Unit-III DNA replication

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Course code: SC301 (Biology)

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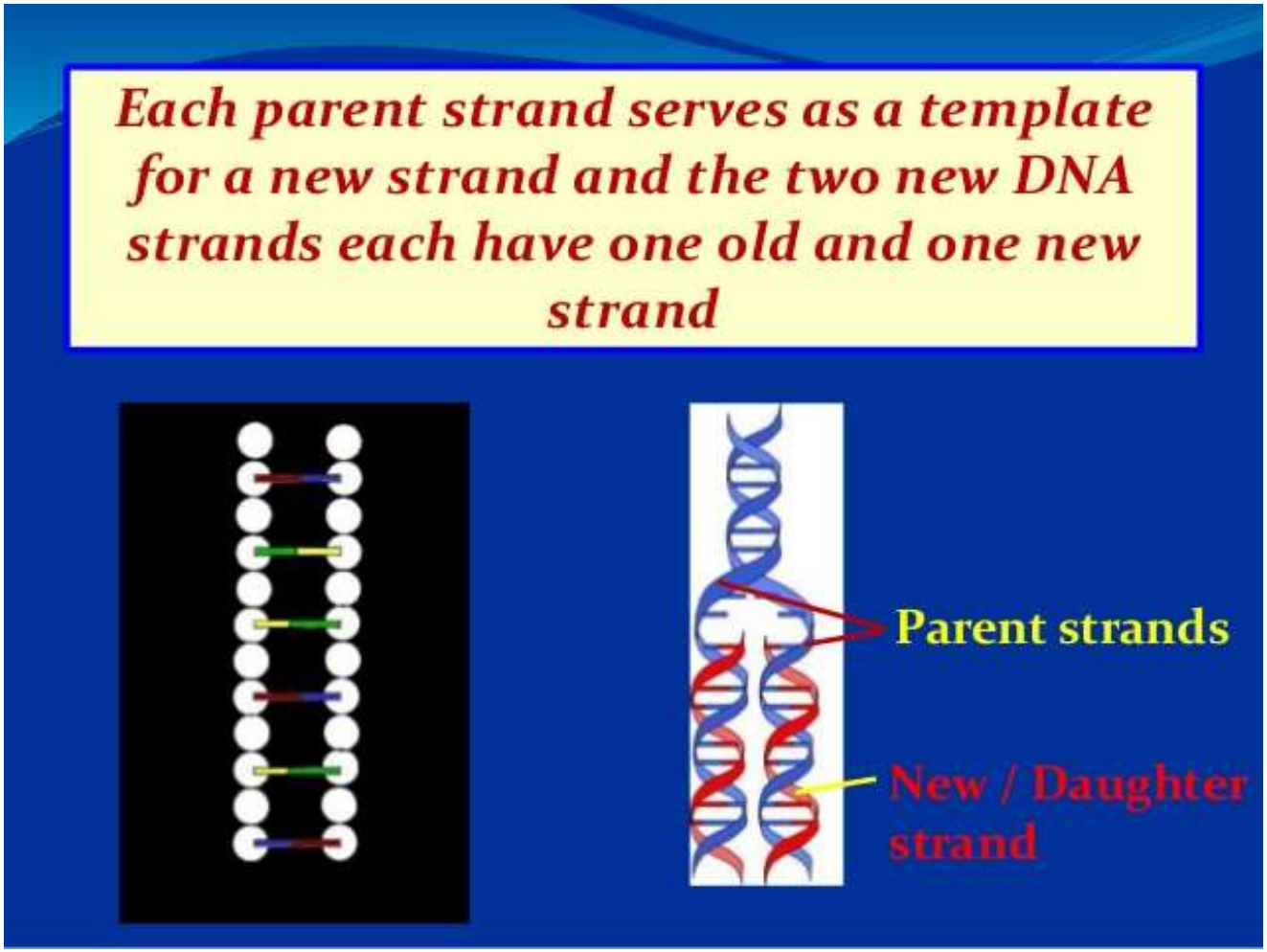
DNA Replication

- DNA replication is a biological process that occurs in all living organisms and copies their exact DNA. It is the basis for biological inheritance.
- Replication is the process of synthesis of daughter DNA from parental DNA by the enzyme DNA Polymerase.
- $(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PPi$

  DNA → Lengthened DNA

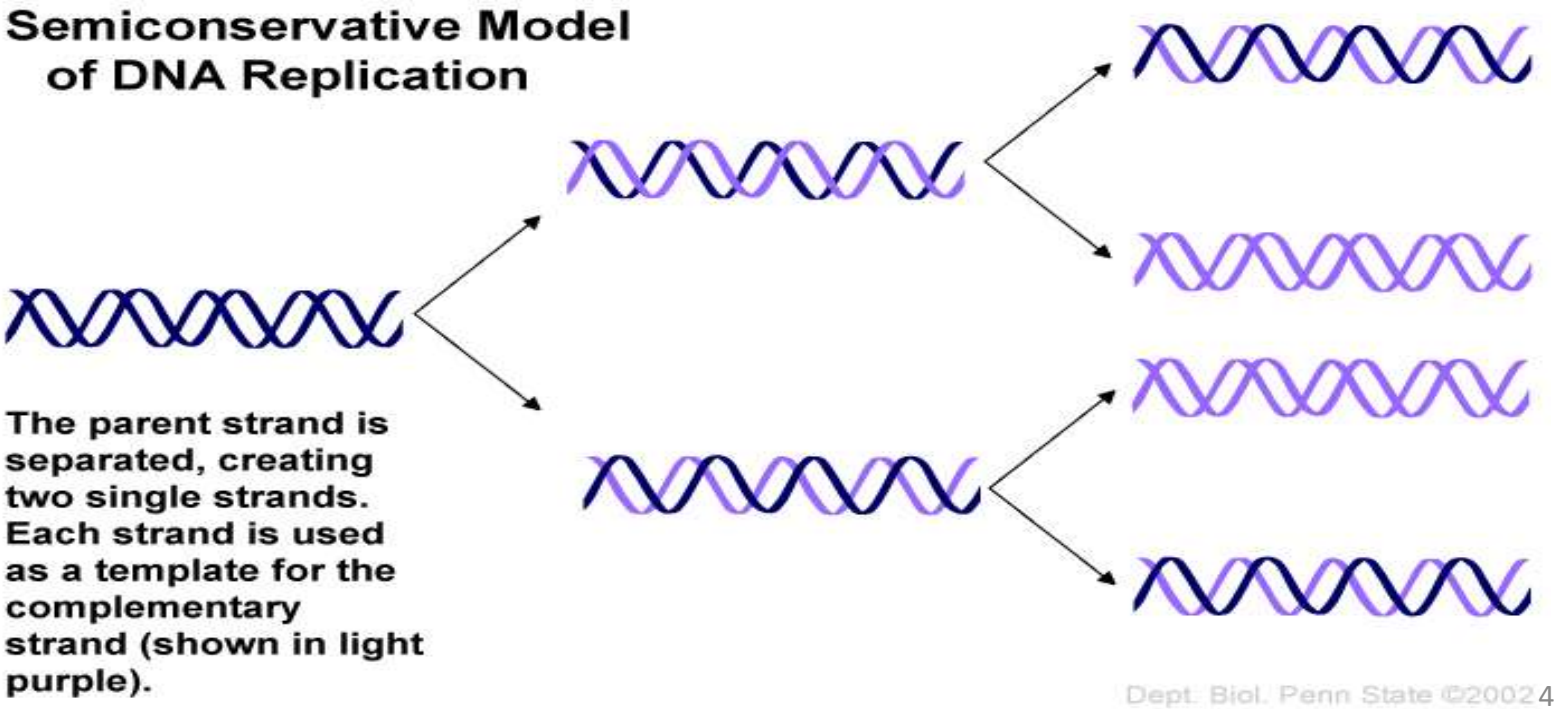
Each cell must replicate its DNA before cell division to transfer genetic information to daughter cells.



Base pairing allows each parental strand to serve as a template for new strand. So, new duplex is half parent template and half new DNA strand.

DNA replication in all organism is Semiconservative.
What is Semiconservative type of DNA 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.

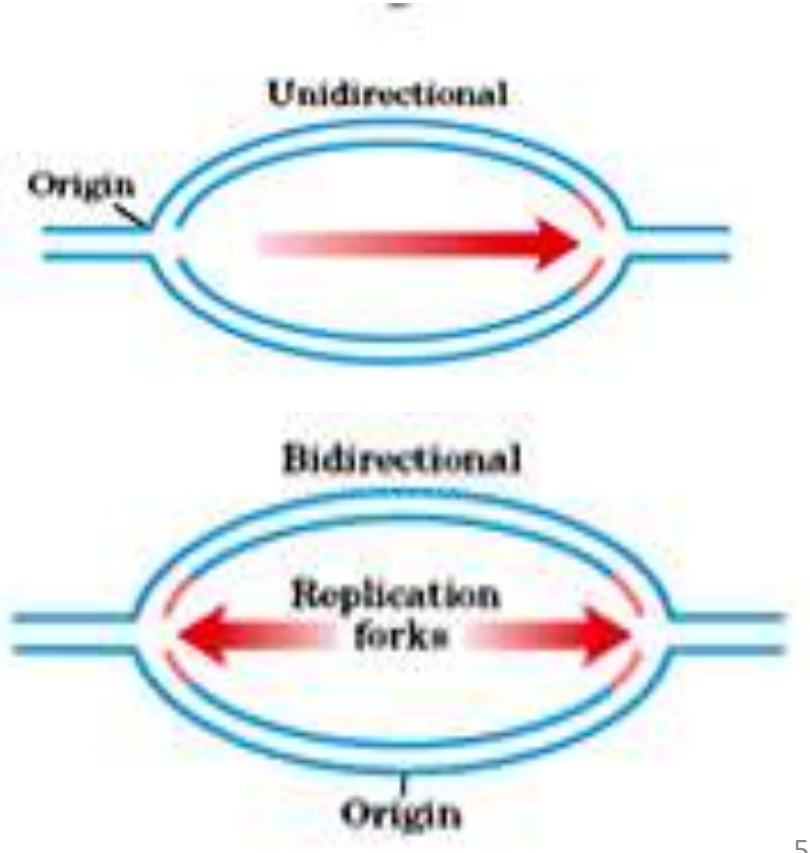


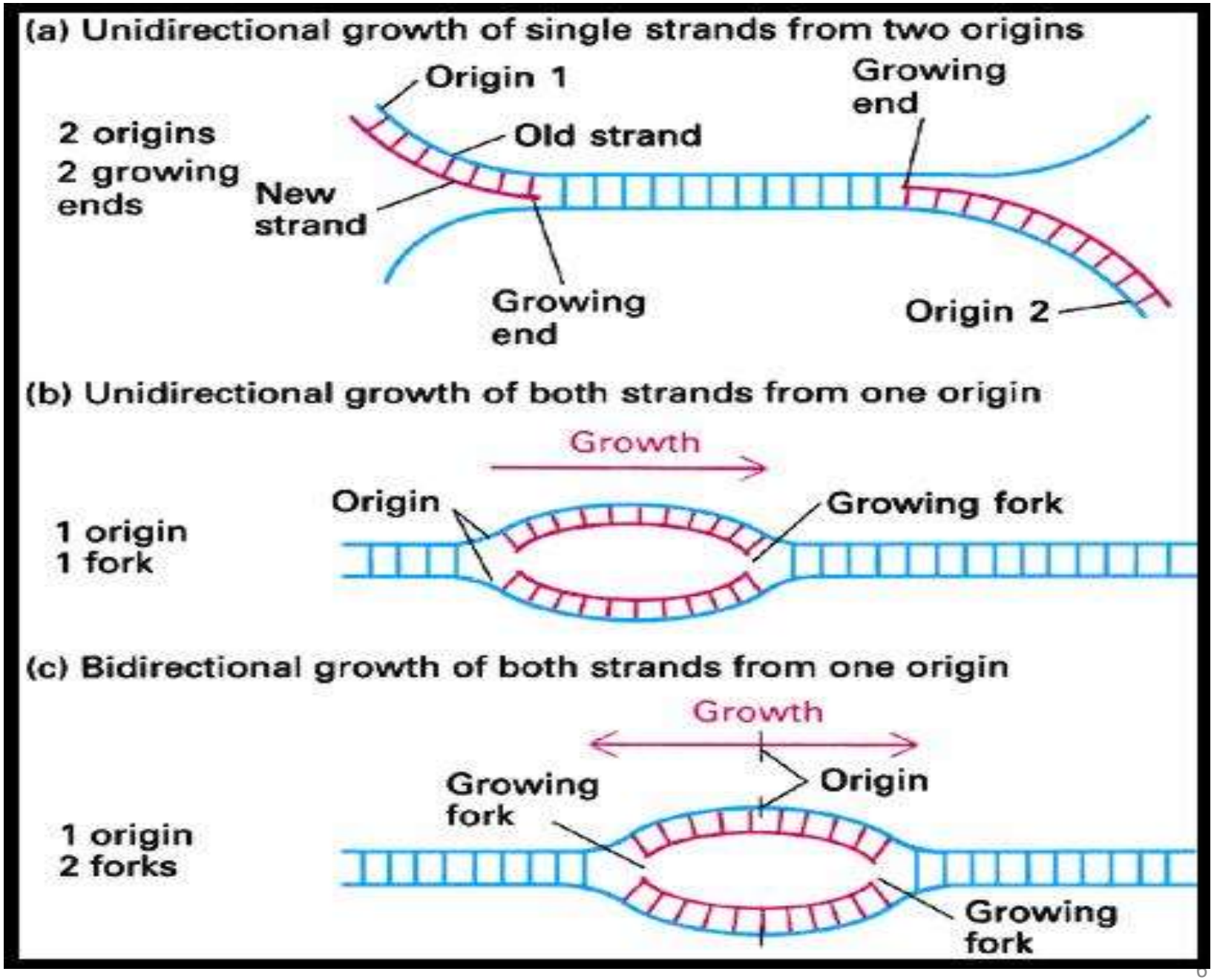
What is unidirectional and bidirectional in semiconservative DNA replication?

DNA replication begins from a particular point of origin called origin of replication (Ori).

1) In **unidirectional replication**, a single growing point moves around the circular **DNA** until **replication** is complete.

2) In **bidirectional replication**, two growing points start at the same site and move in opposite directions until they meet at the opposite side of the circle. Multiple origins of replication

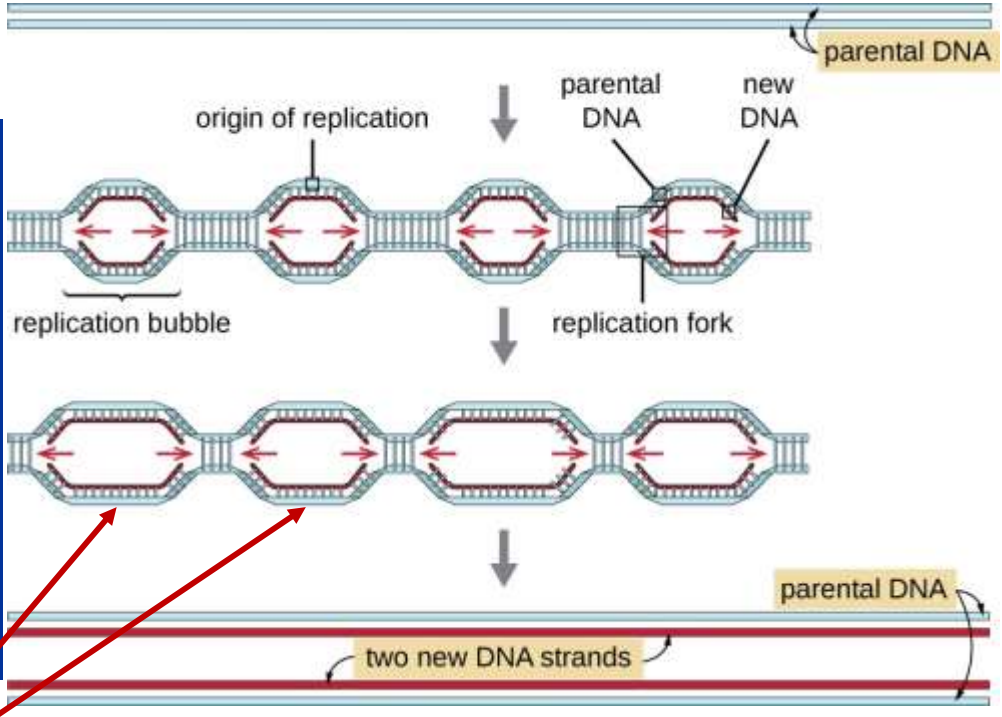



(a) Unidirectional growth of single strands from two origins
Origin 1
Growing end
2 origins
2 growing ends
Old strand
New strand
Growing end
Origin 2
(b) Unidirectional growth of both strands from one origin
Growth
Origin
Growing fork
1 origin
1 fork
(c) Bidirectional growth of both strands from one origin
Growth
Growing fork
Origin
1 origin
2 forks
Growing fork

Bidirectional replication in eukaryotes……human cells

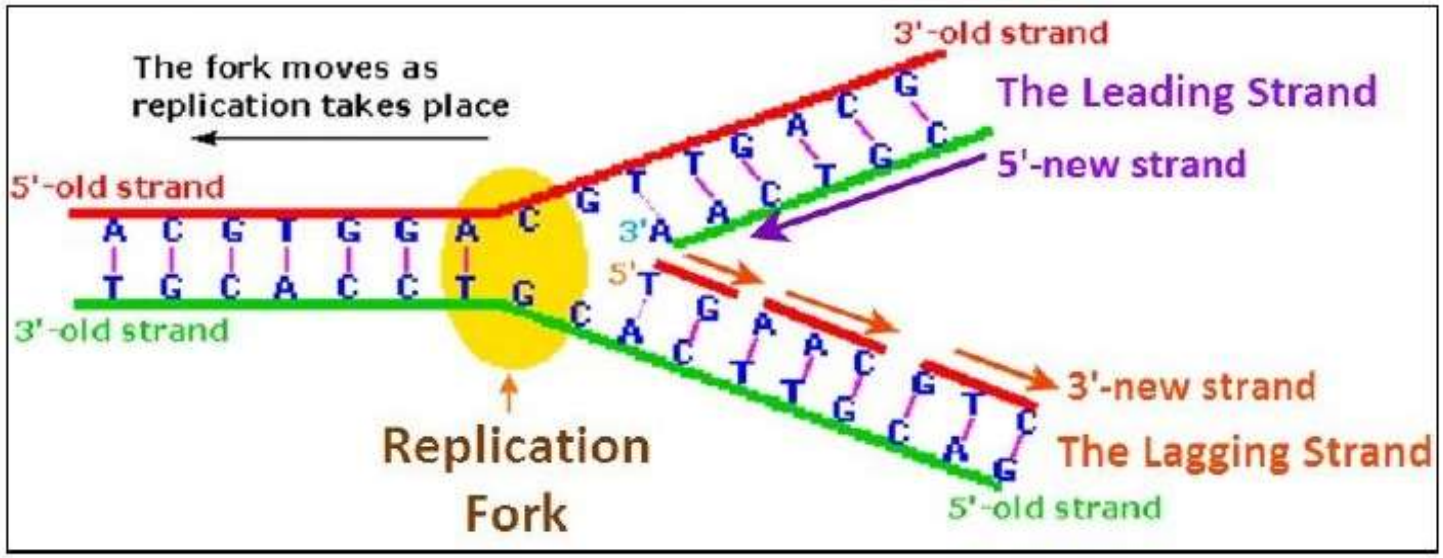
Multiple "*ori* site " is present to start the DNA replication, in eukaryotic DNA and so many replication bubbles are formed in DNA

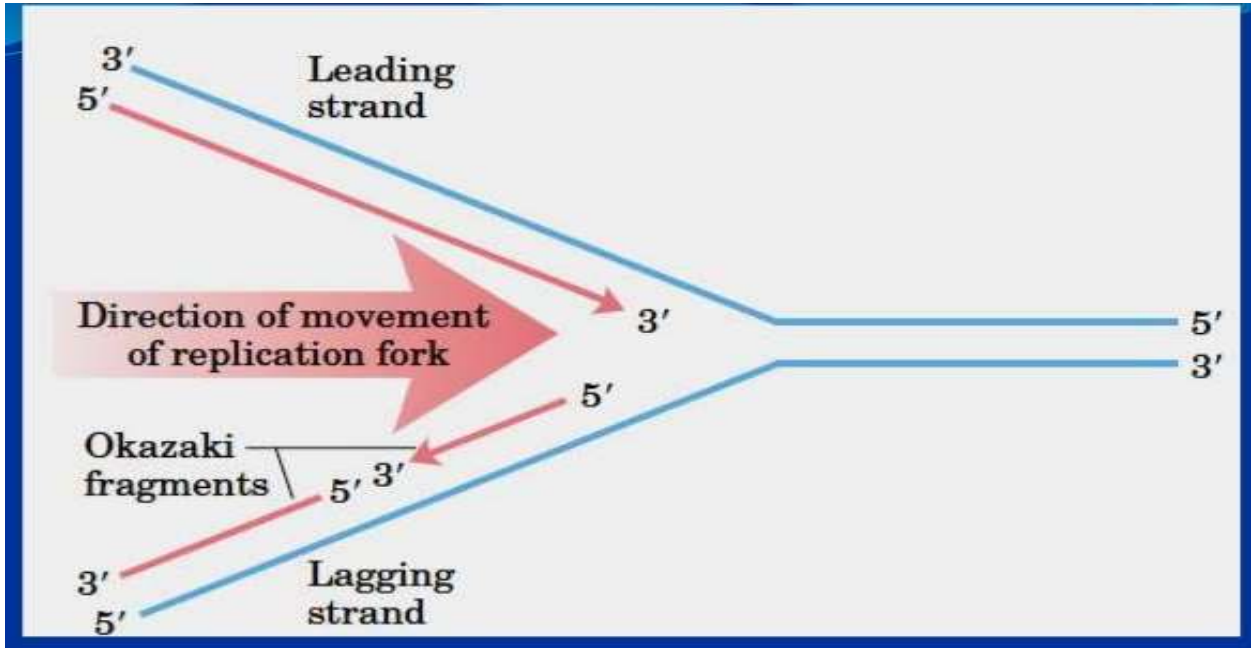
- Replication starts from unwinding the dsDNA at a particular point (called **origin / *ori* site**), followed by the synthesis on each strand.
- The parental dsDNA and two newly formed dsDNA form a Y-shape structure called Replication fork.



Many Replication bubbles are formed during bidirectional replication

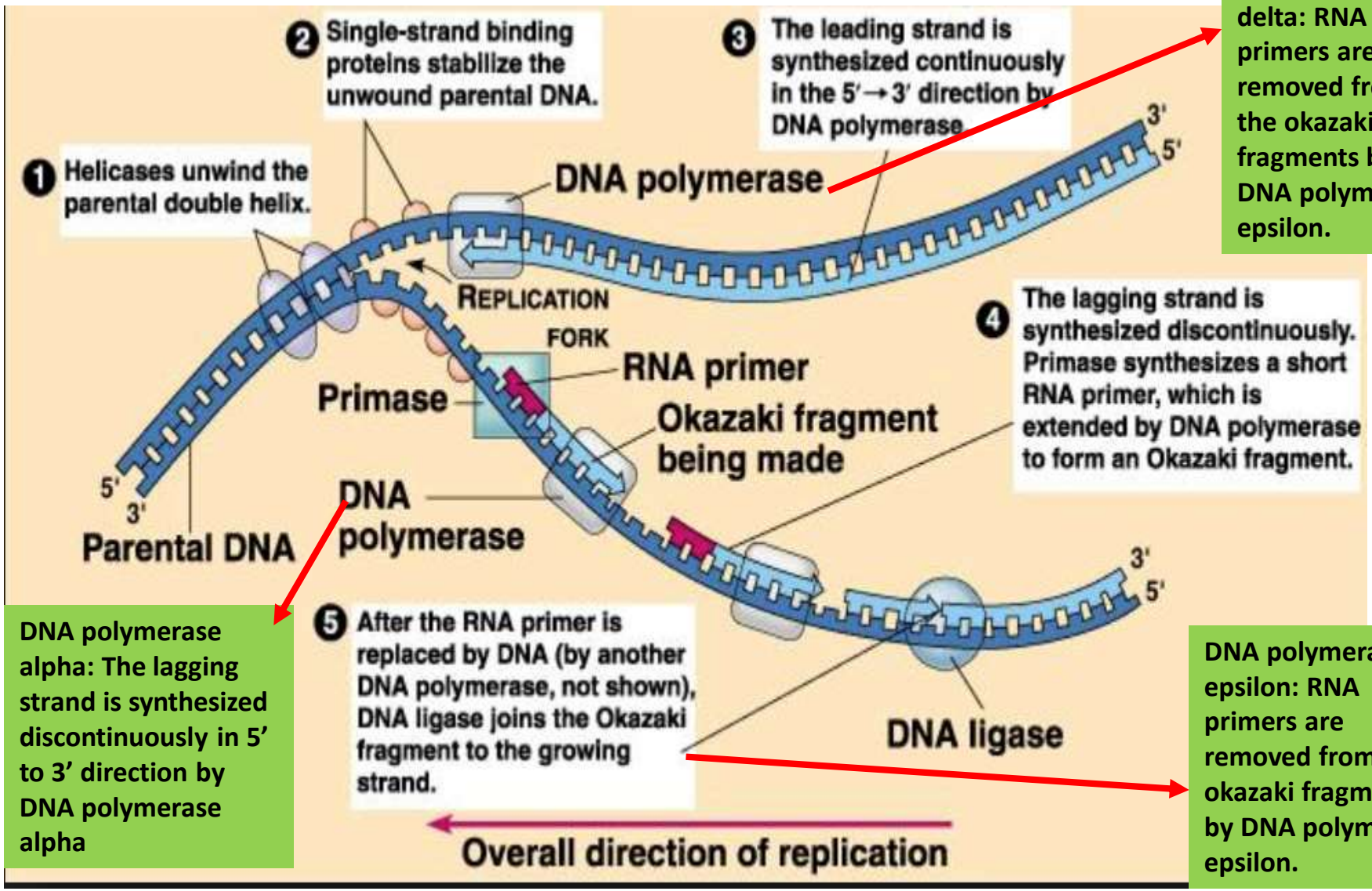
Replication fork is formed during DNA replication





Replication fork: Y-shaped structure is formed at the "*ori* site" where H-bonds between the deoxyribonucleotides are broken or Partially separated. So unzipping at the origin.

Mechanism of DNA replication and enzymes involved



DNA polymerase delta: RNA primers are removed from the okazaki fragments by DNA polymerse epsilon.

DNA polymerase alpha: The lagging strand is synthesized discontinuously in 5’ to 3’ direction by DNA polymerase alpha

DNA polymerase epsilon: RNA primers are removed from the okazaki fragments by DNA polymerse epsilon.

A. Key enzymes that are involved in DNA replication

1. **DNA helicase**: breaks the hydrogen bonds between the DNA strands. Thus, unzipping the DNA double strands
2. **Single strand binding proteins** (SSBs)-keep the parental strands apart.
3. **Topoisomerase** – Enzyme that can break bonds and reforms the bonds. The purpose is to release the twists in DNA that are generated during DNA replication.
4. **Primase**- RNA polymerase which synthesizes the primer by adding ribonucleotides that are complementary to the DNA template.
5. **DNA polymerase delta** -synthesizes a daughter strand of DNA by adding deoxyribonucleotides.
6. **DNA polymerase I in bacteria and DNA polymerase epsilon**-excises the RNA primers and fills in with DNA.
7. **DNA ligase-** covalently links the okazaki fragments. They join the fragments by phosphodiester linkage.

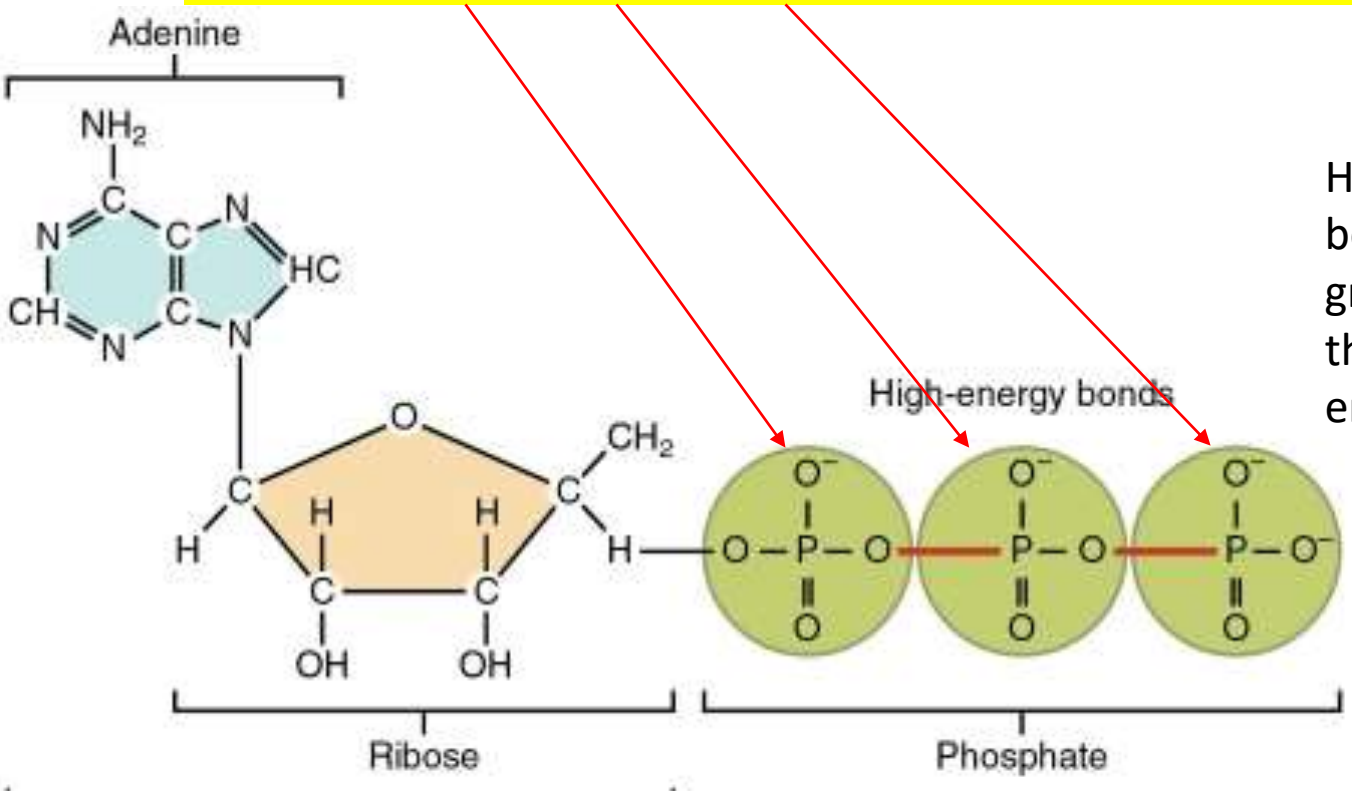
B. Key points in the process of DNA replication or DNA synthesis

1. **Direction of replication**- 5’ to 3’ direction
2. **Leading strand-** synthesized continuously in 5’ to 3’ direction by DNA polymerase delta.
3. **Lagging strands**-synthesized discontinuously by DNA polymerase alpha. The short discontinuous fragments are called “okazaki fragments”.
4. **Replication fork-** partial separation of double helix at the unzipped region of DNA is called as replication fork. Direction of fork movement is also 5’to 3’..
5. **DNA synthesis requires the four deoxynucleoside triphosphates-** dATP, dGTP, dCTP, dTTP. Nucleoside triphospahtes (NTPs) have three phosphoryl groups which are attached via the 5’-hydroxyl of the 2’-deoxyribose. The innermost phosphoryl group is called alpha-phosphate, the middle one is beta & the outermost one is gamma phosphate.
6. **DNA is synthesized by extending the 3’ end of the primer**. The 3’-end contains the –OH group to which new nucleotide is added. The 3’-OH group attacks the alpha phosphoryl group of incoming Nucleoside triphosphate. The leaving group is pyrophosphate which arises from the release of beta and gamma phosphates of the substrate.

The reaction is indicated as XTP + (XMP)n→(XMP)n+1 + P-P (pyrophosphate)

The breaking down of pyrophosphate by an enzyme pyrophosphatase releases energy that forms the driving force of DNA synthesis

dATP as an example-deoxyadenosine triphosphate having alpha, beta, gamma phosphate



High energy bonds between phosphate groups…..breakage of these bonds provide energy for DNA synthesis.

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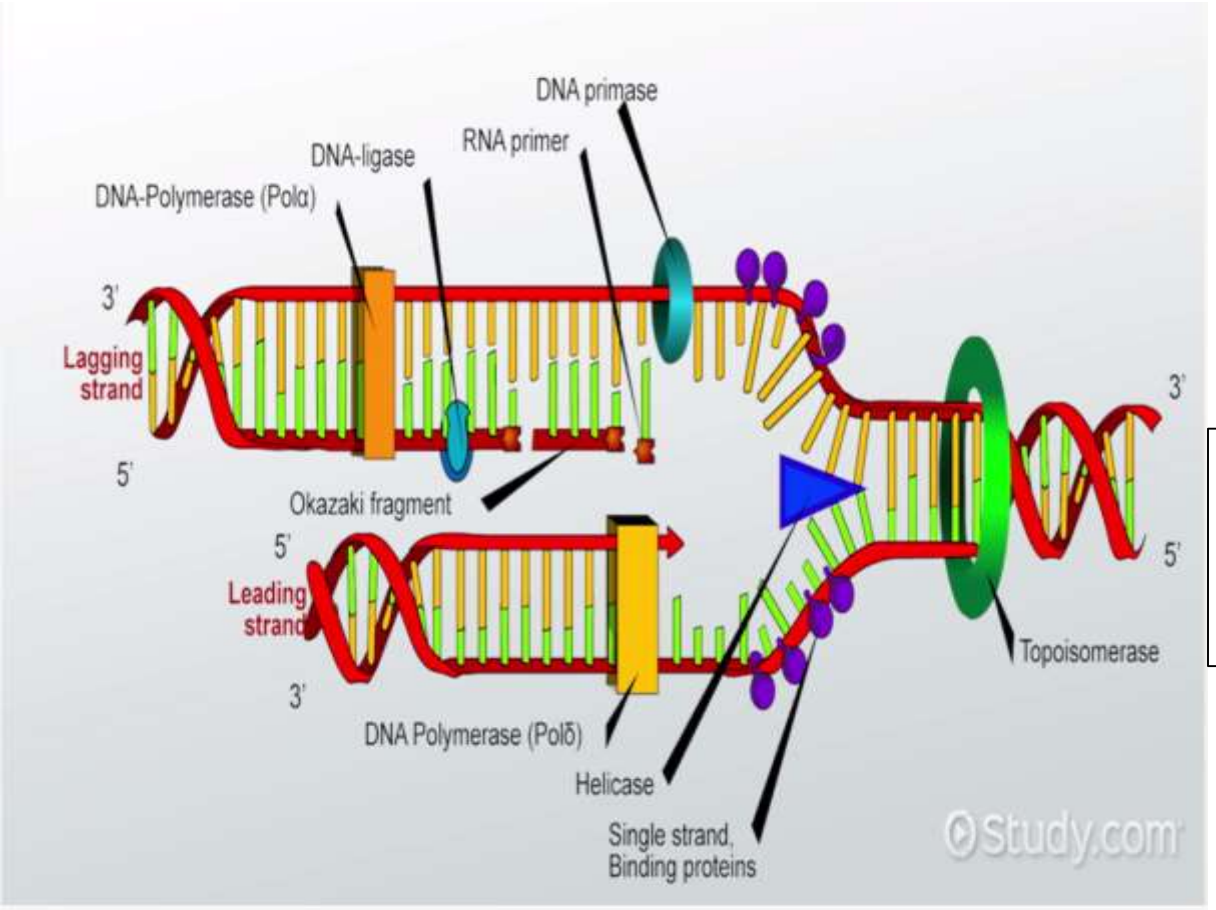


1. **DNA helicase and topoisomerase**
Unwinds the DNA double helix

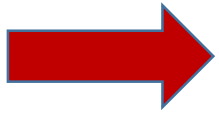
2. SSBs stabilizes the unwound parental strands

3. The leading strand is synthesized continuously in 5' to 3' direction by DNA polymerase delta. The short starting fragment is the RNA primer

4. The lagging strand is synthesized discontinuously in 5' to 3' direction by DNA polymerase alpha. Primase synthesizes short RNA primer which is extended by DNA polymaerse to form okazaki fragments

5. RNA primers are removed from the okazaki fragments by DNA polymerse epsilon. The fragments are joined by DNA ligase

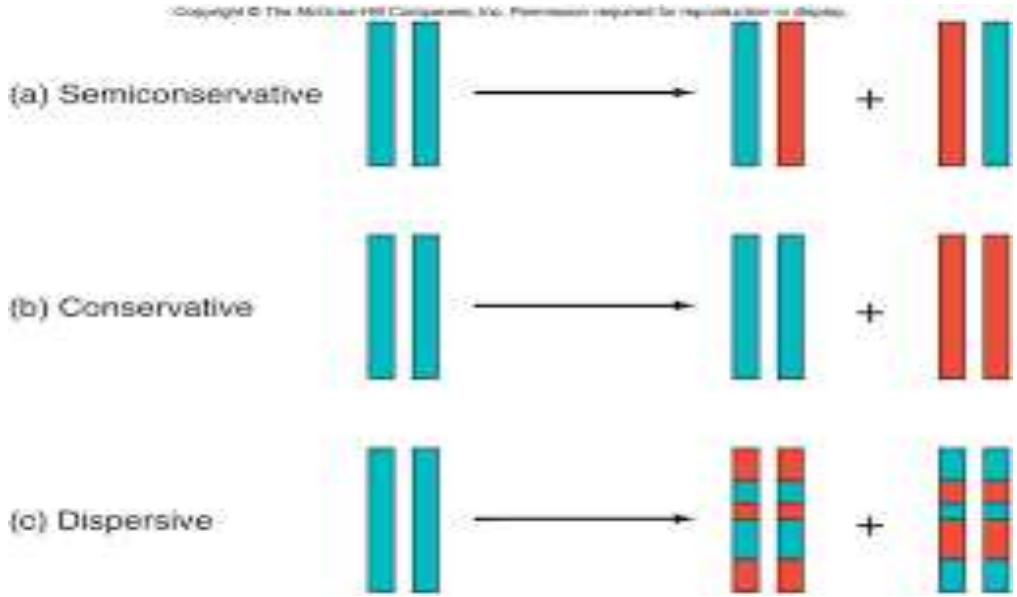
Meselson and Stahl Experiment to prove the semiconservative mode of DNA replication



Proposed Models of DNA Replication

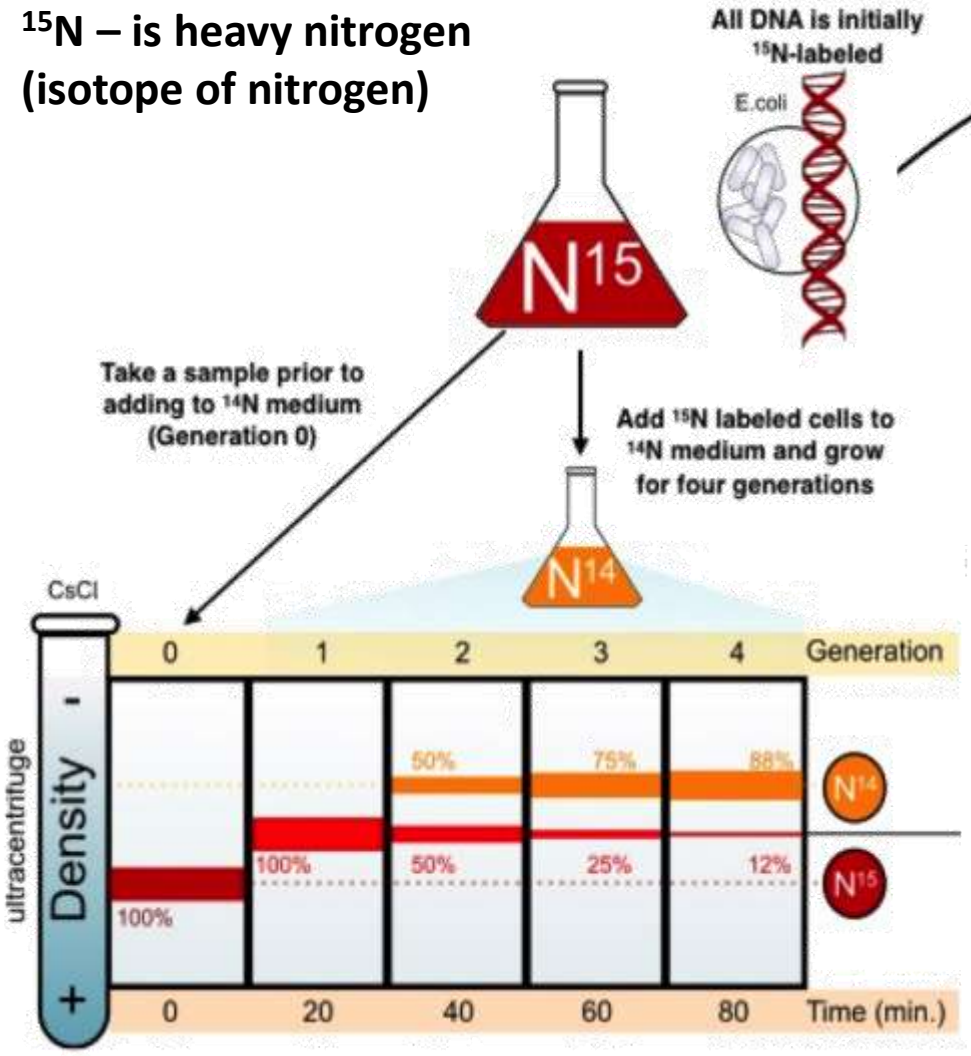
- In the late 1950s, three different mechanisms were proposed for the replication of DNA
  - **Conservative model**
    - Both parental strands stay together after DNA replication
  - **Semiconservative model**
    - The double-stranded DNA contains one parental and one daughter strand following replication
  - **Dispersive model**
    - Parental and daughter DNA are interspersed in both strands following replication

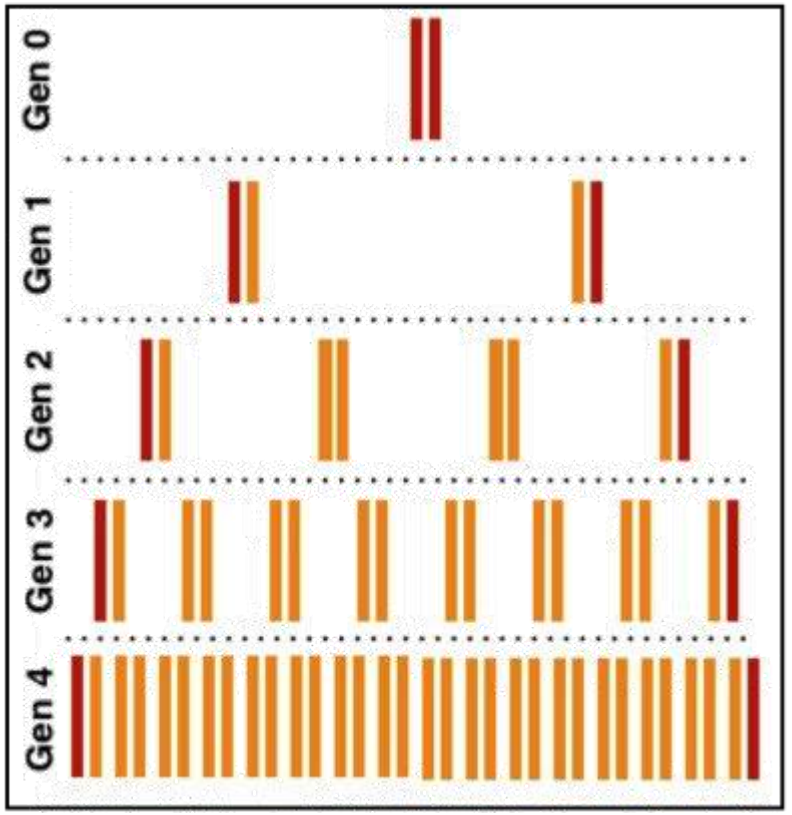
Three possible replication patterns



^{14}N - is light nitrogen
^{15}N – is heavy nitrogen (isotope of nitrogen)

Semiconservative model of replication

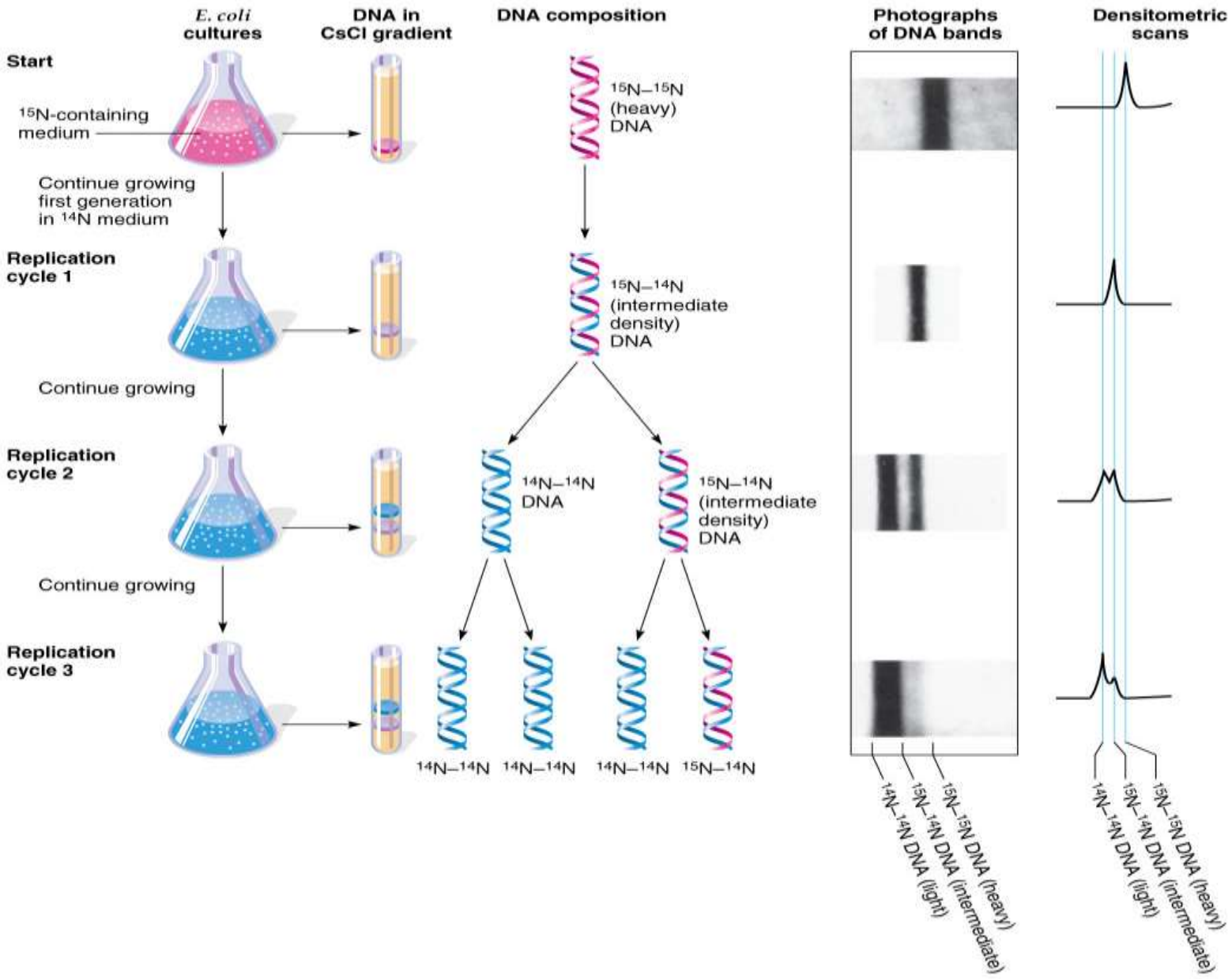




Brief explanation of model of DNA replication

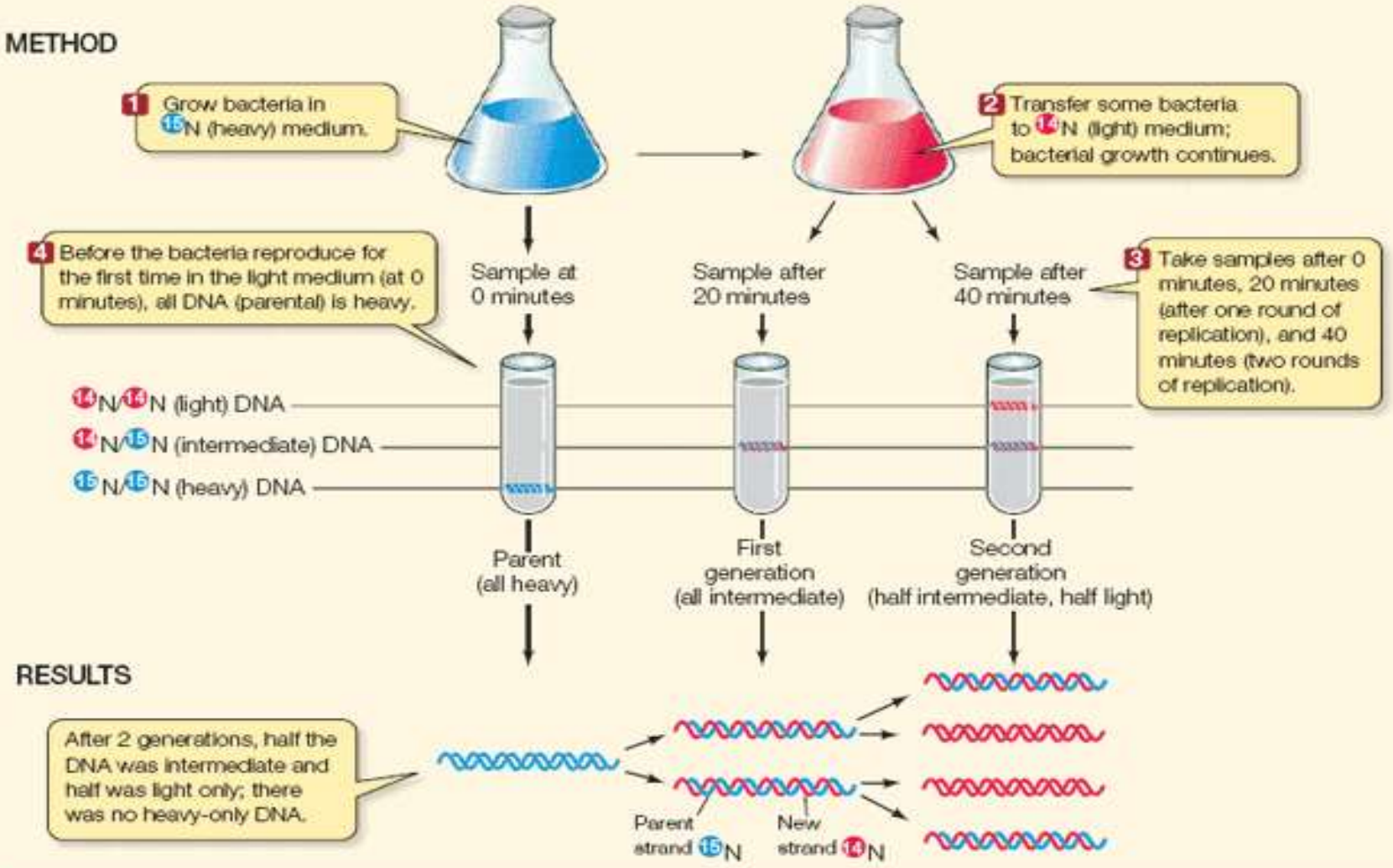
- 1. Bacteria were grown in a medium containing nitrogen 15 (N^{15}) for several generation
- 2. If the medium contains no other nitrogen source, the E. coli will use N^{15} and incorporate it into their DNA
- 3. Eventually, they will only have N^{15}
- 4. Once the E. coli had only N^{15} they were put into a growing medium contain only N^{14}
- 5. N^{15} is heavier than N^{14} making new incorporation of nitrogen easy to distinguish
- 6. The differences were measured according to the densities of the new strands

Semiconservative model of replication



EXPERIMENT

HYPOTHESIS: DNA replicates semiconservatively.



CONCLUSION: This pattern could only have been observed if each DNA molecule contains a template strand from the parental DNA; thus DNA replication is semiconservative.

https://www.youtube.com/watch?v=TNKWgcFPHqw