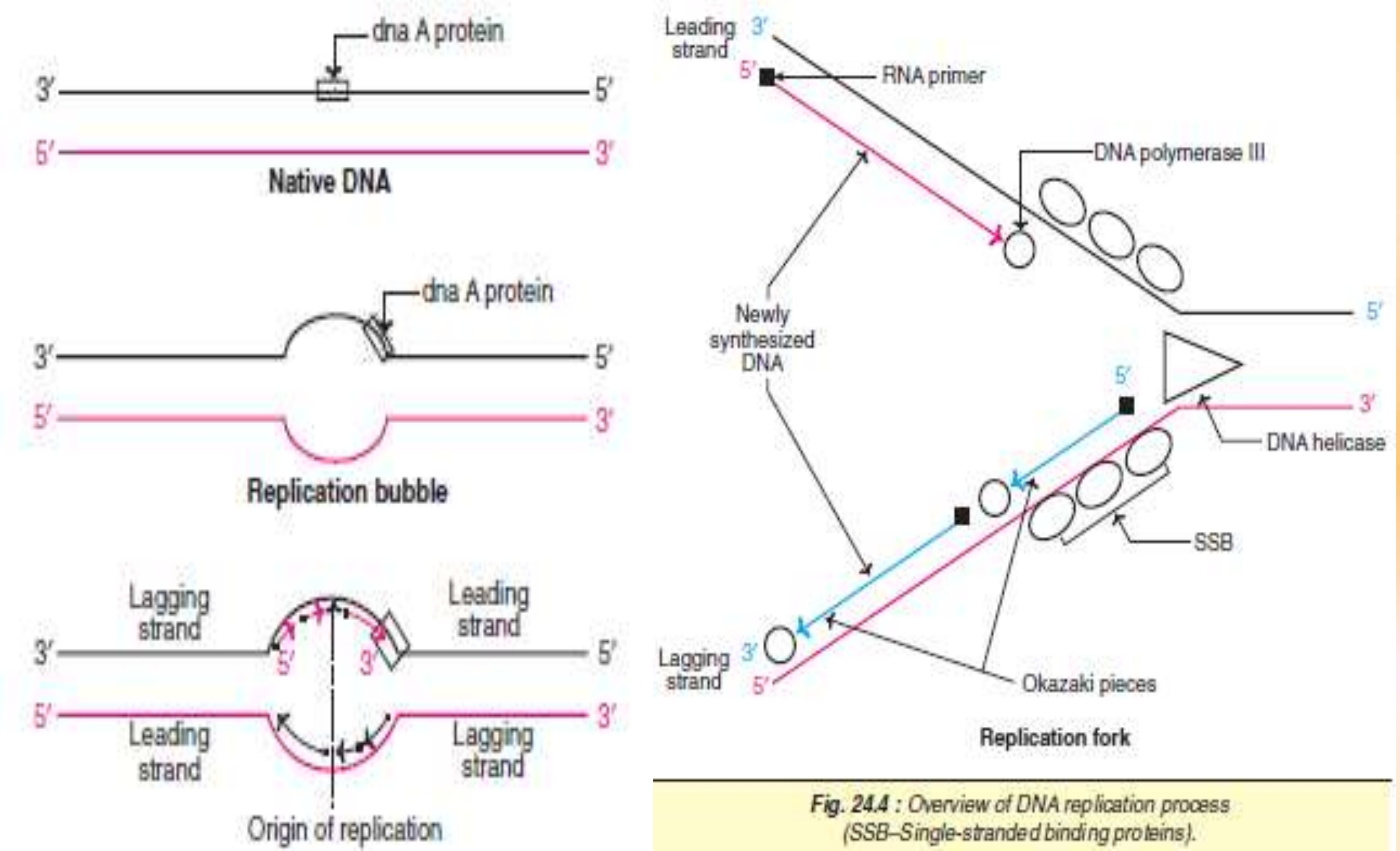



*Fig. 24.4 : Overview of DNA replication process (SSB–Single-stranded binding proteins).*


Thank
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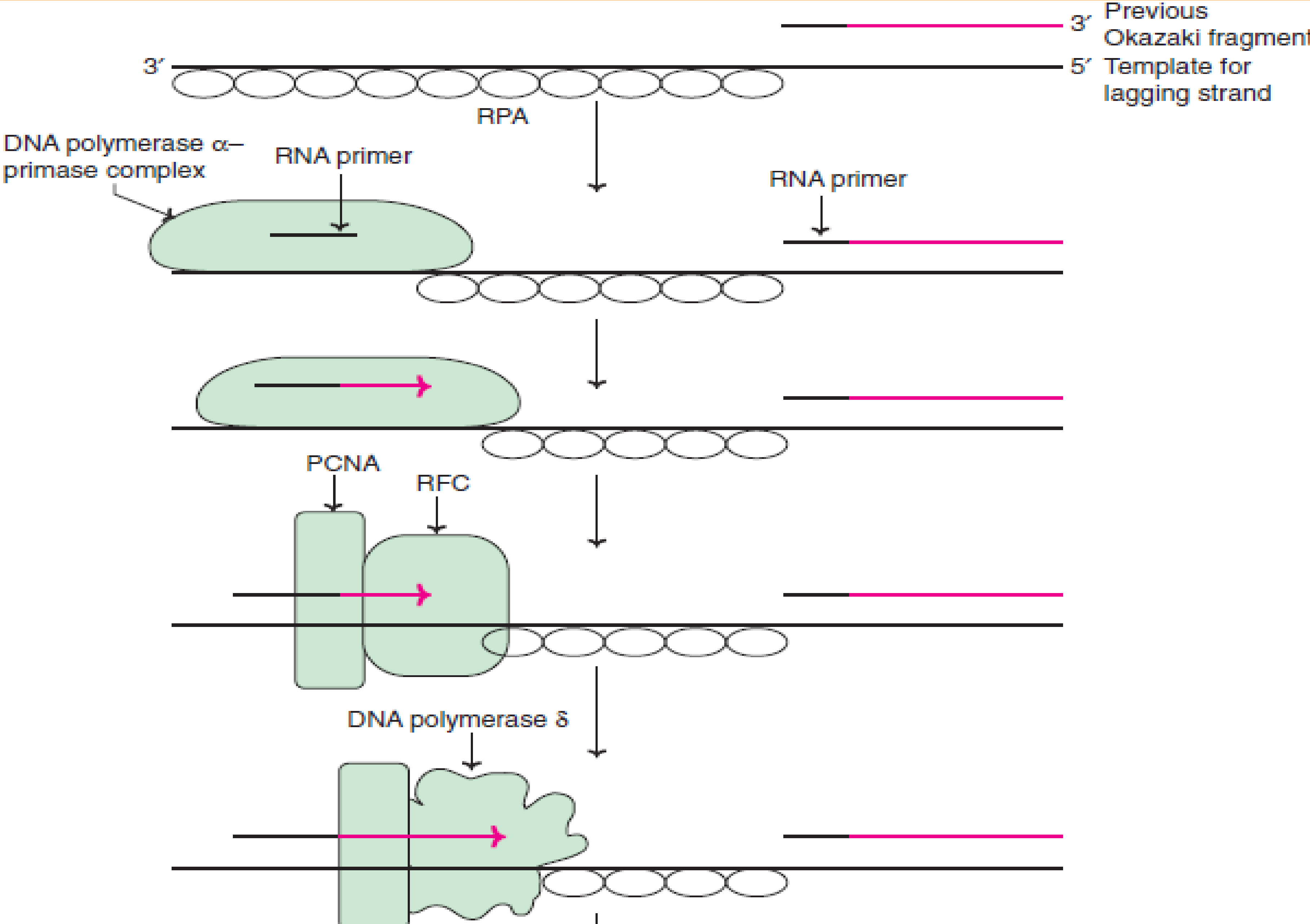
# REPLICATION IN EUKARYOTES

- Replication of DNA in eukaryotes closely resembles that of prokaryotes. Certain differences, however, exist. **Multiple origins of replication is** a characteristic feature of eukaryotic cell. Further, at least **five distinct DNA polymerases are known** in eukaryotes. Greek letters are used to number these enzymes.

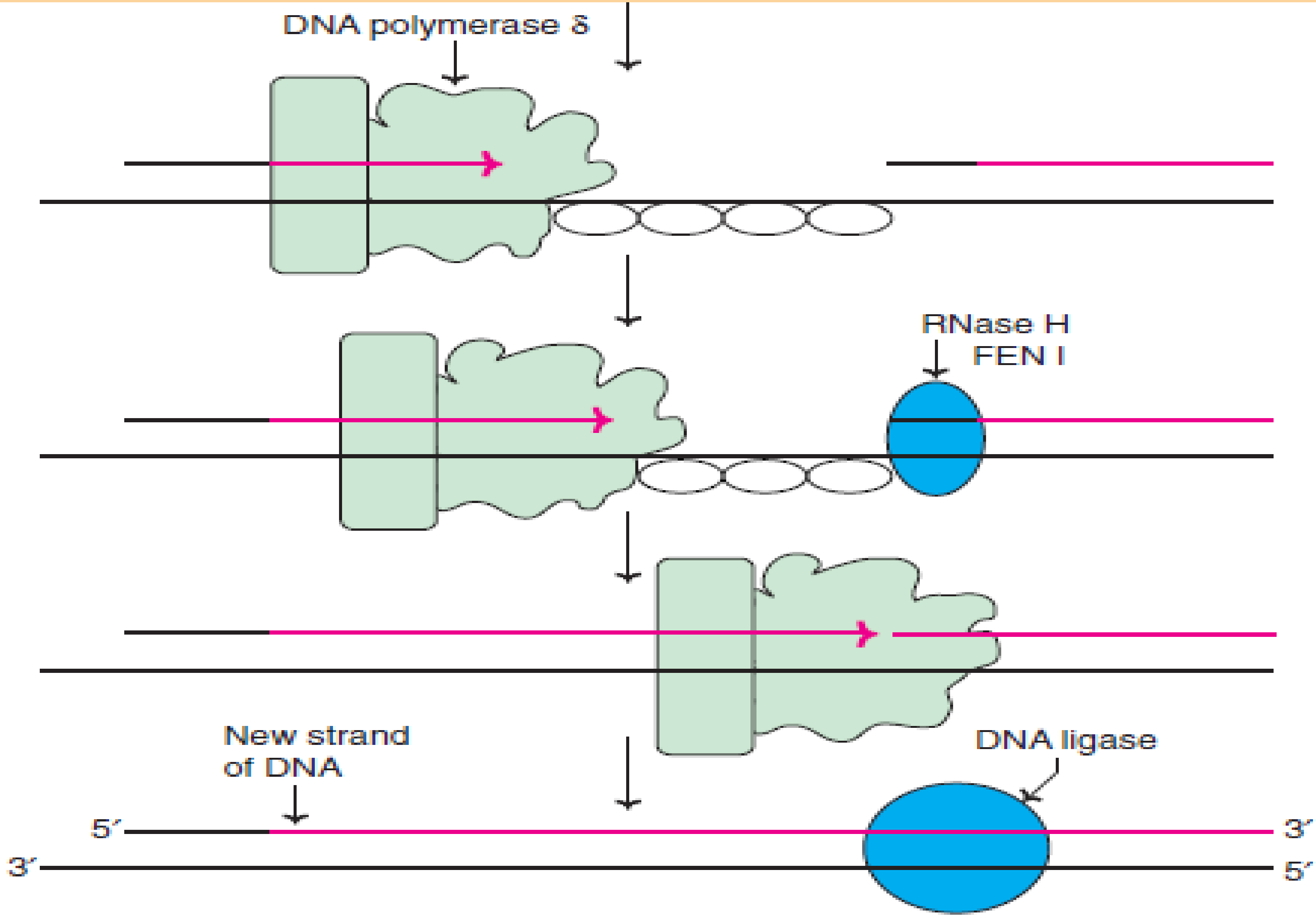
# REPLICATION IN EUKARYOTES

I. **DNA polymerase α is responsible for the** synthesis of RNA primer for both the leading and lagging strands of DNA.
II. **DNA polymerase β is involved in the** repair of DNA. Its function is comparable with DNA polymerase I found in prokaryotes.
III. **DNA polymerase γ participates in the** replication of mitochondrial DNA.
IV. **DNA polymerase δ is responsible for the** replication on the leading strand of DNA. It also possesses proof-reading activity.
V. **DNA polymerase ε is involved in DNA** synthesis on the lagging strand and proof-reading function.

# PROCESS OF REPLICATION IN EUKARYOTES



# PROCESS OF REPLICATION IN EUKARYOTES



# INHIBITORS OF DNA REPLICATION

- Bacteria contain a specific type II topoisomerase namely **gyrase.**
- **This enzyme cuts** and reseals the circular DNA (of bacteria), and thus overcomes the problem of supercoils.
- Bacterial gyrase is inhibited by the antibiotics **ciprofloxacin, novobiocin and nalidixic acid**.

# INHIBITORS OF DNA REPLICATION

- Certain compounds that **inhibit human topoisomerases are used as anticancer agents** e.g. adriamycin, etoposide, doxorubicin.
- The nucleotide analogs that inhibit DNA replication are also used as anticancer drugs e.g. 6-mercaptopurine, 5-fluorouracil.


Thank
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