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# Lecture – 17

## Replication of DNA

by

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# Four requirements for DNA to be genetic material

## ❖ Must carry information

Cracking the genetic code

## ❖ Must replicate

DNA replication

## ❖ Must allow for information to change

Mutation

## ❖ Must govern the expression of the phenotype

Gene function



# DNA REPLICATION

The process by which a DNA molecule makes its identical copies is called DNA replication.

## Replication Facts

- Basis for inheritance
- DNA has to be copied **before a cell divides**
- DNA is copied during the **S** or synthesis phase of **interphase**
- New cells will need **identical** DNA strands



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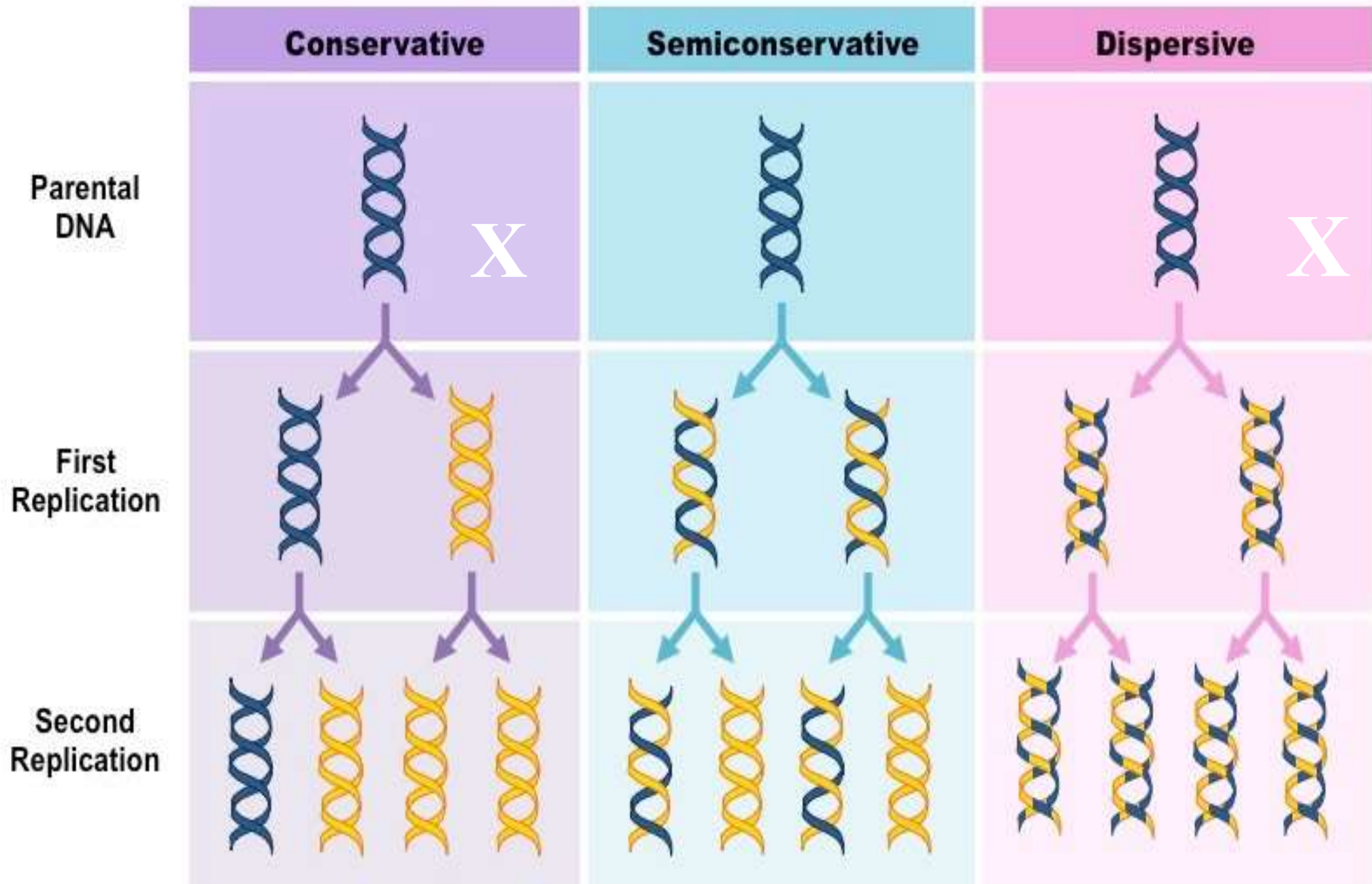
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# Basic rules of replication

- A. Semi-conservative**
- B. Starts at the 'origin'**
- C. Synthesis always in the 5'-3' direction**
- D. Can be uni or bidirectional**
- E. Semi-discontinuous**
- F. RNA primers required**



# Modes of DNA Replication:





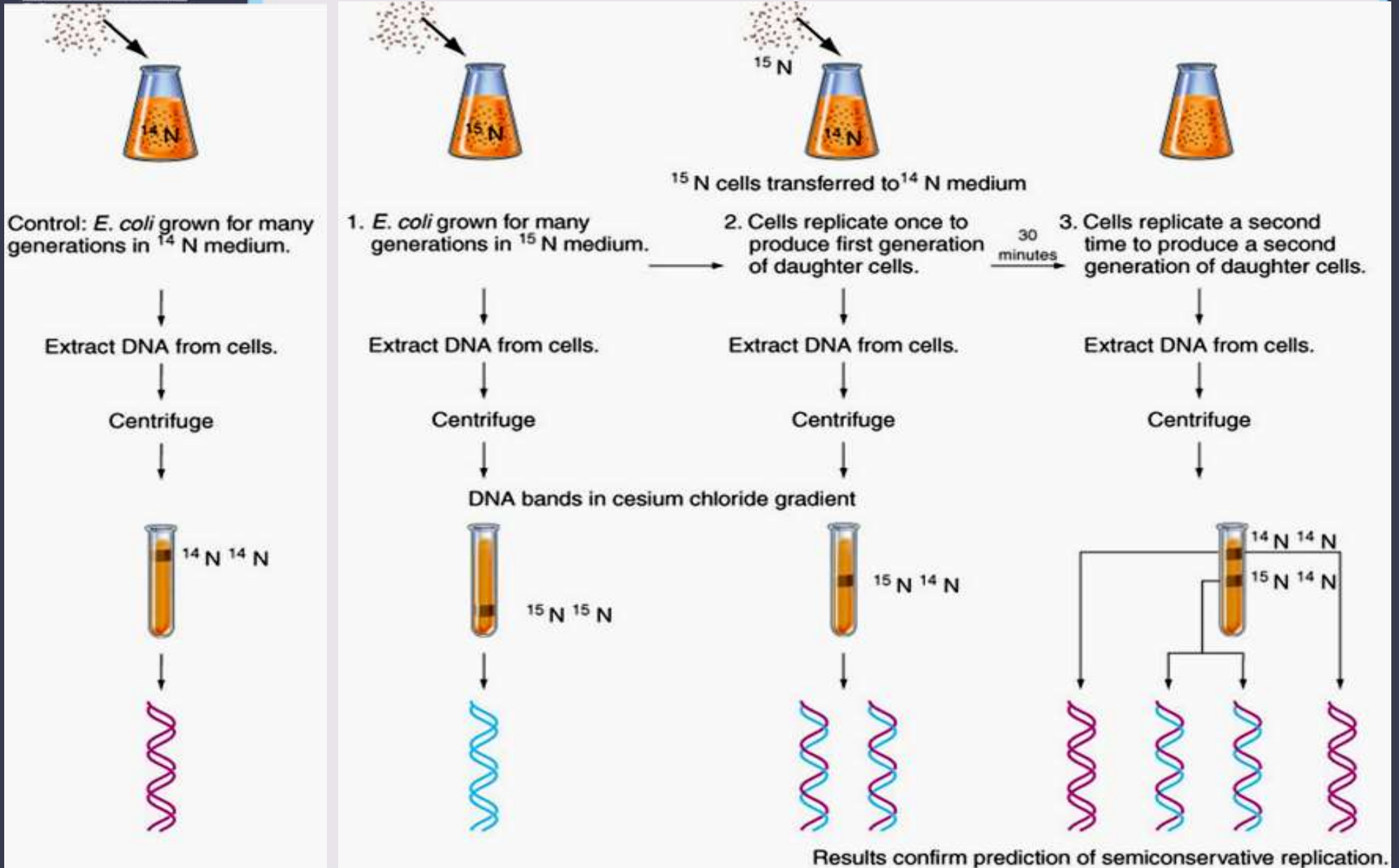
# Evidence for Semi conservative replication

- ❖ **Matthew Meselson and Franklin Stahl in 1958 on *E. coli*** provided a conclusive proof that replication of DNA is by semi conservative model.
- ❖ Meselson and Stahl labelled DNA of *E. coli* bacteria with **heavy nitrogen** i.e.  $^{15}\text{N}$  by growing them on a medium containing  $^{15}\text{N}$  for 14 generations to replace the normal nitrogen ( $^{14}\text{N}$ ).
- ❖ The density of normal and heavy nitrogen differs. The  $^{14}\text{N}$  is lighter ( $1.710 \text{ g/cm}^3$ ) than  $^{15}\text{N}$  ( $1.724 \text{ g/cm}^3$ ).
- ❖ to detect such minute differences in density through **density gradient centrifugation** (used **6M CsCl** a heavy salt solution)
- ❖ Distinct bands are formed in the centrifuge tube for different density DNA. DNA extracts of *E. coli* with  $^{15}\text{N}$  gave a characteristic heavy band at one end of a tube that had been centrifuged at a high speed in an ultra-centrifuge.



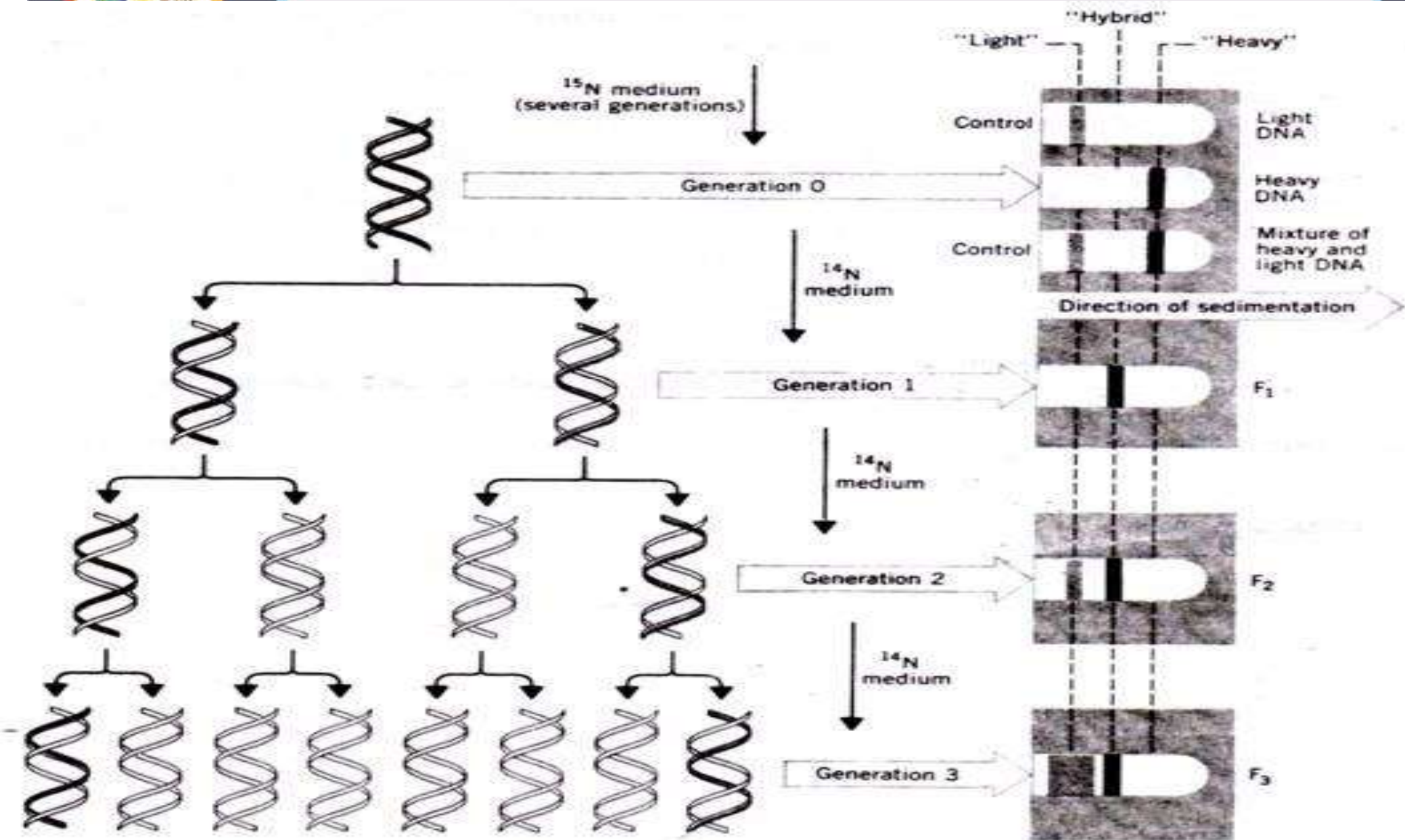
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# How do we know that DNA replication is semiconservative? Meselson-Stahl experiments






## Evidence for Semi conservative replication



**FIGURE 21-3** Results of the Meselson-Stahl experiments (right) and their interpretation (left) (see text for explanation). (From E. J. Gardner and D. P. Snustad, *Principles of Genetics* (7th ed.), John Wiley & Sons, 1984, p. 99.)



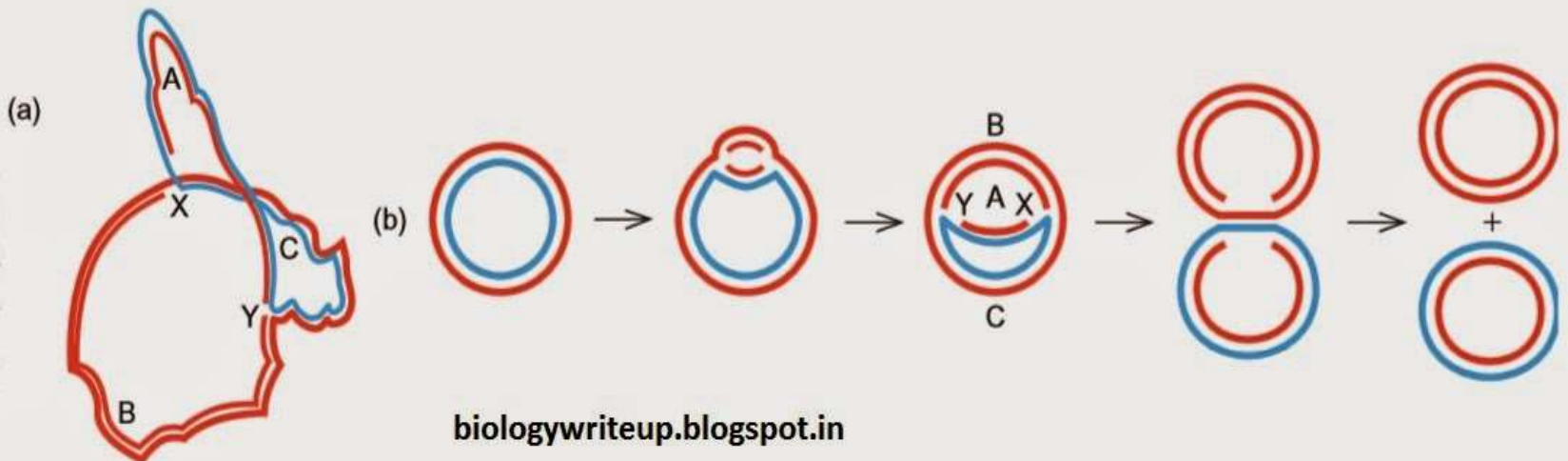
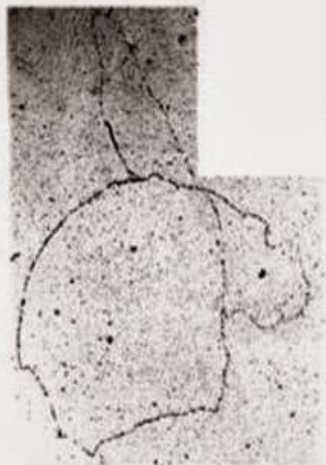
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- ❖ These labelled cells were then grown on a normal unlabelled media containing  $^{14}\text{N}$  for one generation. DNA was again extracted and processed, and it was found to consist of a hybrid DNA containing both  $^{14}\text{N}$  and  $^{15}\text{N}$  at the same time
- ❖ The next generation of growth on unlabelled DNA was found to be in amounts equal to the partially labelled hybrid DNA. Additional generation of growth on unlabelled media gave a relative increase in the amount of unlabelled DNA. After two generations, half the DNA was with intermediate density and half with light bands which further confirm semi-conservative mode of DNA replication. After third generation,  $\frac{3}{4}$  DNA was found with  $^{14}\text{N}$  and  $\frac{1}{4}$  with hybrid nitrogen ( $^{14}\text{N}+^{15}\text{N}$ ).
- ❖ When the hybrid DNA was denatured by heating up to  $100^\circ\text{C}$  it was found to produce two separate single strands and in the ultracentrifuge density gradient, it was observed to form two separate bands; one band containing  $^{15}\text{N}$  and the other  $^{14}\text{N}$ . Thus, it was concluded that DNA replication was by semi-conservative mode.



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# Autoradiography of replicating bacterial chromosome

Cairns (1963) provided evidence for this in his experiments with radio-active labelled ( $^3\text{H}$ - Thymidine) *E. coli* chromosomes. The *E. coli* chromosome is a double stranded circular chromosome. It was shown that the two circular component strands separate during replication with each strand duplicating individually producing a  $\theta$  shaped structures during replication. This indicates that unwinding and replication proceed simultaneously.



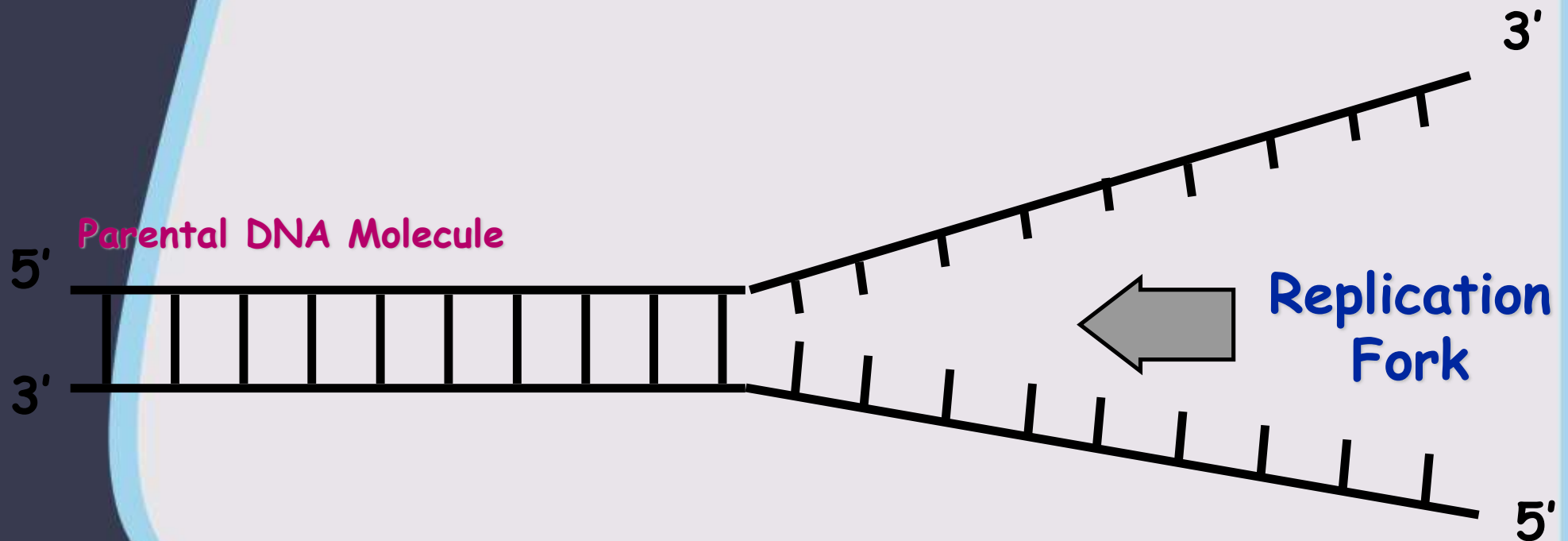


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## B) Starts at origin

Initiator proteins identify specific base sequences on DNA called **sites of origin**

- Two strands open forming **Replication Forks** (Y-shaped region)
- **New strands grow at the forks**

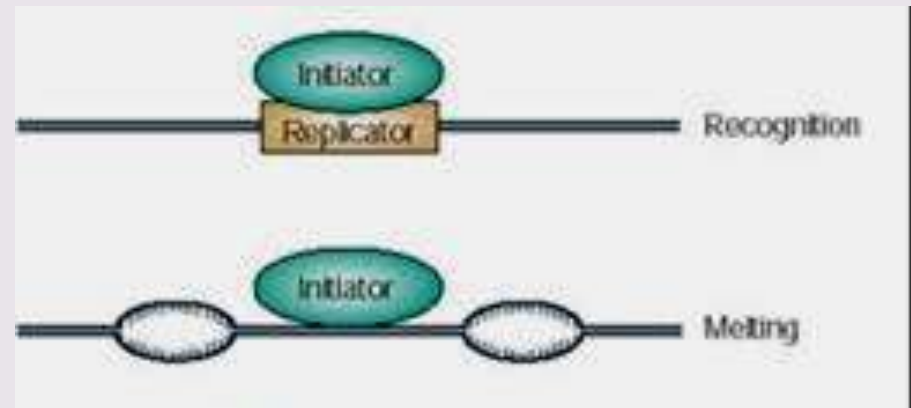
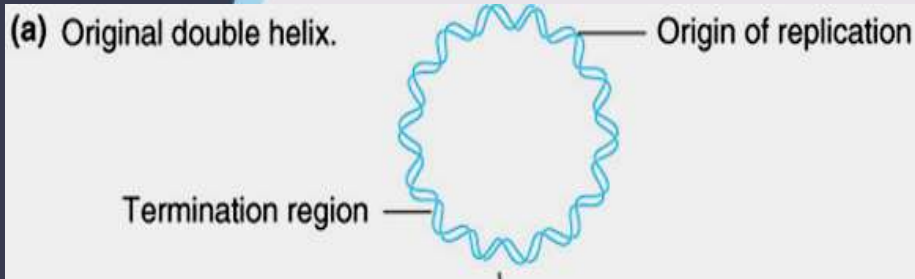


As the 2 DNA strands open at the origin, Replication Bubbles form

Prokaryotes - single origin site E.g *E.coli* - *oriC*

Eukaryotes - multiple sites of origin (replicator)

E.g. yeast - ARS (autonomously replicating sequences)

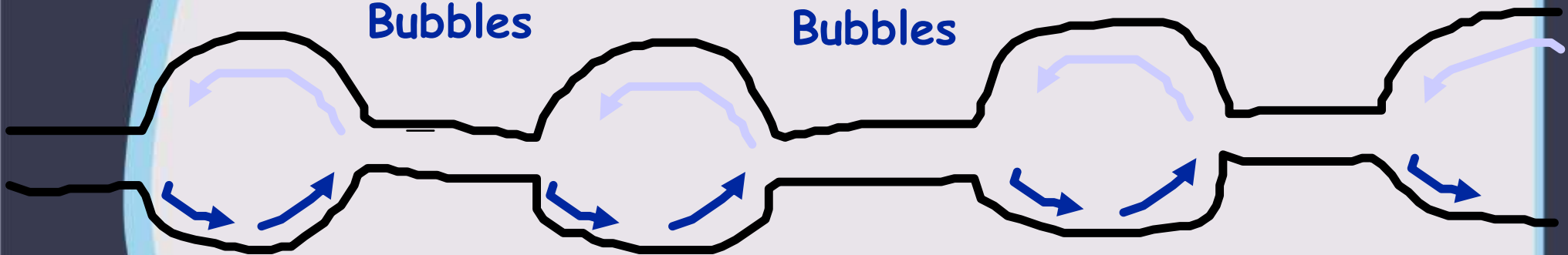


Prokaryotes

Eukaryotes

Bubbles

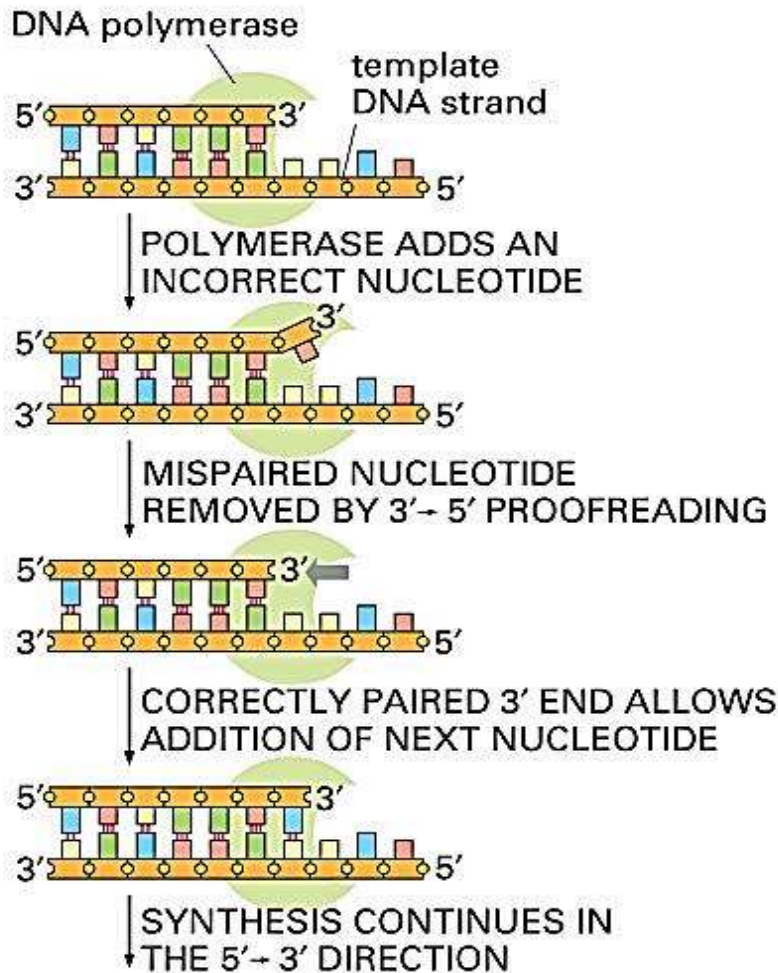
Bubbles



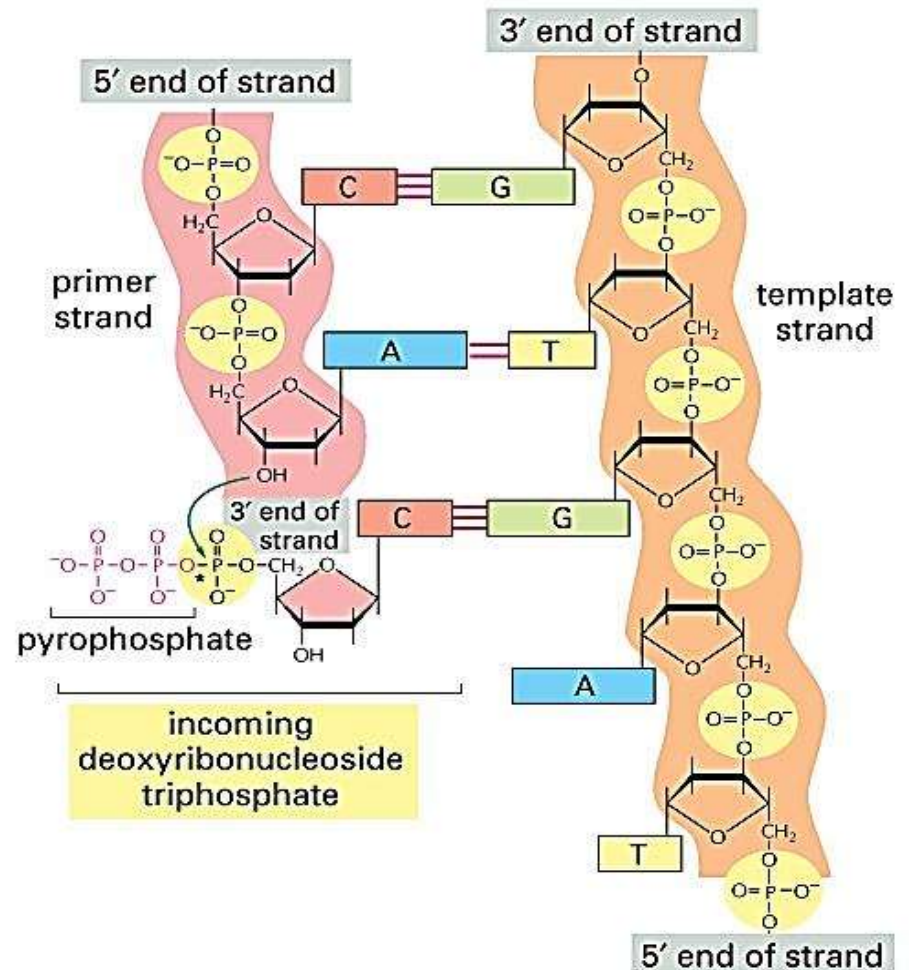
# In what direction does DNA replication occur?

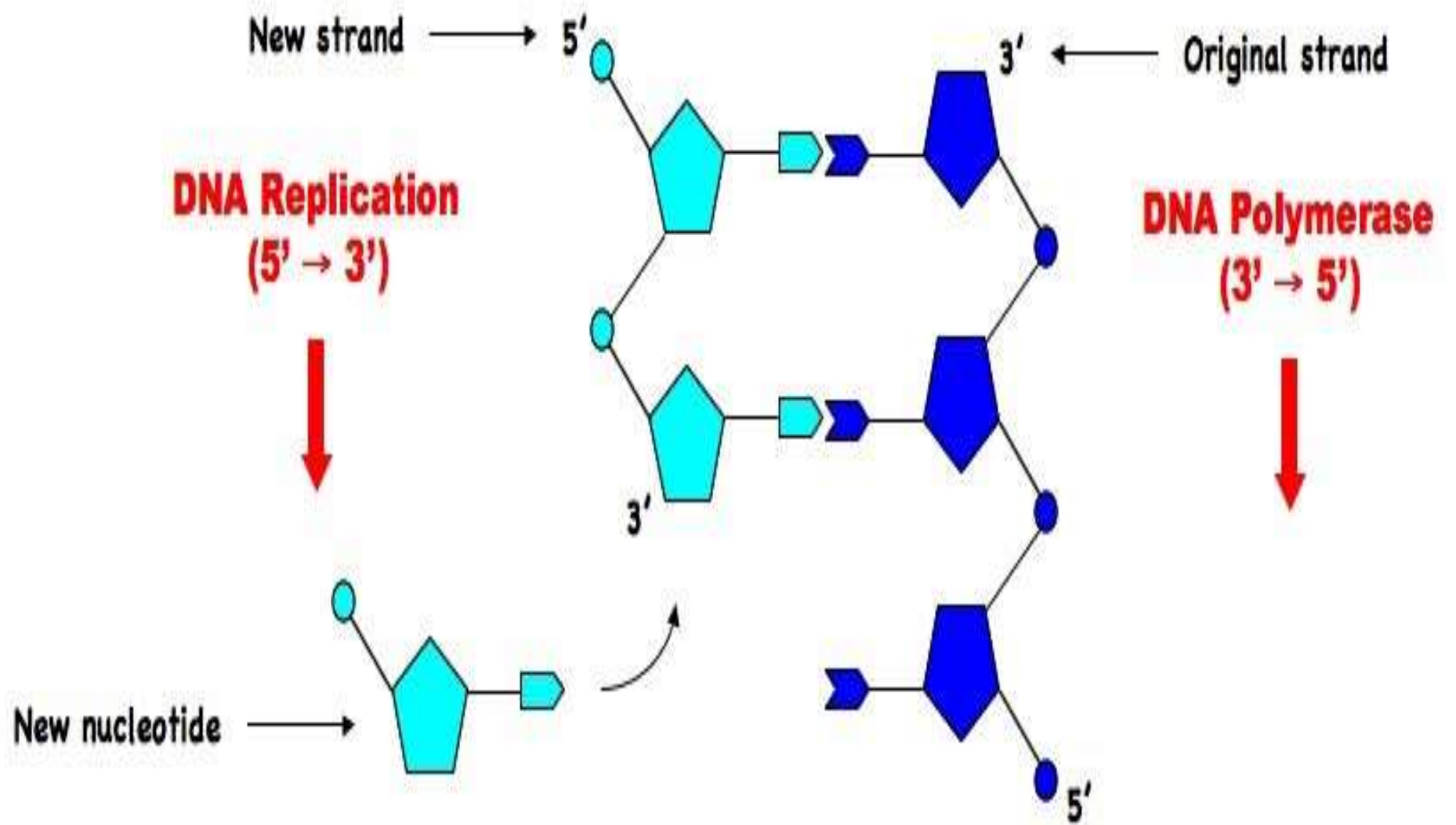
C) Synthesis is ALWAYS in the 5'-3' direction

What happens if a base mismatch occurs?



Where does energy for addition of nucleotide come from?







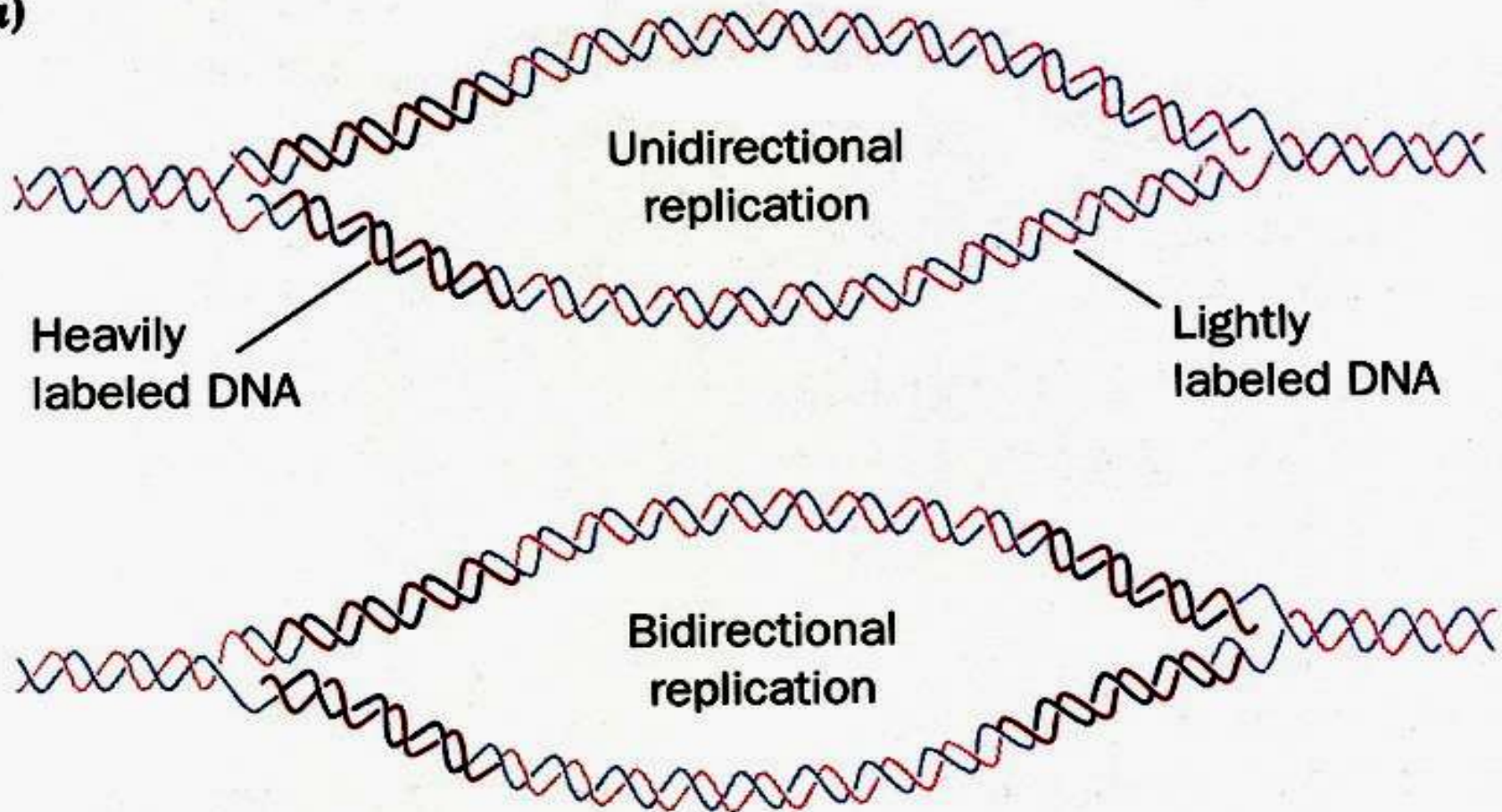
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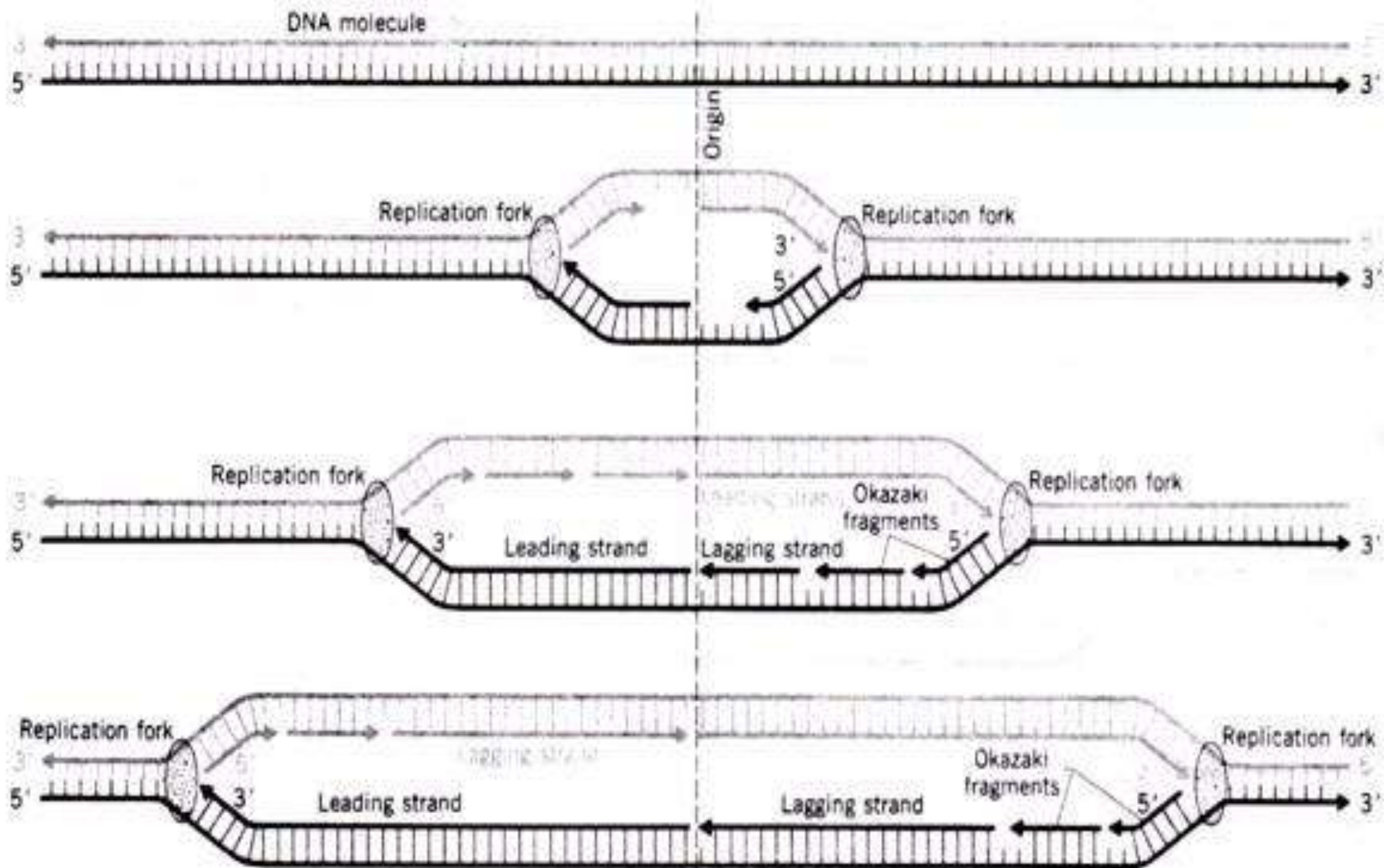
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# D) Uni or bidirectional

- Replication forks move in one or opposite directions

(a)





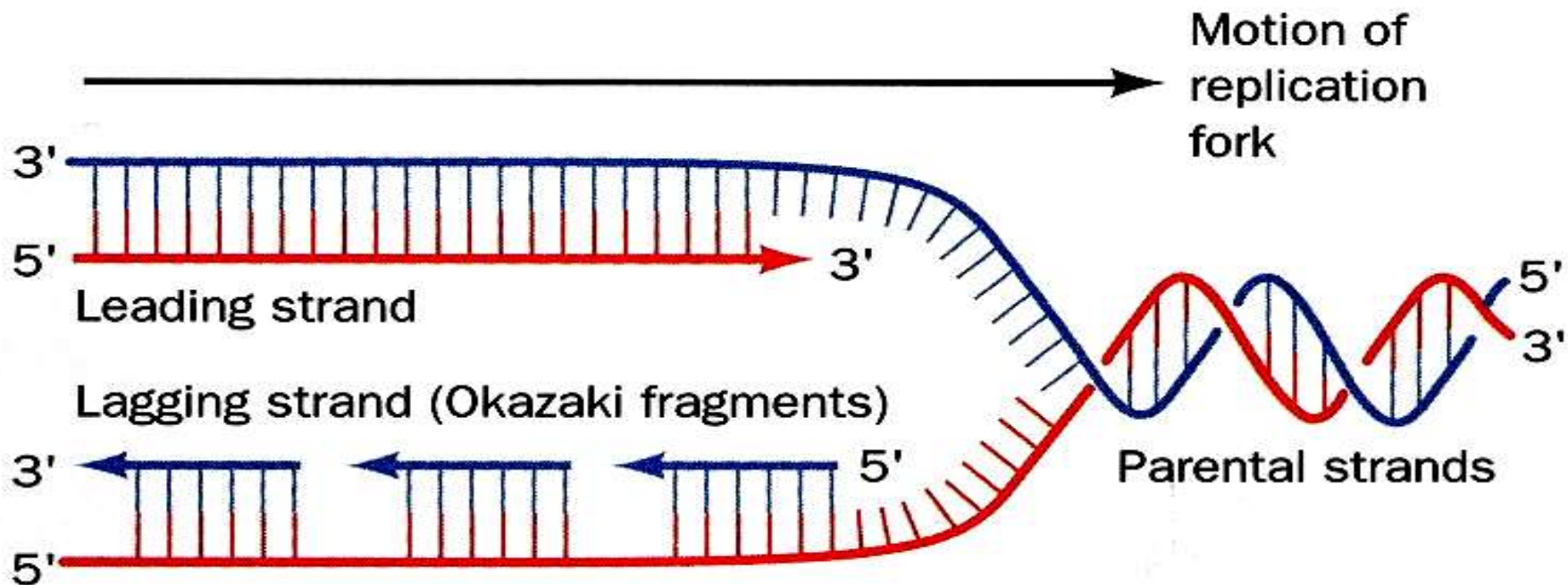
**FIGURE 21-5** *Bidirectional* replication of DNA. Two replication forks move away from the origin in opposite directions. Behind each fork, one new DNA strand grows continuously and the other grows as a series of fragments that are eventually linked together. The overall growth of both sets of new strands is in the direction of the moving forks.



# E) Semi-discontinuous replication

Anti parallel strands replicated simultaneously

- ❑ Leading strand synthesis continuously in 5'-3'
- ❑ Lagging strand synthesis is discontinuous in fragments (Okazaki fragments) in 5'-3'
- ❑ In prokaryotes Okazaki fragments- 1000-2000 nucleotide long
- ❑ In eukaryotes Okazaki fragments- 100-200 nucleotide long

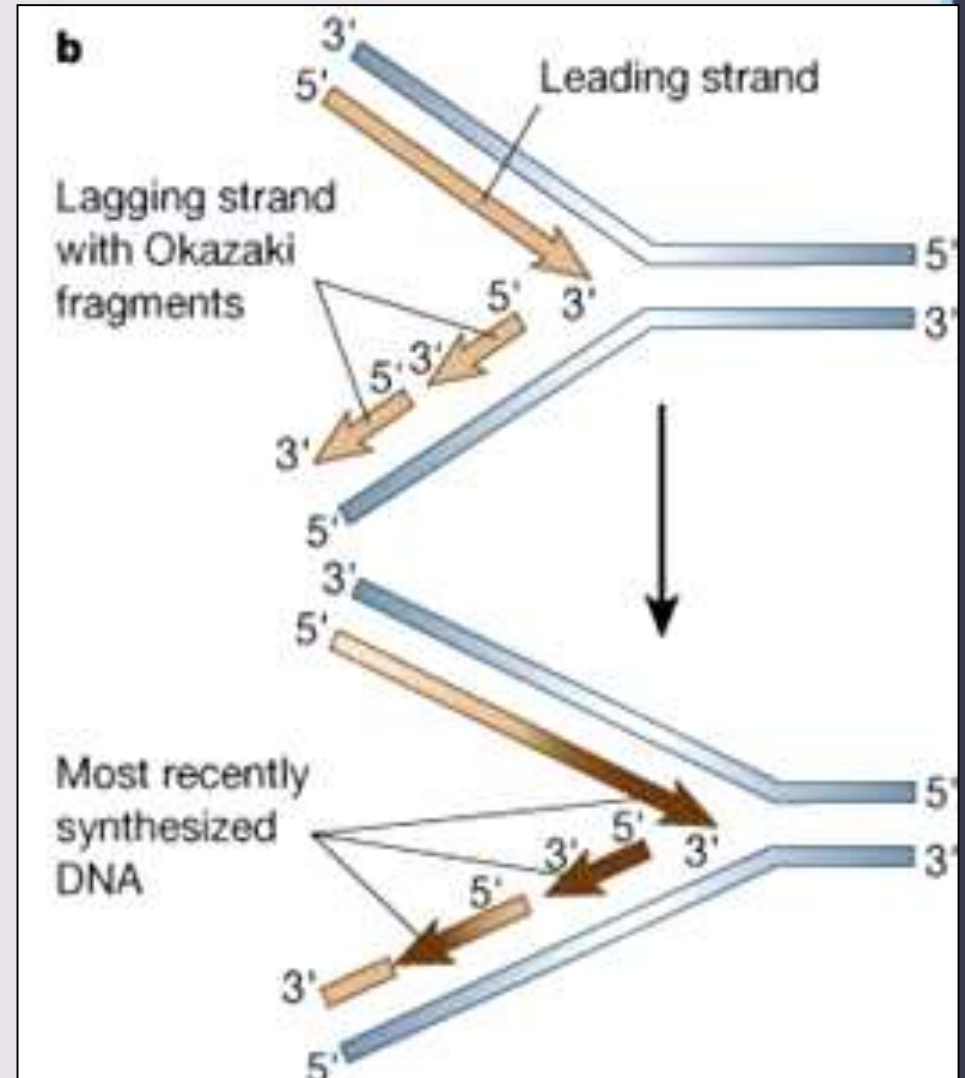
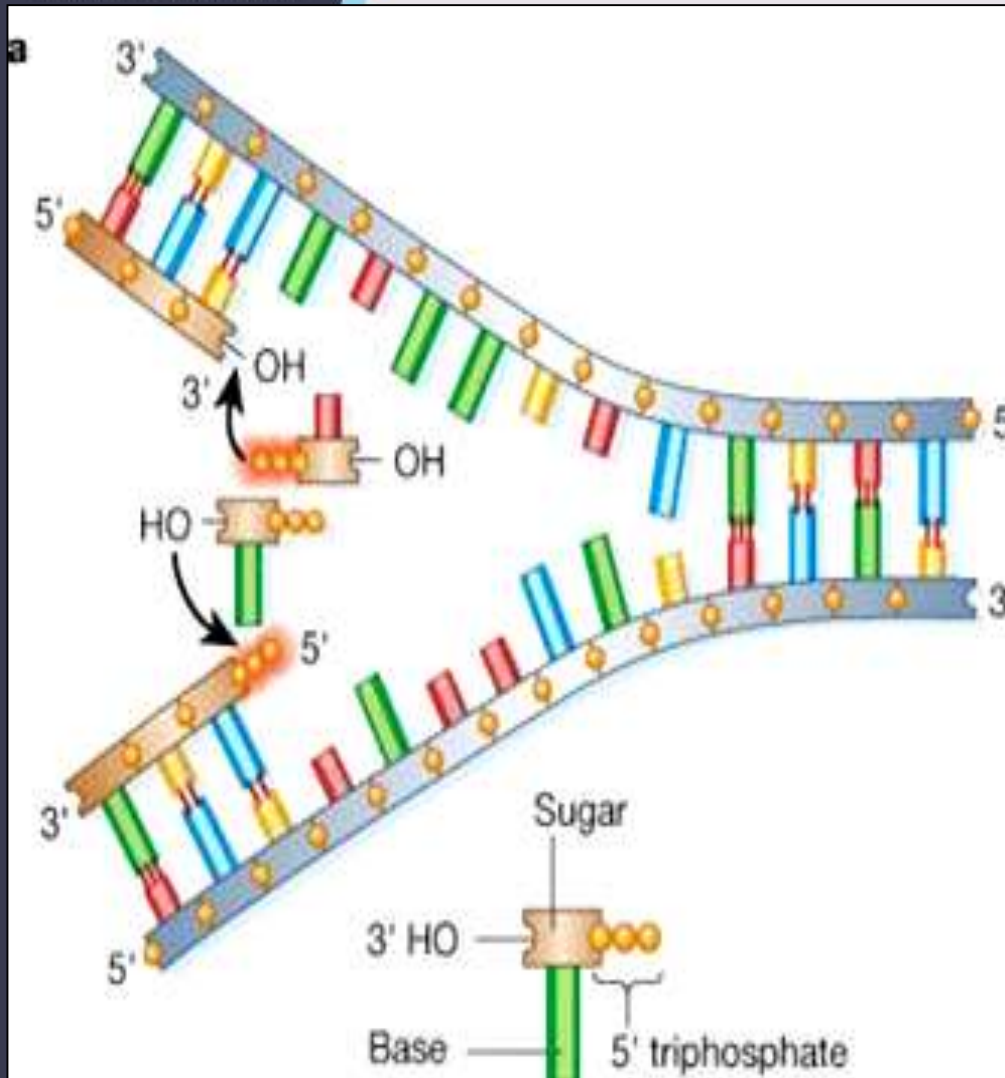




# Semi-discontinuous replication

always

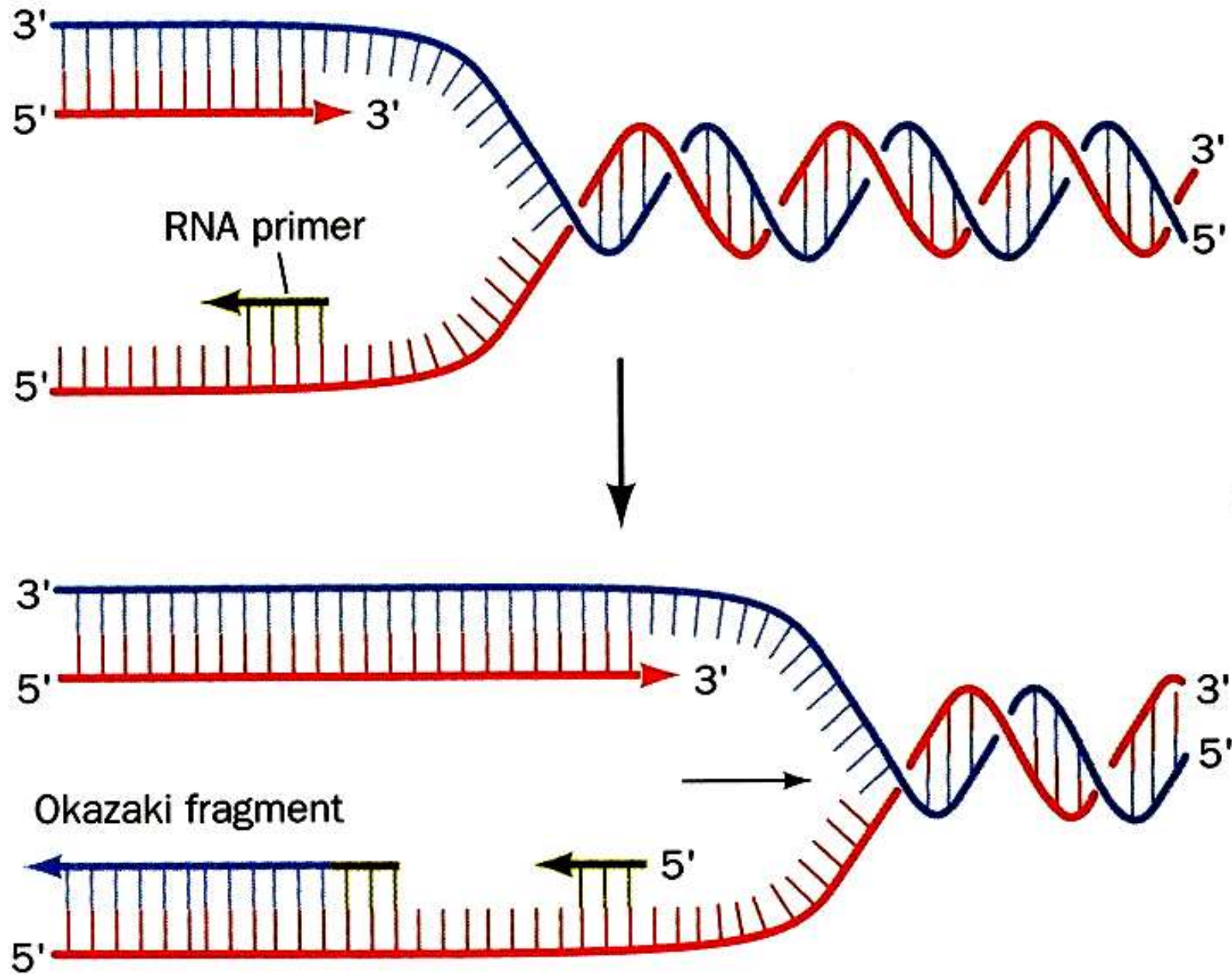
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# F) RNA primers required



# Core proteins at the replication fork

- ❖ **Topoisomerases**
  - Prevents torsion by DNA breaks
- ❖ **Helicases**
  - separates 2 strands
- ❖ **Primase**
  - RNA primer synthesis
- ❖ **Single strand binding proteins**
  - prevent reannealing of single strands
- ❖ **DNA polymerase**
  - synthesis of new strand
- ❖ **Tethering protein**
  - stabilises polymerase
- ❖ **DNA ligase**
  - seals nick via phosphodiester linkage



# The DNA Polymerase Complex

A number of different DNA polymerase molecules engage in DNA replication. These share three important properties

- ❖ **Chain elongation** accounts for the rate (in nucleotides per second) at which polymerization occurs.
- ❖ **Processivity** is an expression of the number of nucleotides added to the nascent chain before the polymerase disengages from the template.
- ❖ **The proofreading** function identifies copying errors and corrects them



# 3 types of DNA Polymerase important for DNA replication

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- ❑ In *E. coli*, **polymerase III (pol III)** functions at the replication fork. Of all polymerases, it catalyzes the highest rate of chain elongation and is the most processive.
- ❑ **Polymerase II (pol II)** is mostly involved in proofreading and DNA repair.
- ❑ **Polymerase I (pol I)** completes chain synthesis between Okazaki fragments on the lagging strand.

# The mechanism of DNA replication

Arthur Kornberg, a Nobel prize winner and other biochemists deduced steps of replication

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DNA replication is complex process, carried out by multienzyme complex- Replication apparatus or Replisome

## DNA Replication-Steps

- Identification of the origins of replication
- Unwinding (denaturation) of dsDNA to provide an ssDNA template
- Formation of the replication fork
- Initiation of DNA synthesis and elongation
- Primer removal and ligation of the newly synthesized DNA segments
- Reconstitution of chromatin structure



❖ At the origin of replication (ori), there is an association of sequence-specific dsDNA-binding proteins with a series of direct repeat DNA sequences (generally **A-T repeat**)

❖ Three different types of proteins involve in unbinding of DNA double helix.

1. **DNA Helicase** allows for processive unwinding of DNA.

2. **Single-stranded DNA-binding proteins (SSBs)** stabilize this complex and remain established as single strand.

3. **DNA Gyrase** catalysed the formation - ve super coiling in DNA.





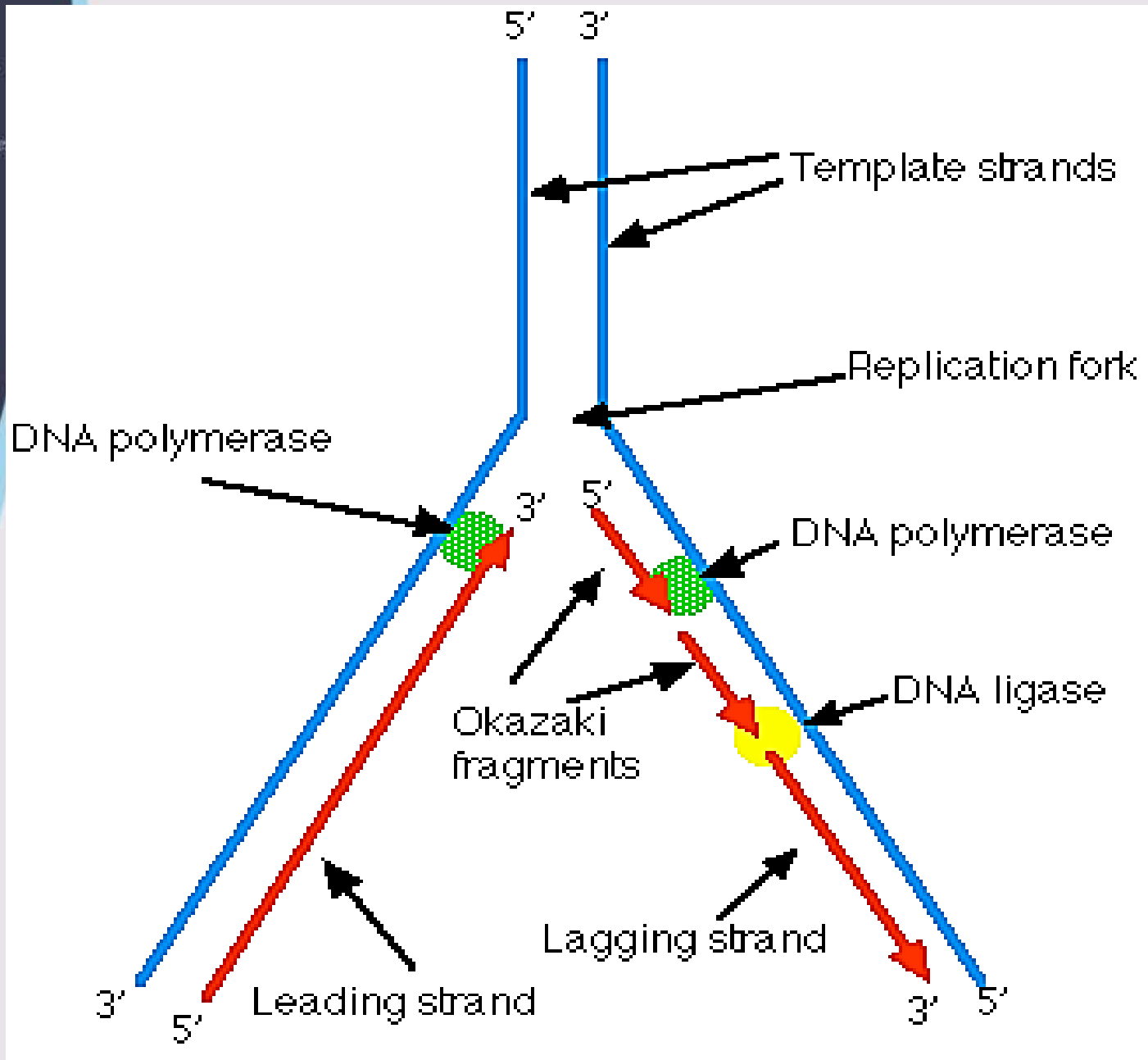
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- ❖ **Enzyme Primase** Synthesize RNA primer first at leading strand and than at Okazaki fragments (Lagging strand) to provide a free -OH group for DNA Polymerase binding.
- ❖ **DNA Polymerase III** catalyzed covalent extension of primed DNA chain with 5'-3' polymerase activity
- ❖ **DNA Polymerase I** catalyzed the removal of RNA primer by 5'-3' exonuclease activity and also simultaneously perform 5'-3' polymerase activity.
- ❖ **DNA Ligase** join the okazaki fragments in lagging strand and RNA primer region and in leading strand after digestion of RNA primer and gap filling as a result of DNA polymerase I activity.

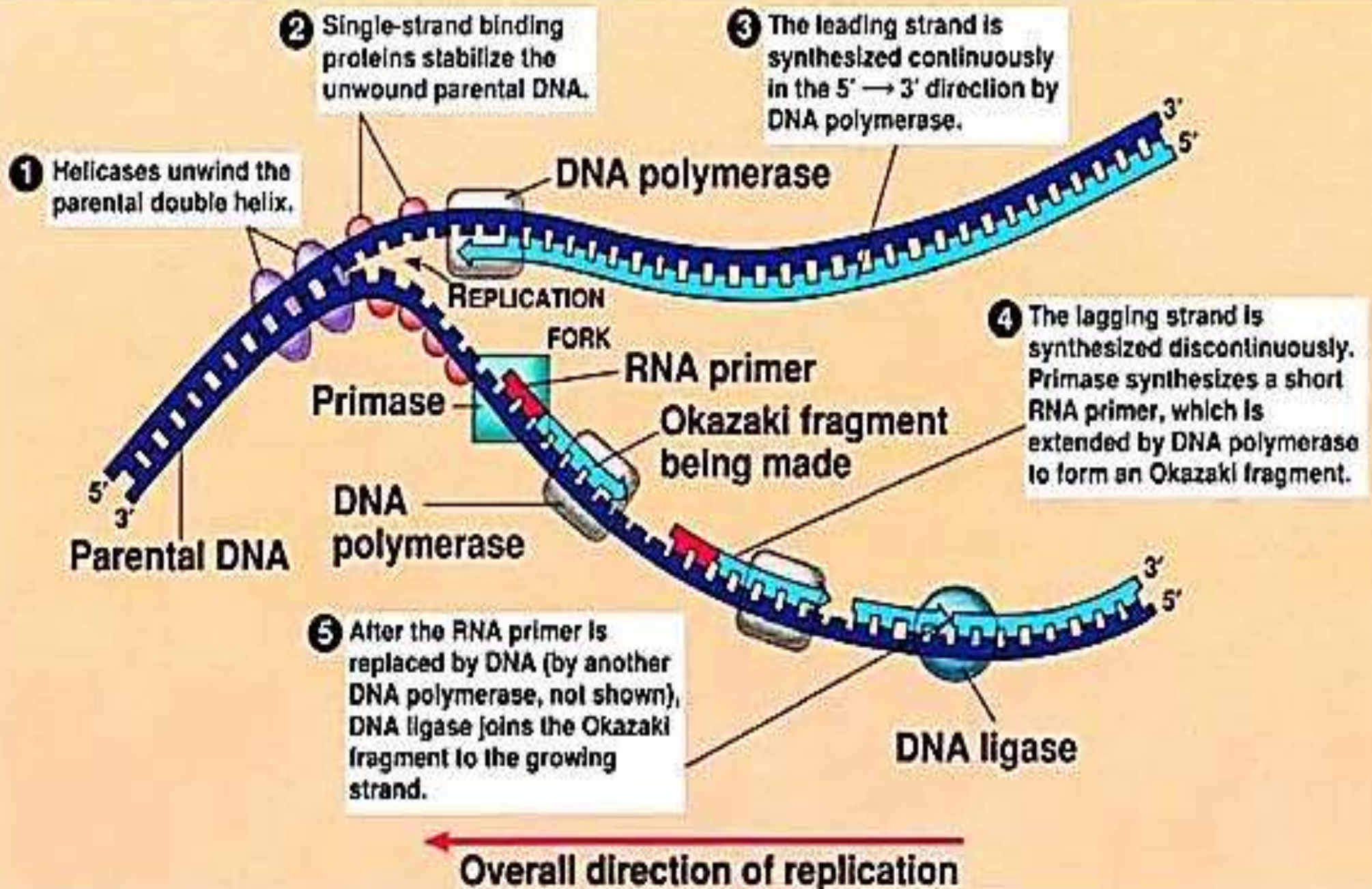
# Generalised model of semi-conservative replication of DNA showing different enzymes involved during replication.



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# A SUMMARY OF DNA REPLICATION





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