



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Lecture – 17

Replication of DNA

by

Dr. Praveen Kumar

*Asst. Prof. **GPB***

MSSSoA, CUTM, Odisha



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

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



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

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



Centurion
UNIVERSITY

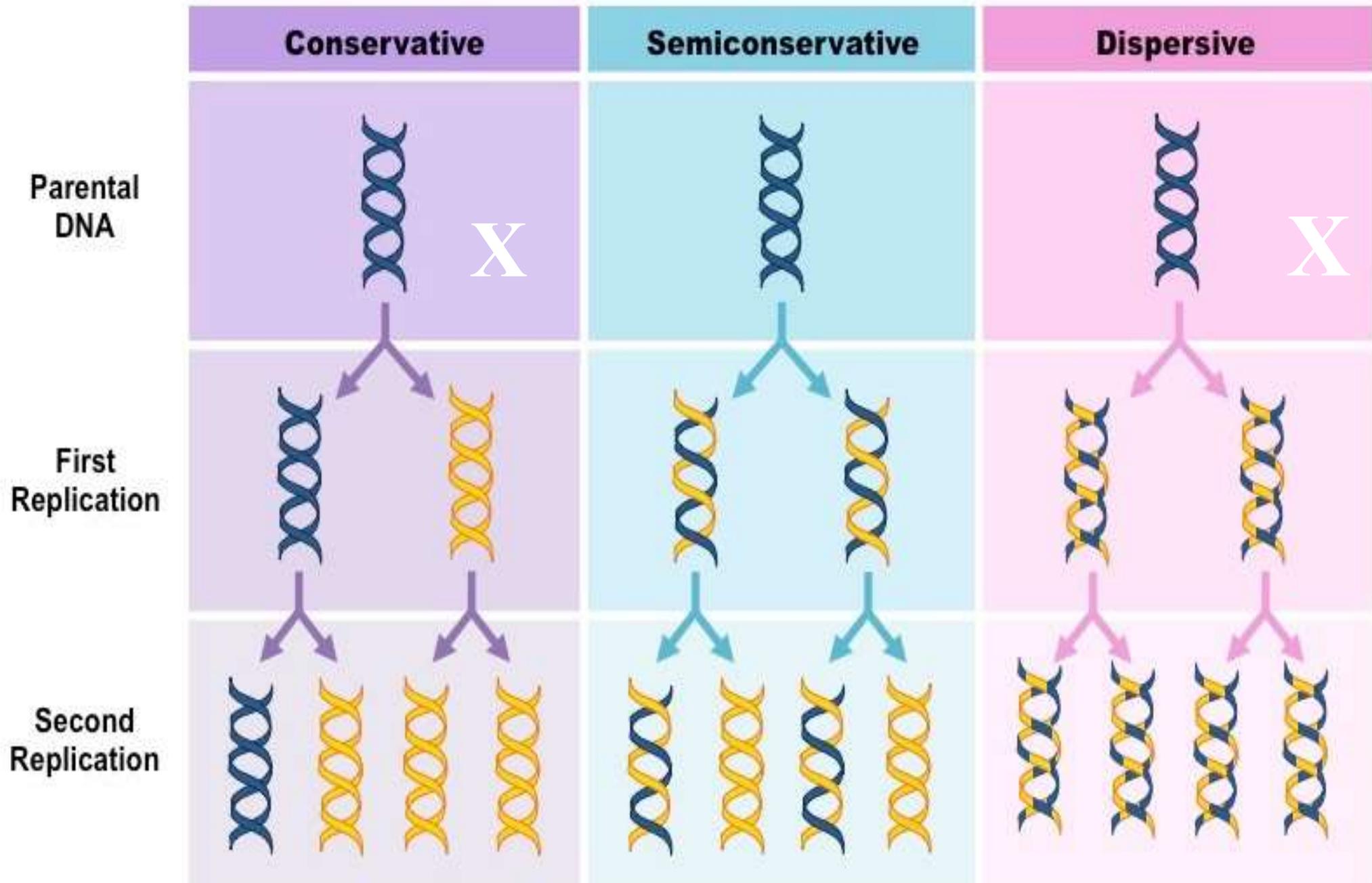
Shaping Lives...
Improving Communities

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:





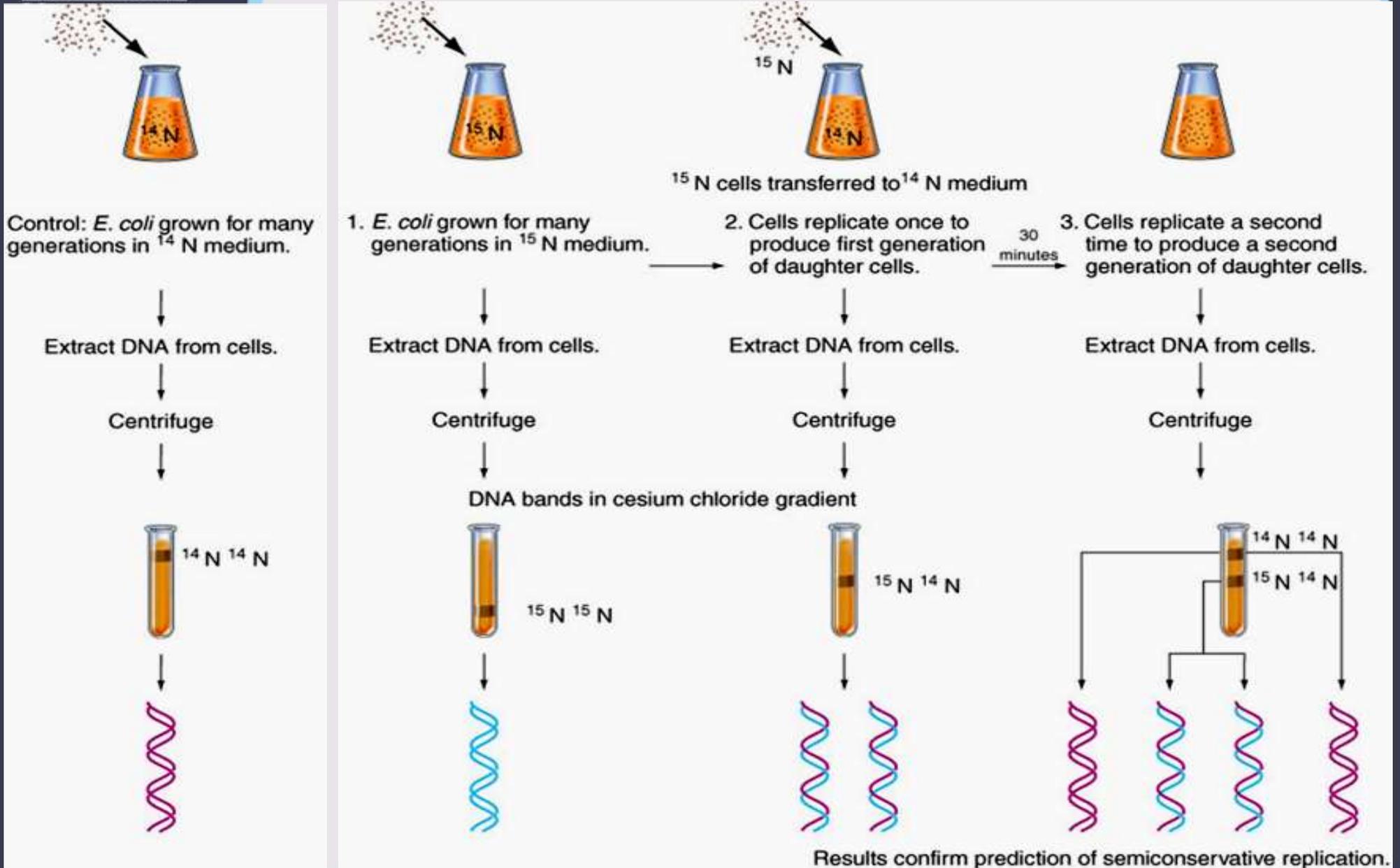
Evidence for Semi conservative replication

- ❖ **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}N by growing them on a medium containing ^{15}N for 14 generations to replace the normal nitrogen (^{14}N).
- ❖ The density of normal and heavy nitrogen differs. The ^{14}N is lighter (1.710 g/cm^3) than ^{15}N (1.724 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}N gave a characteristic heavy band at one end of a tube that had been centrifuged at a high speed in an ultra-centrifuge.



Centurion

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





Evidence for Semi conservative replication

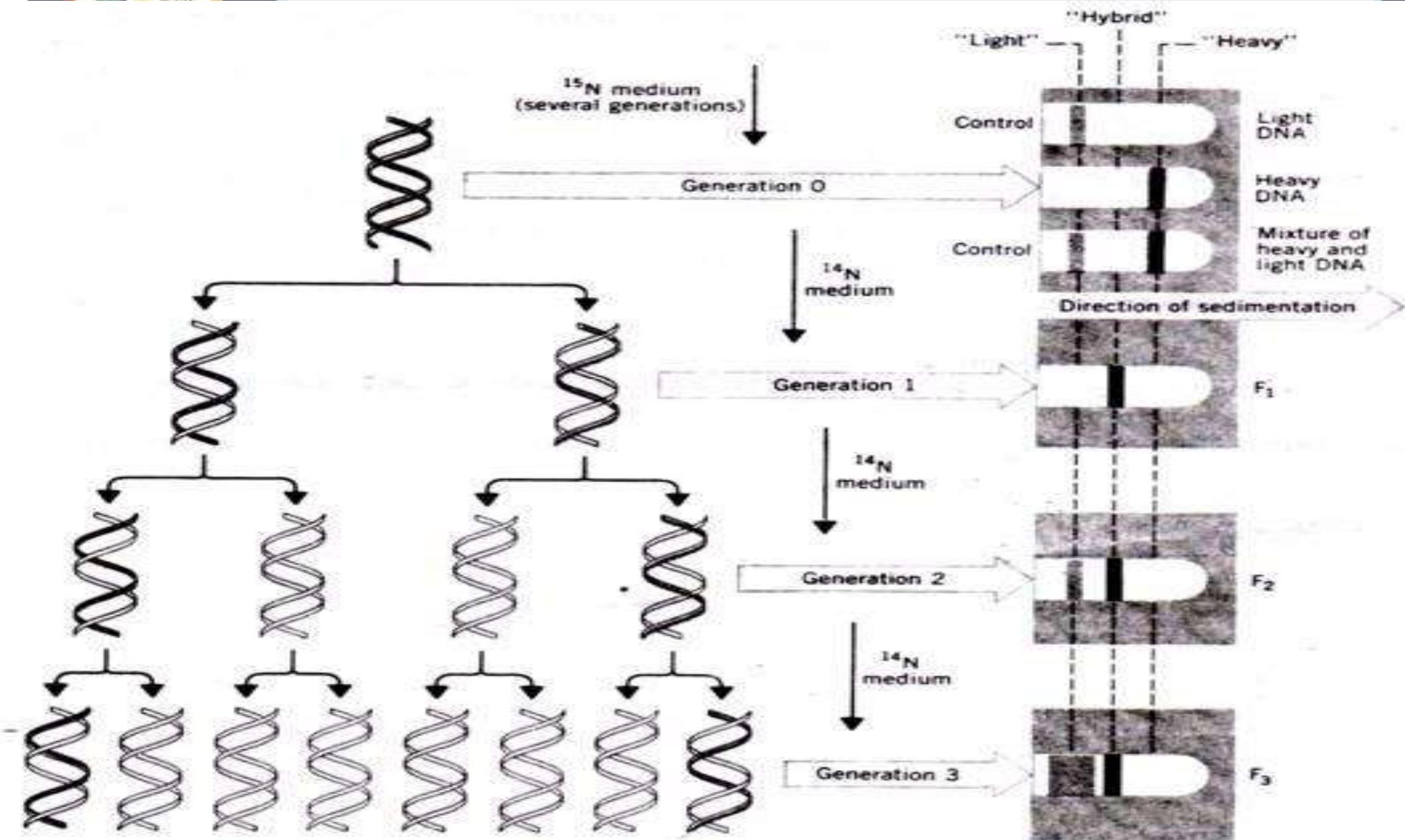


FIGURE 21-3 Results of the Meselson-Stahl experiments (right) and their interpretation (left) (see text for explanation). (From E. J. Gardner and D. P. Snustad, *Principles of Genetics* (7th ed.), John Wiley & Sons, 1984, p. 99.)

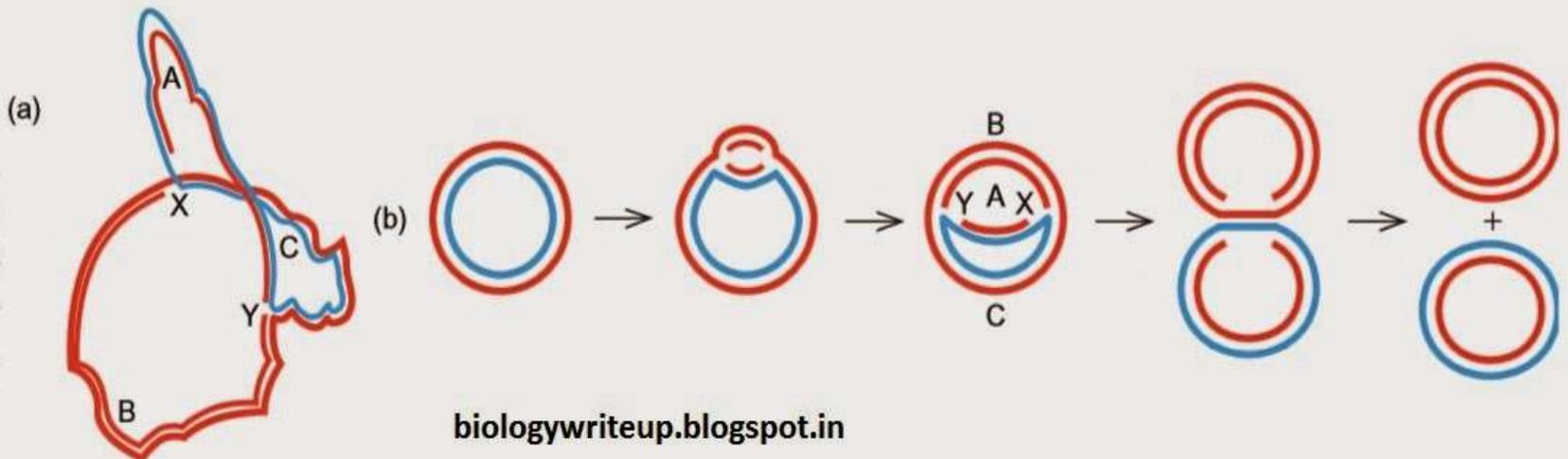
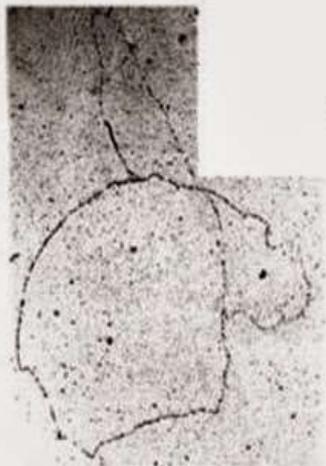
- 
- ❖ These labelled cells were then grown on a normal unlabelled media containing ^{14}N for one generation. DNA was again extracted and processed, and it was found to consist of a hybrid DNA containing both ^{14}N and ^{15}N at the same time
- ❖ The next generation of growth on unlabelled DNA was 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}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°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}N and the other ^{14}N . Thus, it was concluded that DNA replication was by semi-conservative mode.



Centurion
UNIVERSITY
Empowering Communities...

Autoradiography of replicating bacterial chromosome

Cairns (1963) provided evidence for this in his experiments with radio-active labelled (^3H - 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 θ shaped structures during replication. This indicates that unwinding and replication proceed simultaneously.



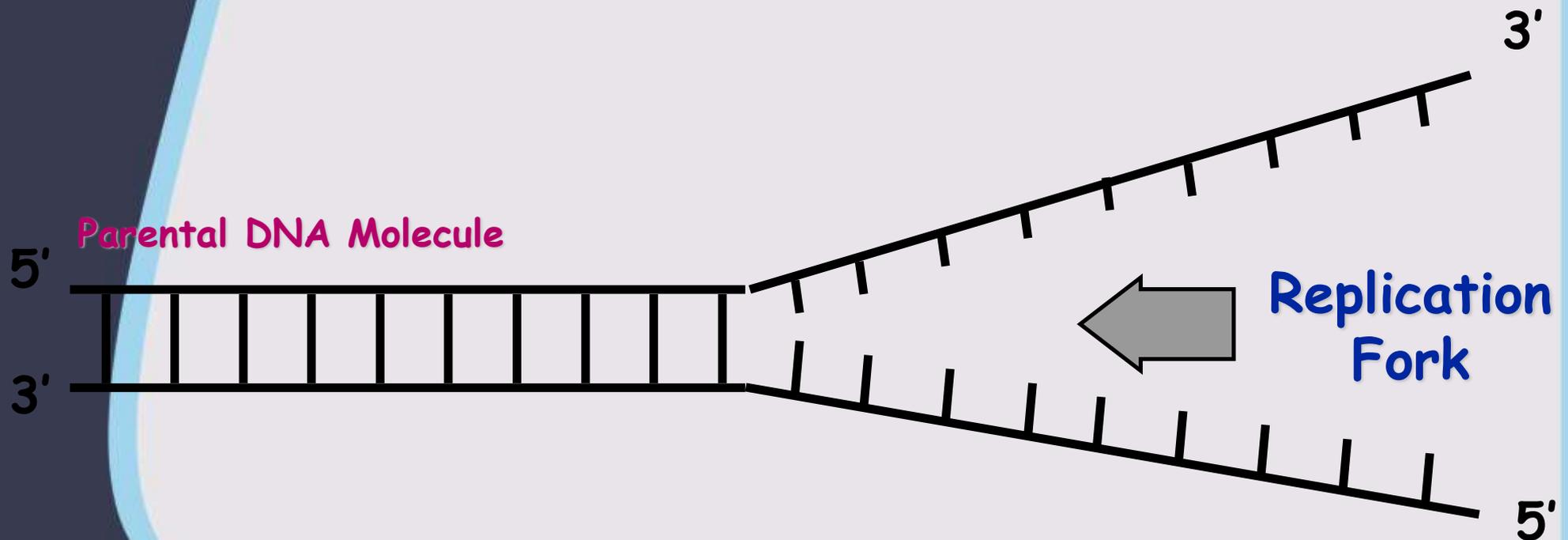


Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities...

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**

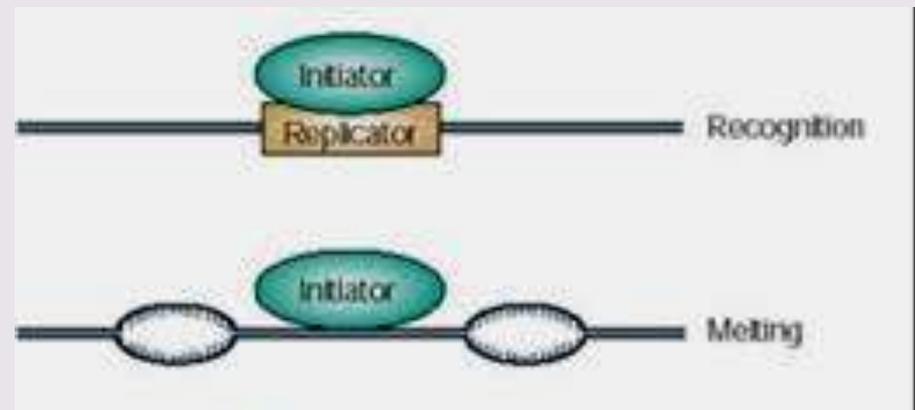
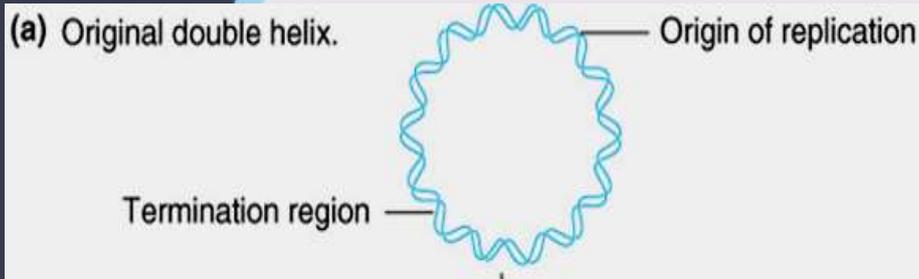


As the 2 DNA strands open at the origin, Replication Bubbles form

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

Eukaryotes - multiple sites of origin (replicator)

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

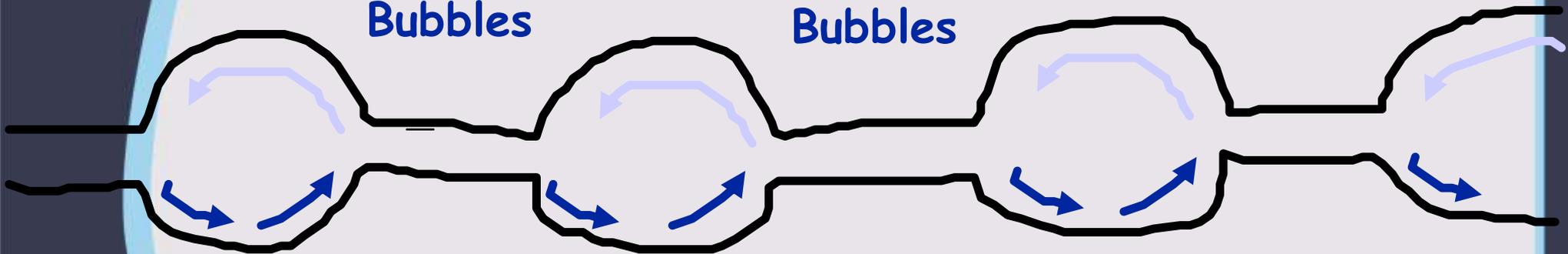


Prokaryotes

Eukaryotes

Bubbles

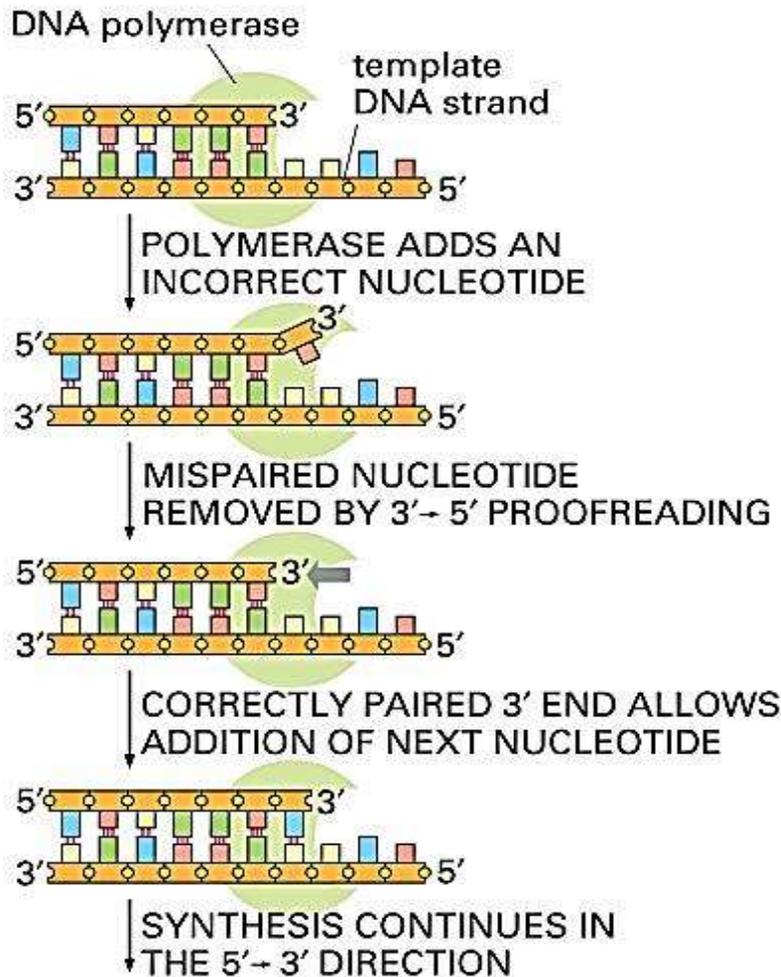
Bubbles



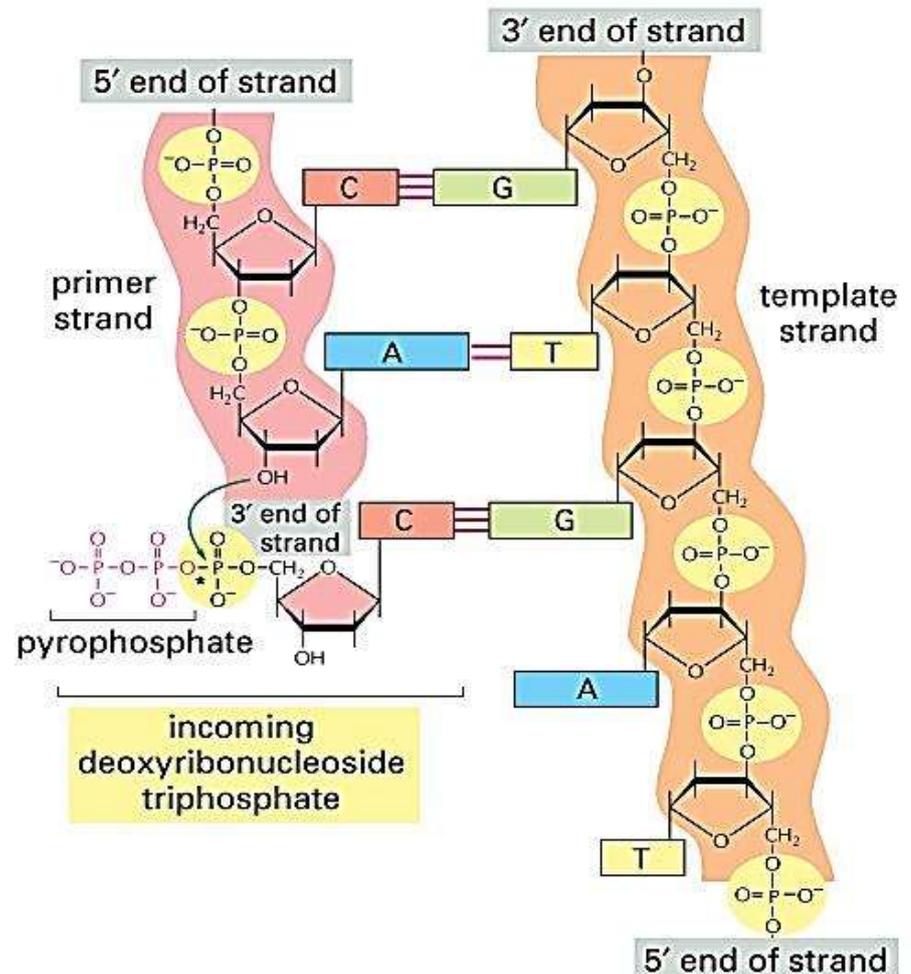
In what direction does DNA replication occur?

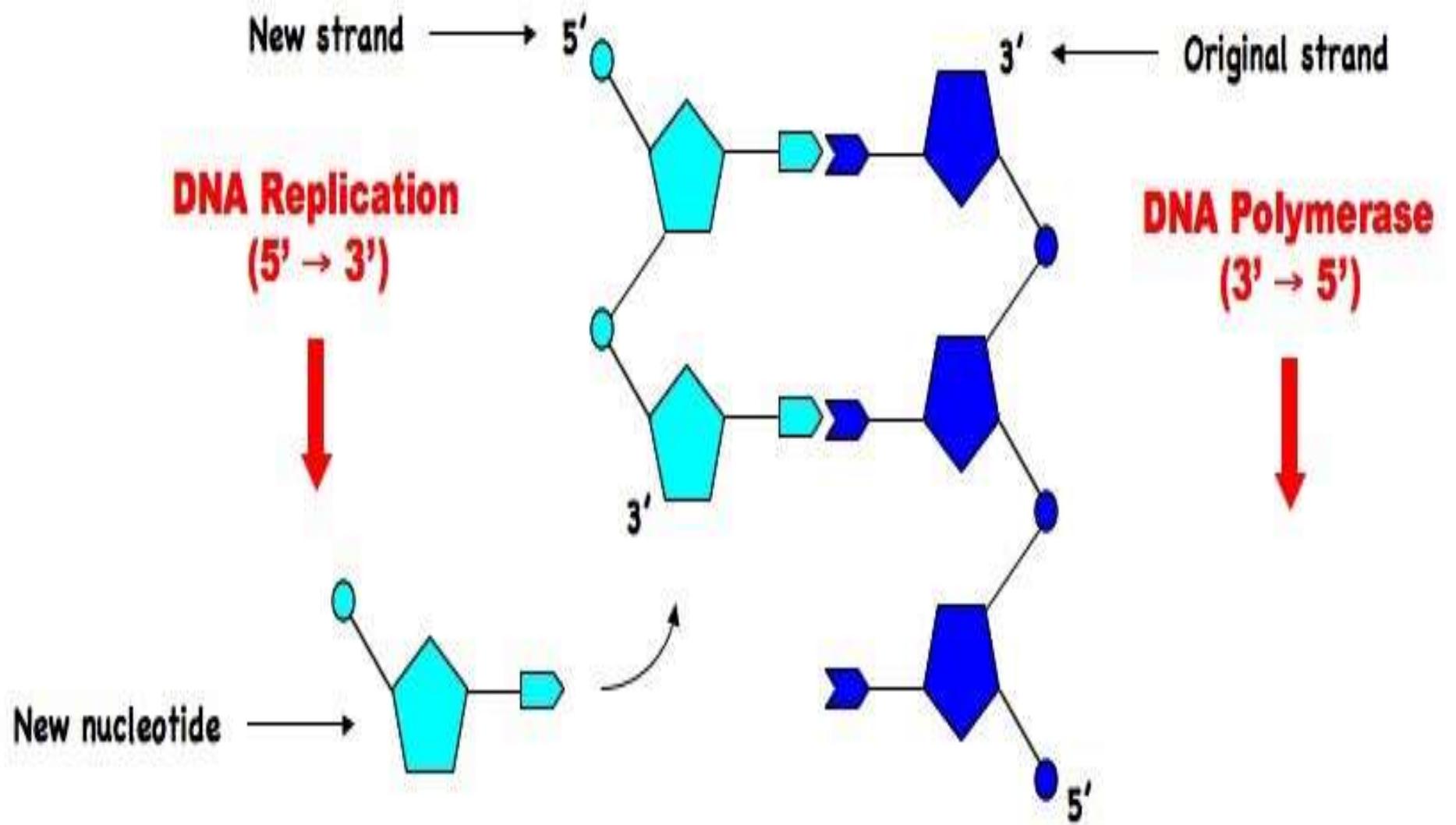
C) Synthesis is ALWAYS in the 5'-3' direction

What happens if a base mismatch occurs?



Where does energy for addition of nucleotide come from?







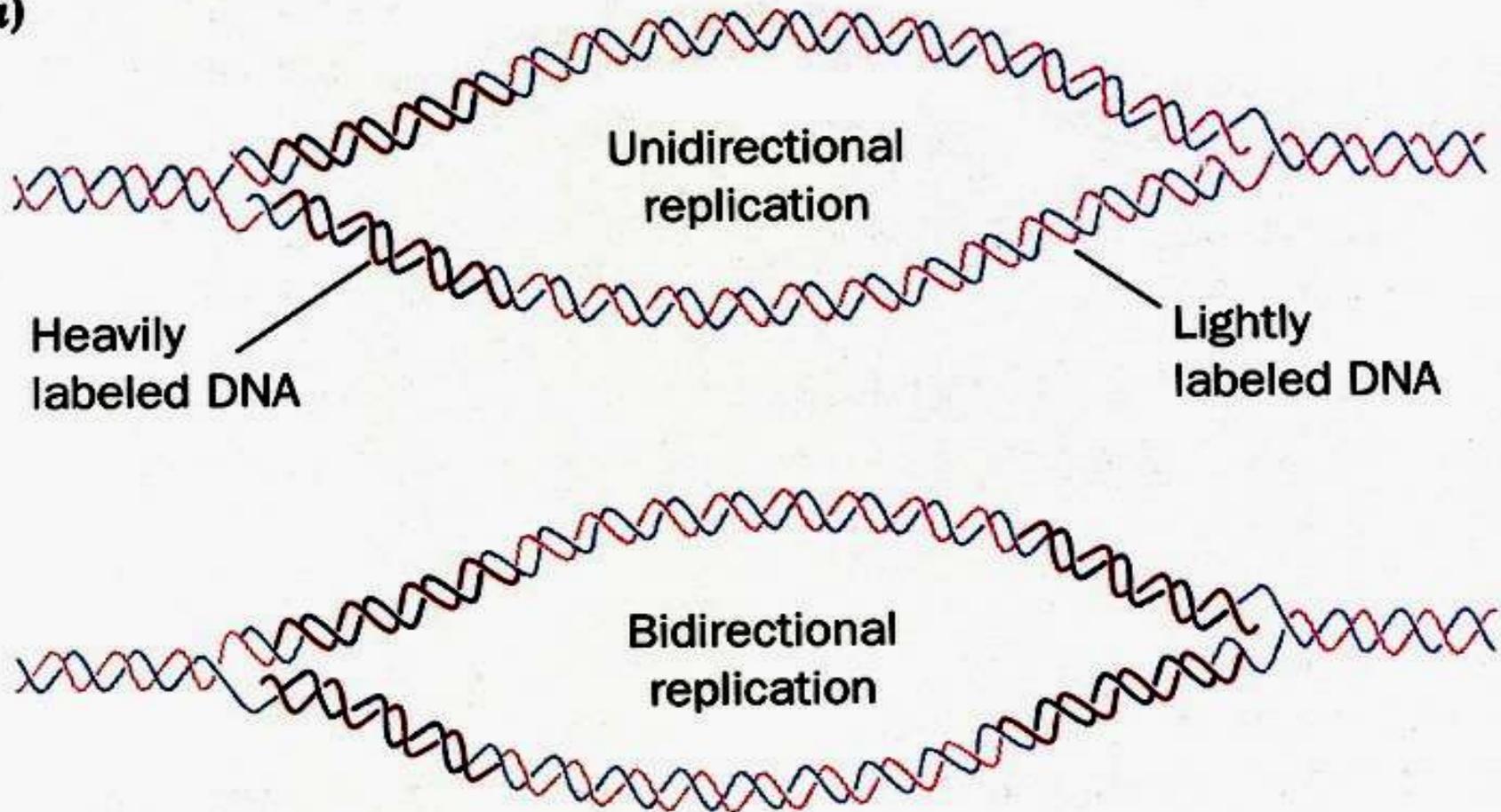
Centurion
UNIVERSITY

Shri...
Emp...

D) Uni or bidirectional

- Replication forks move in one or opposite directions

(a)



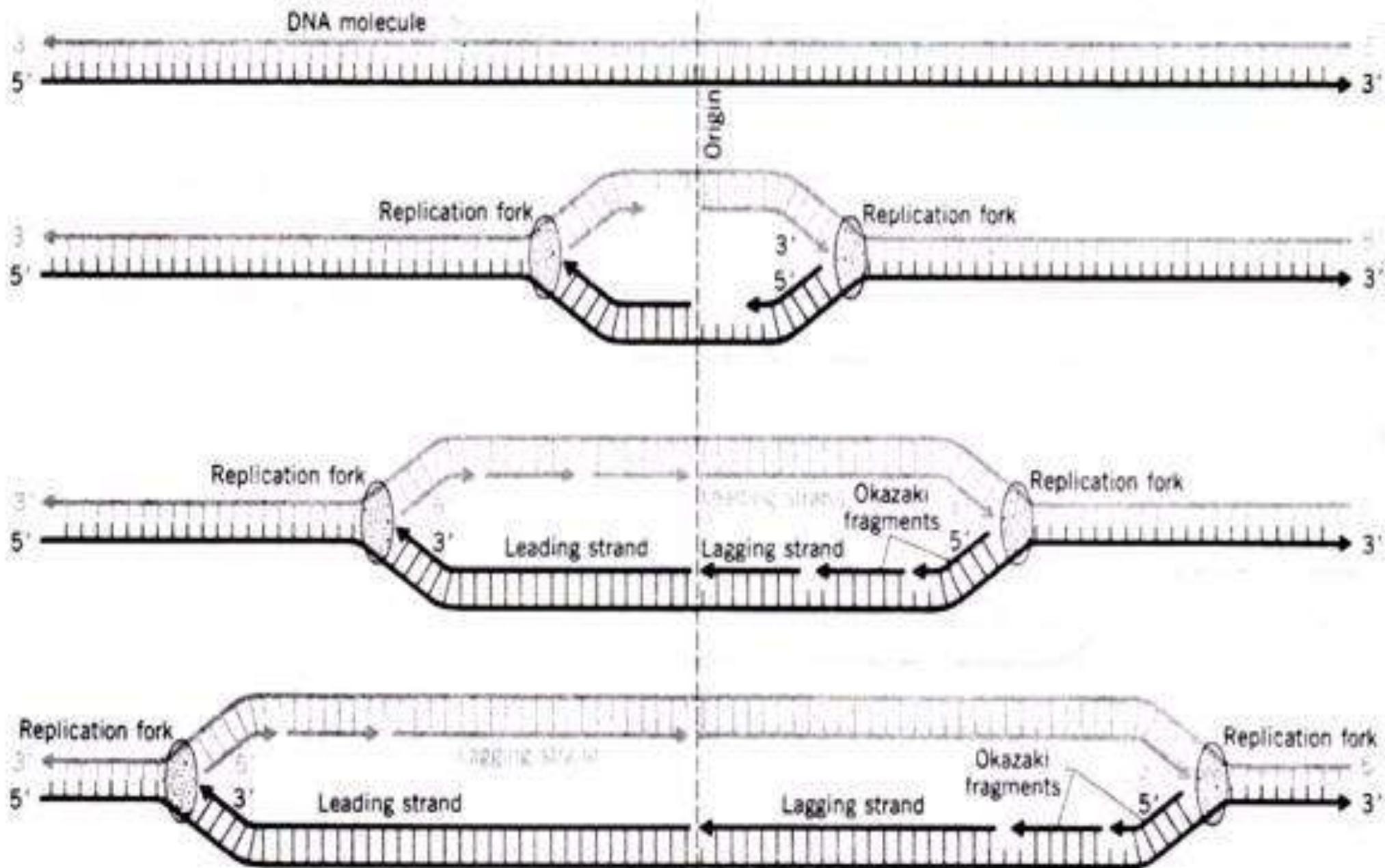
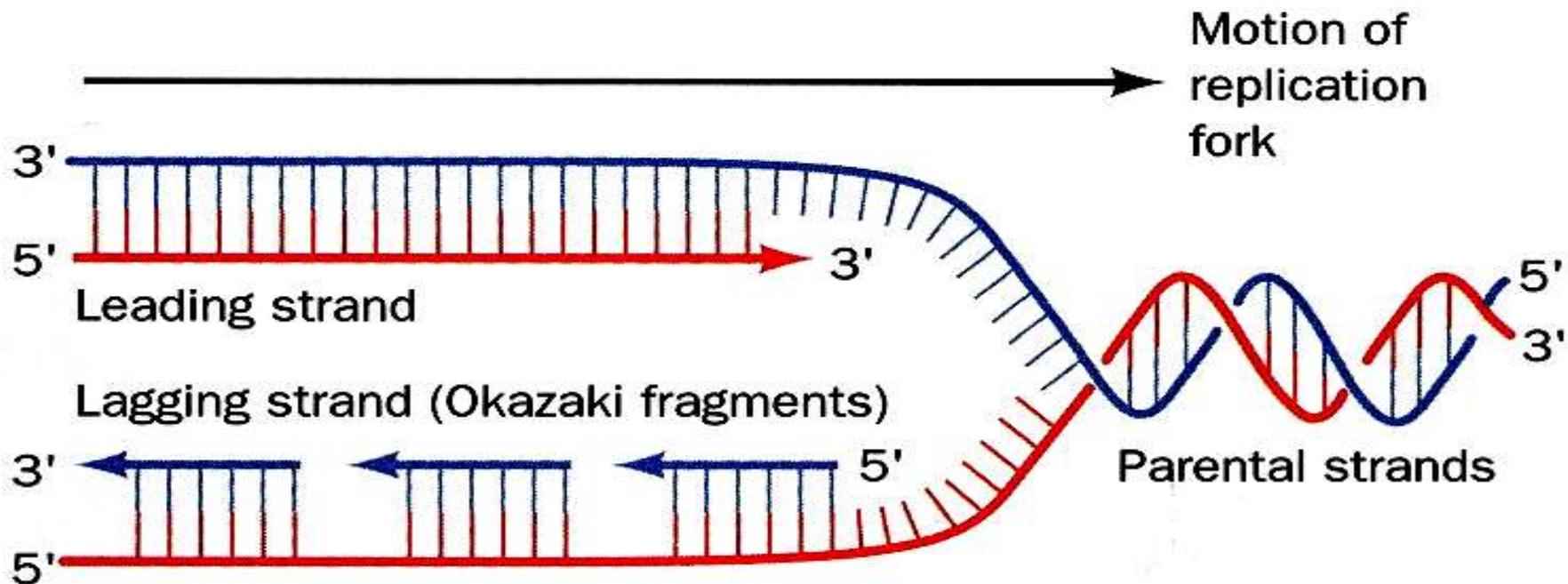


FIGURE 21-5 Bidirectional replication of DNA. Two replication forks move away from the origin in opposite directions. Behind each fork, one new DNA strand grows continuously and the other grows as a series of fragments that are eventually linked together. The overall growth of both sets of new strands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

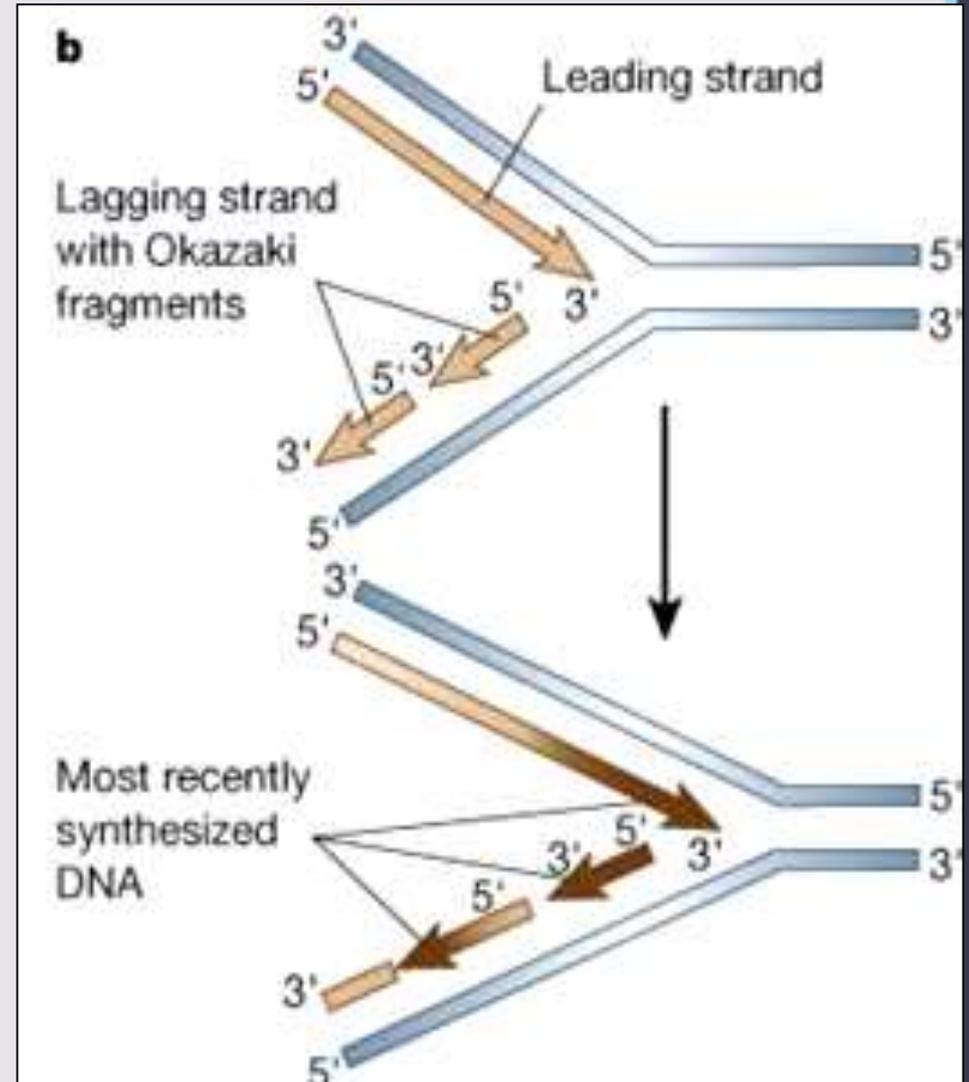
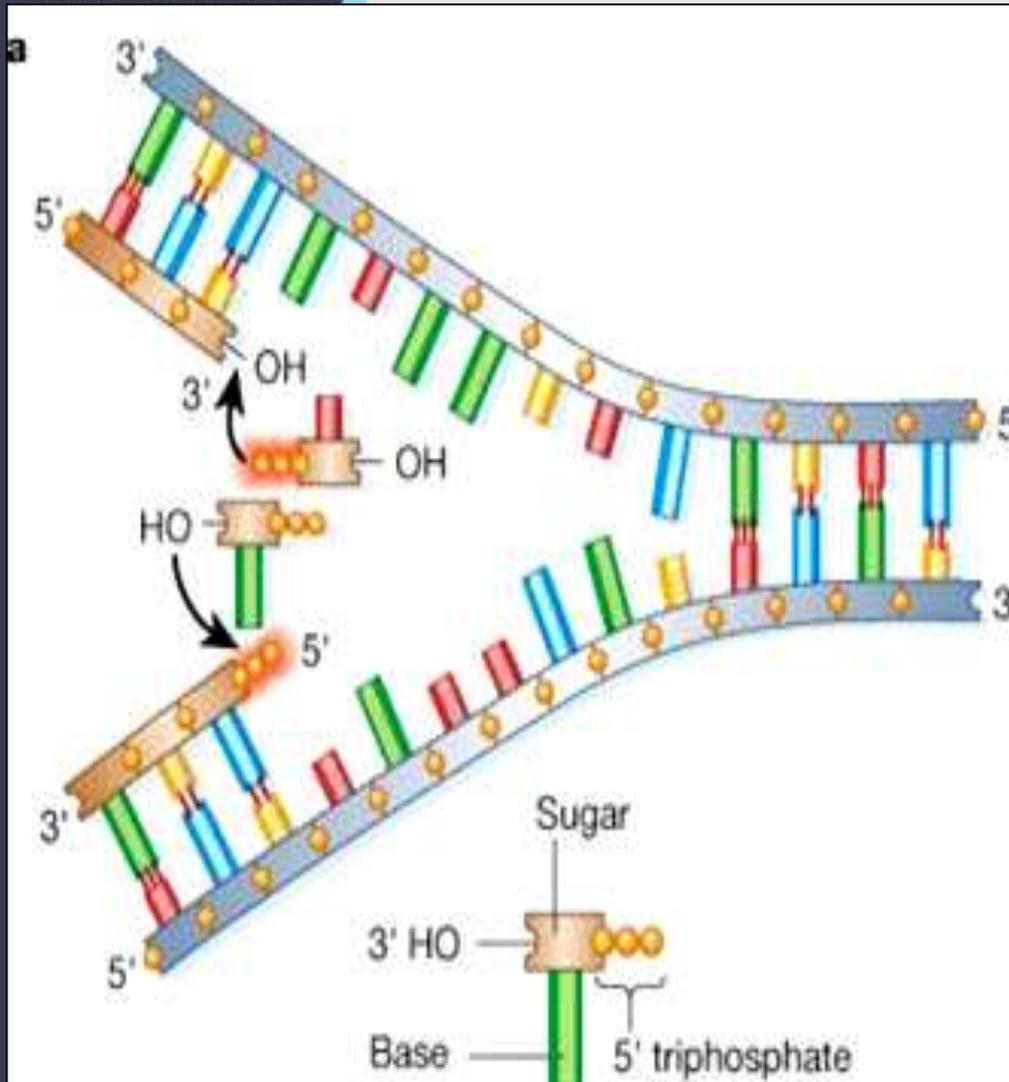




Semi-discontinuous replication

always

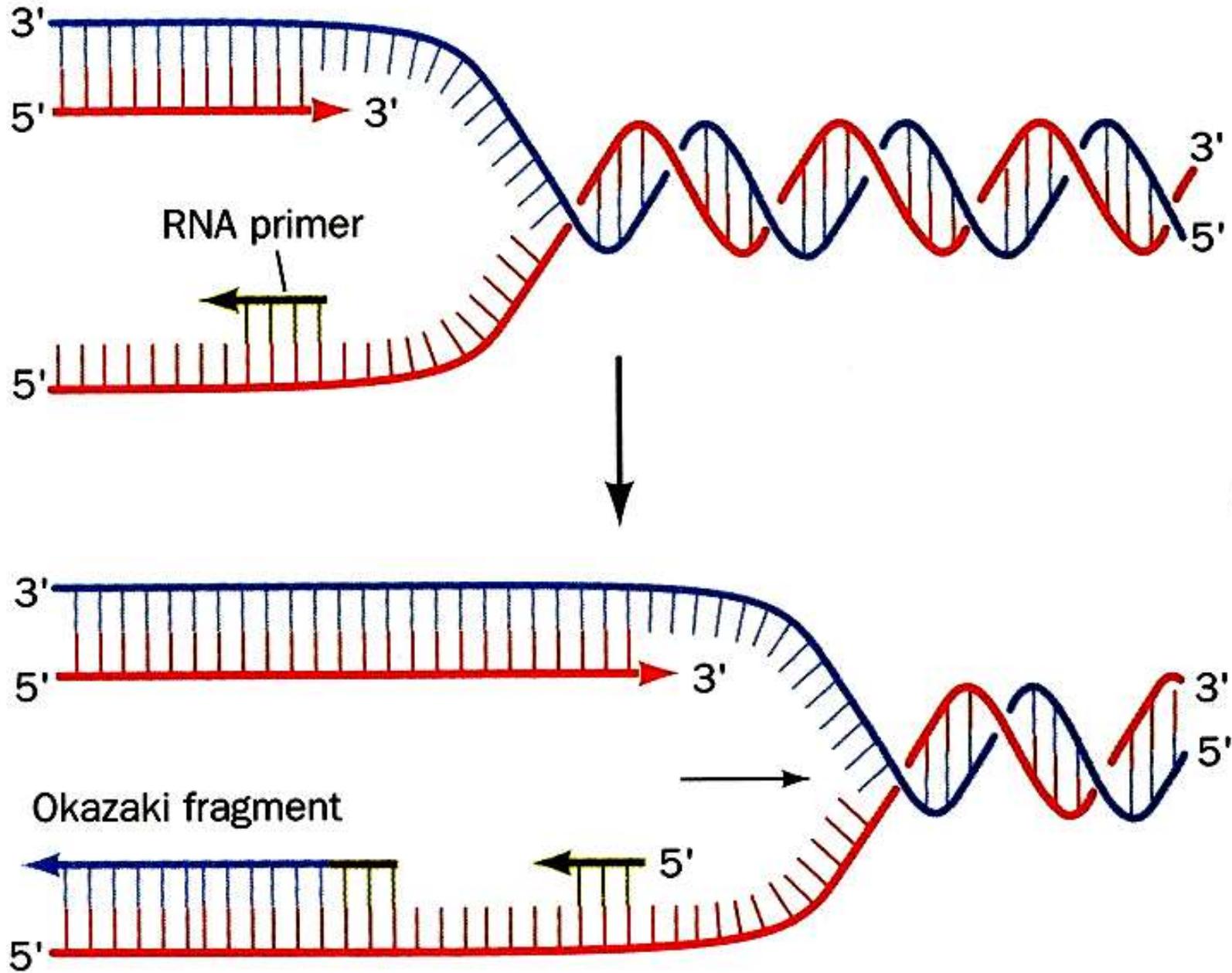
UNIVERSITY
Shaping Lives...
Empowering Communities...





Cent
UNIV
Shaping Liv
Empowering

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



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities

3 types of DNA Polymerase important for DNA replication

- ❑ 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

Centurion
UNIVERSITY

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



Centurion
UNIVERSITY
Shaping the Future
Empowering Communities...

- ❖ 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.



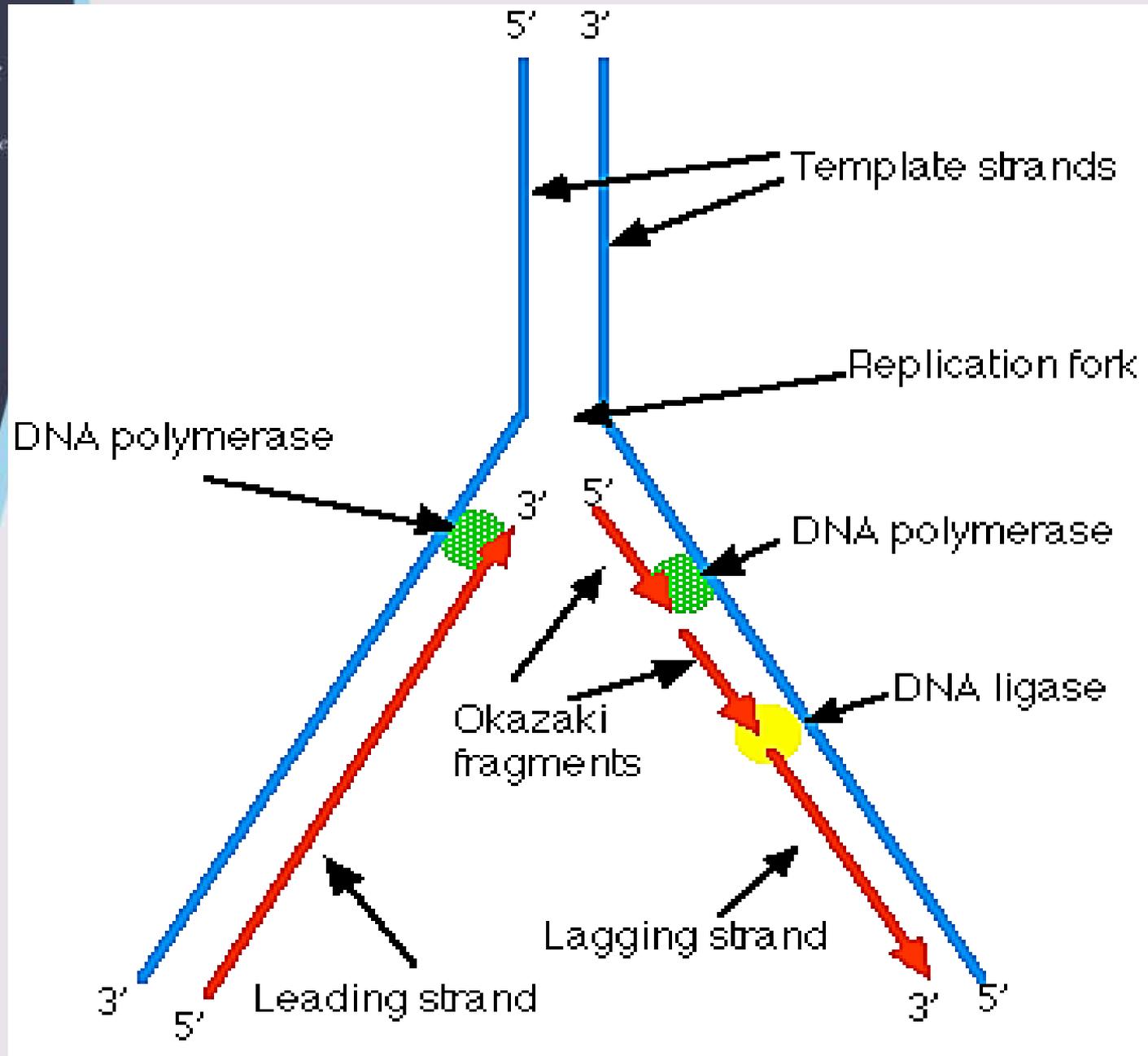
Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities

- ❖ **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.

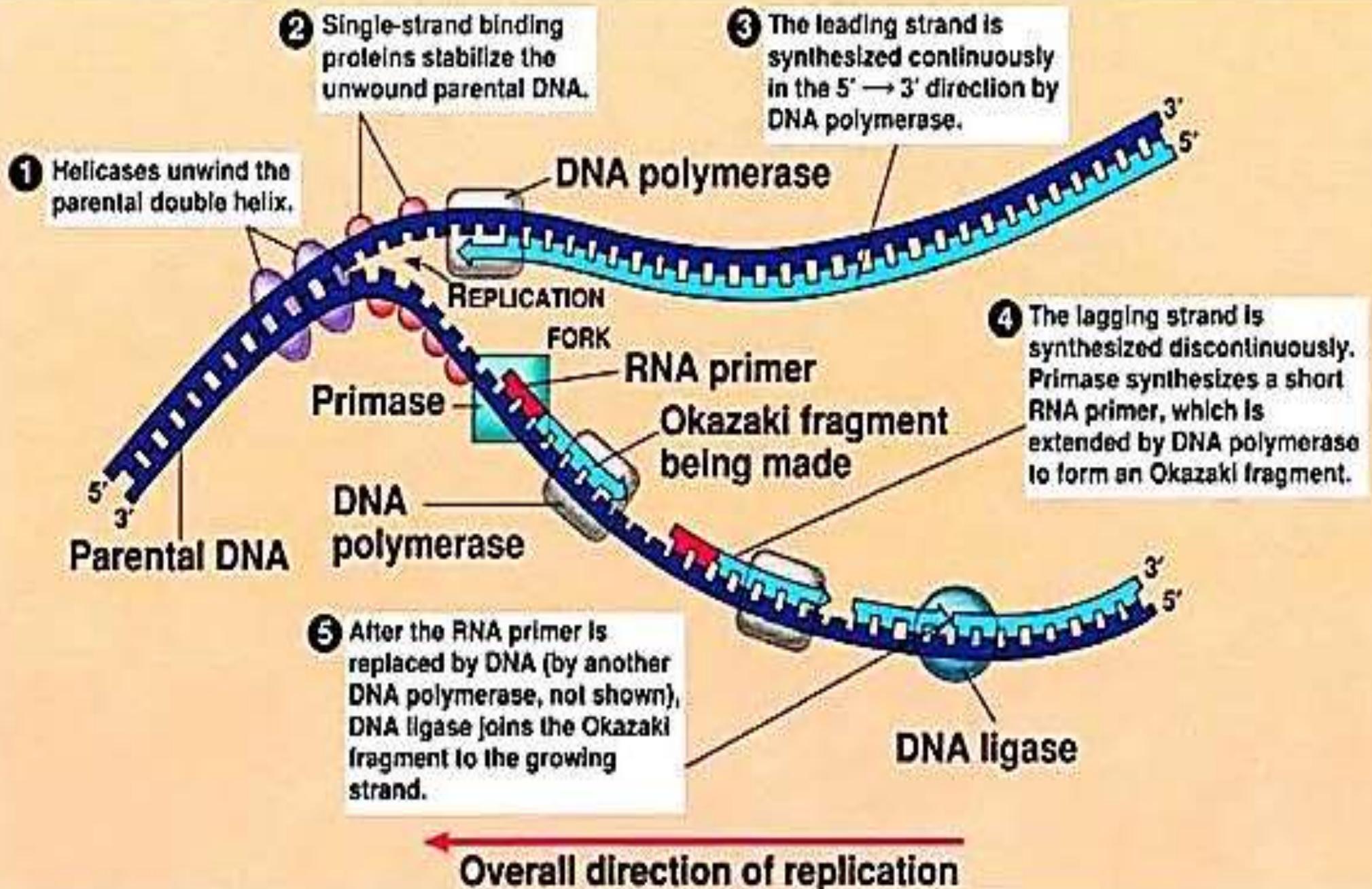
Generalised model of semi-conservative replication of DNA showing different enzymes involved during replication.



Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities



A SUMMARY OF DNA REPLICATION





Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

THANK YOU