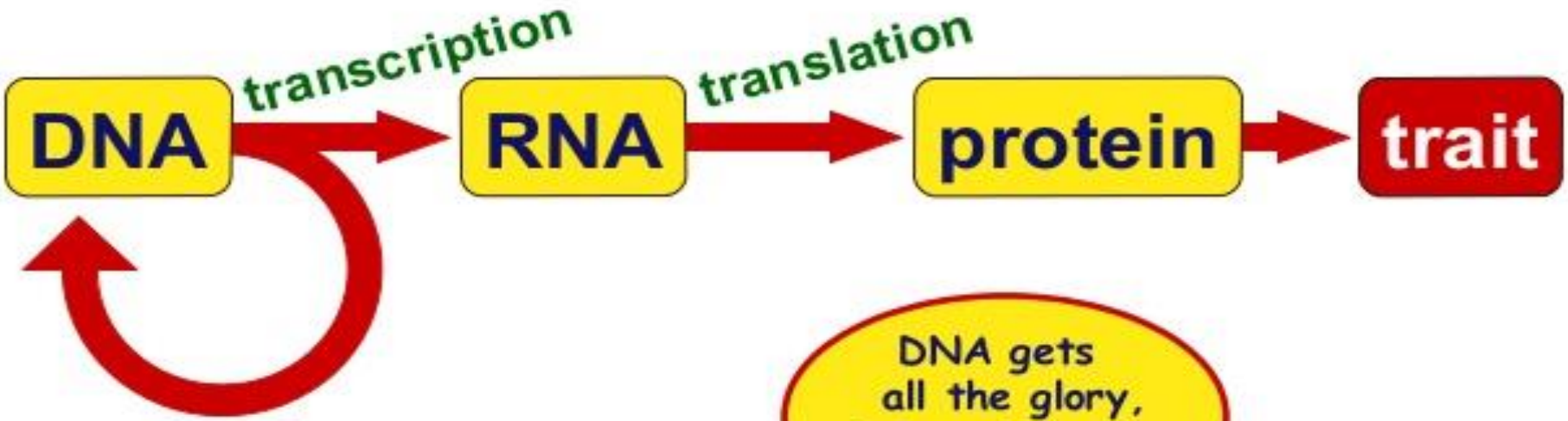
Flow of information: from DNA (genes) to proteins

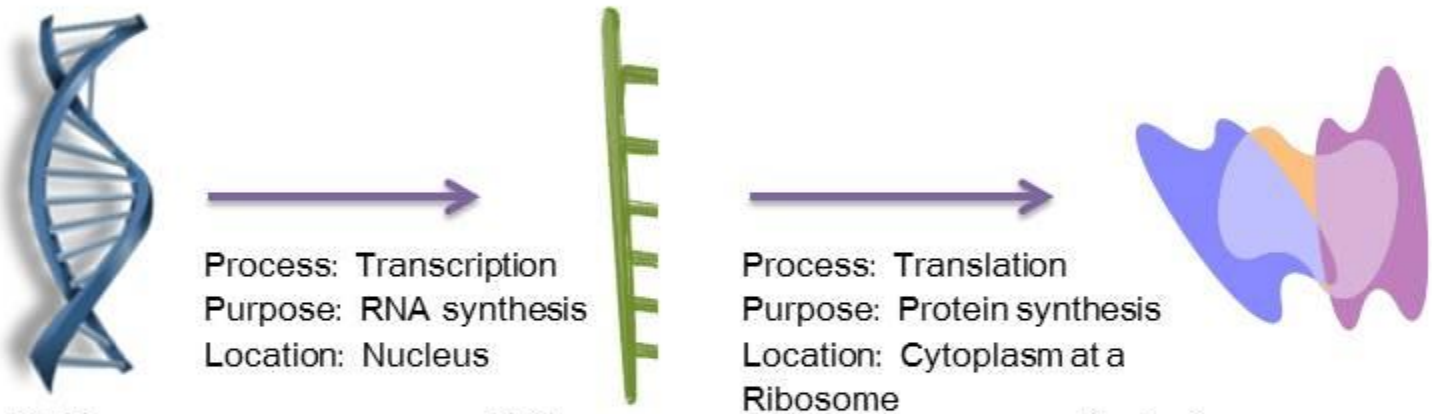
❖ Expression of genes

The "Central Dogma"

- Flow of genetic information in a cell
  - How do we move information from DNA to proteins?



The Central Dogma



DNA contains the original codes for making the proteins that living cells need. mRNA is a copy of a gene located on the DNA molecule. mRNA will leave the nucleus of the cell and the ribosome will read its coding sequences and put the appropriate amino acids together.

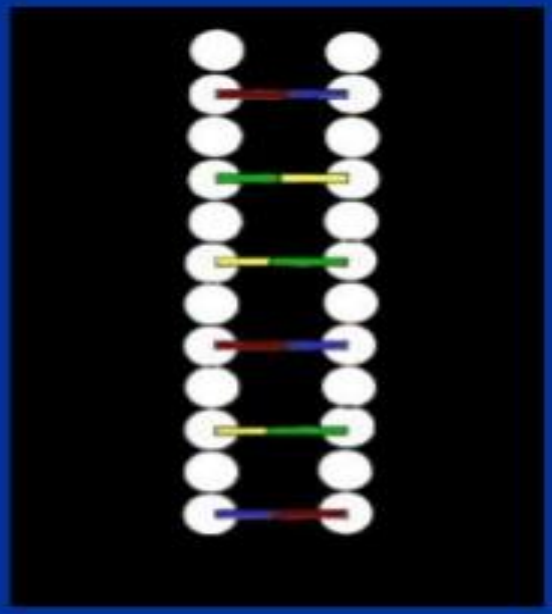
DNA Replication

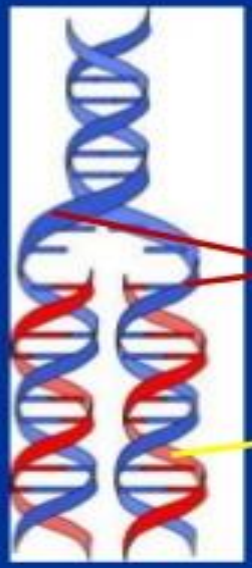
- DNA replication is a biological process that occurs in all living organisms and copies their exact DNA. It is the basis for biological inheritance.
- **Replication** is the process of synthesis of daughter DNA from parental DNA by the enzyme **DNA Polymerase.**
- $(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1}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

  DNA → Lengthened DNA

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

Each parent strand serves as a template for a new strand and the two new DNA strands each have one old and one new strand





- **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.**

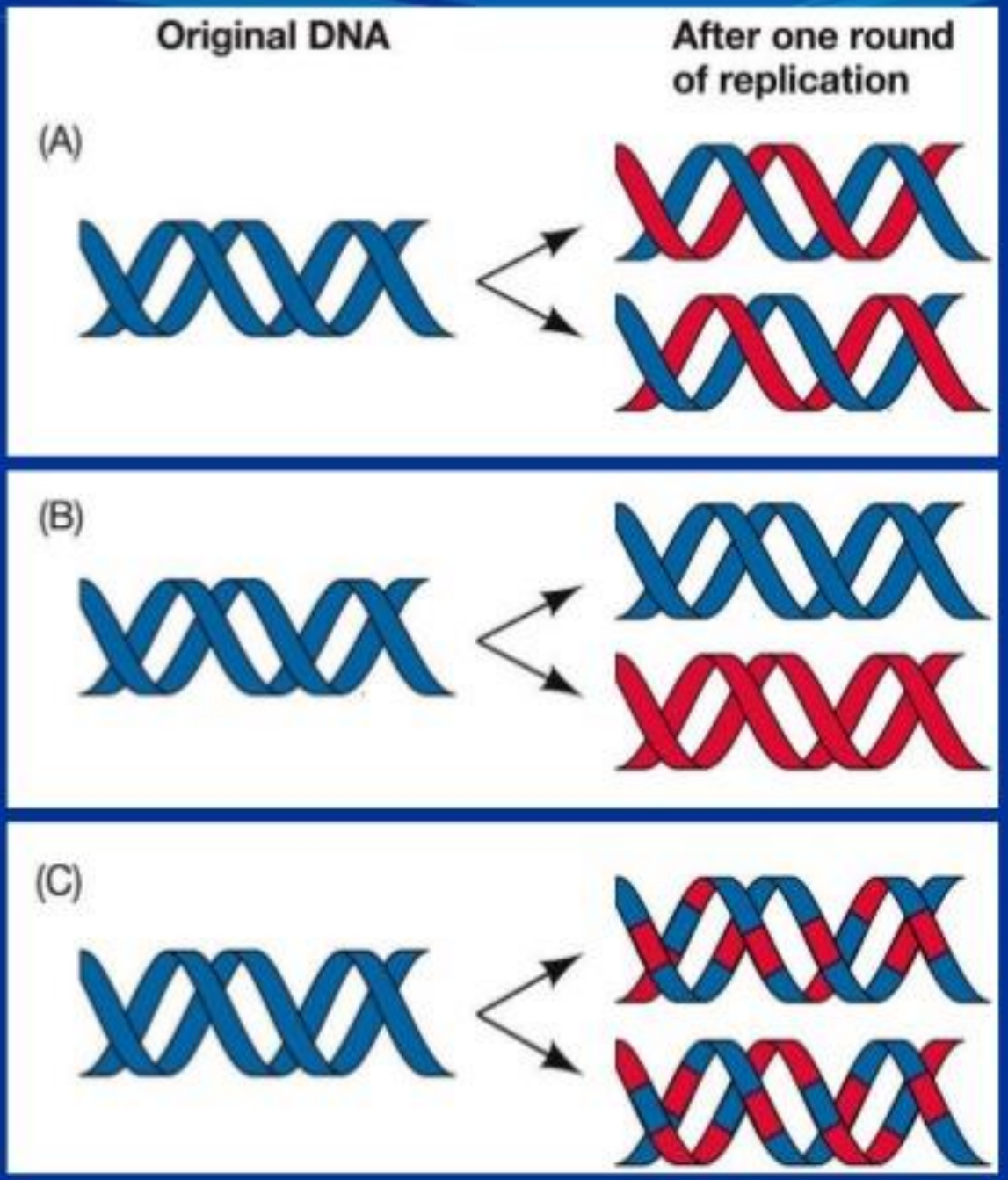
Three possible replication patterns

1. *Semiconservative replication*
2. *Conservative replication*
3. *Dispersive replication*

Semiconservative replication

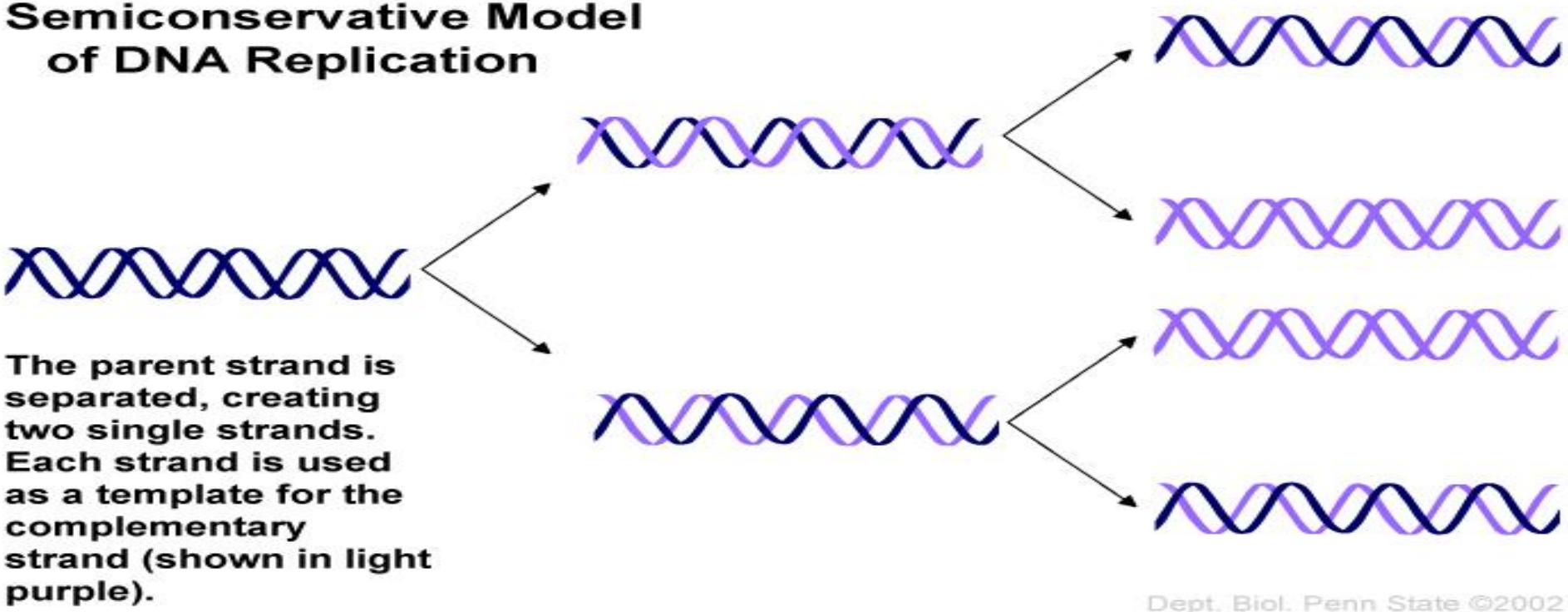
Conservative replication

Dispersive replication



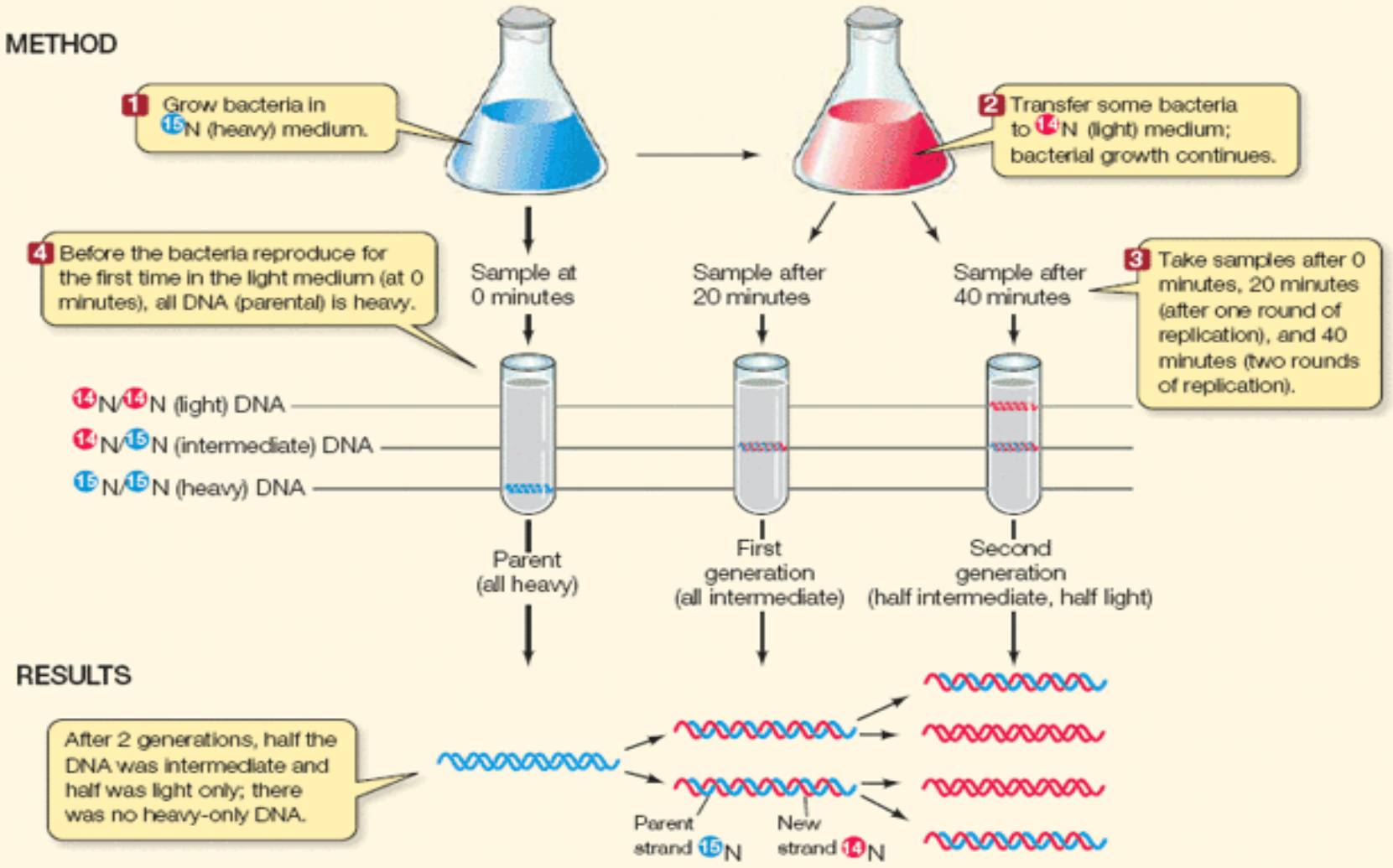
Semiconservative Replication

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.



Meselson & Stahl Experiment

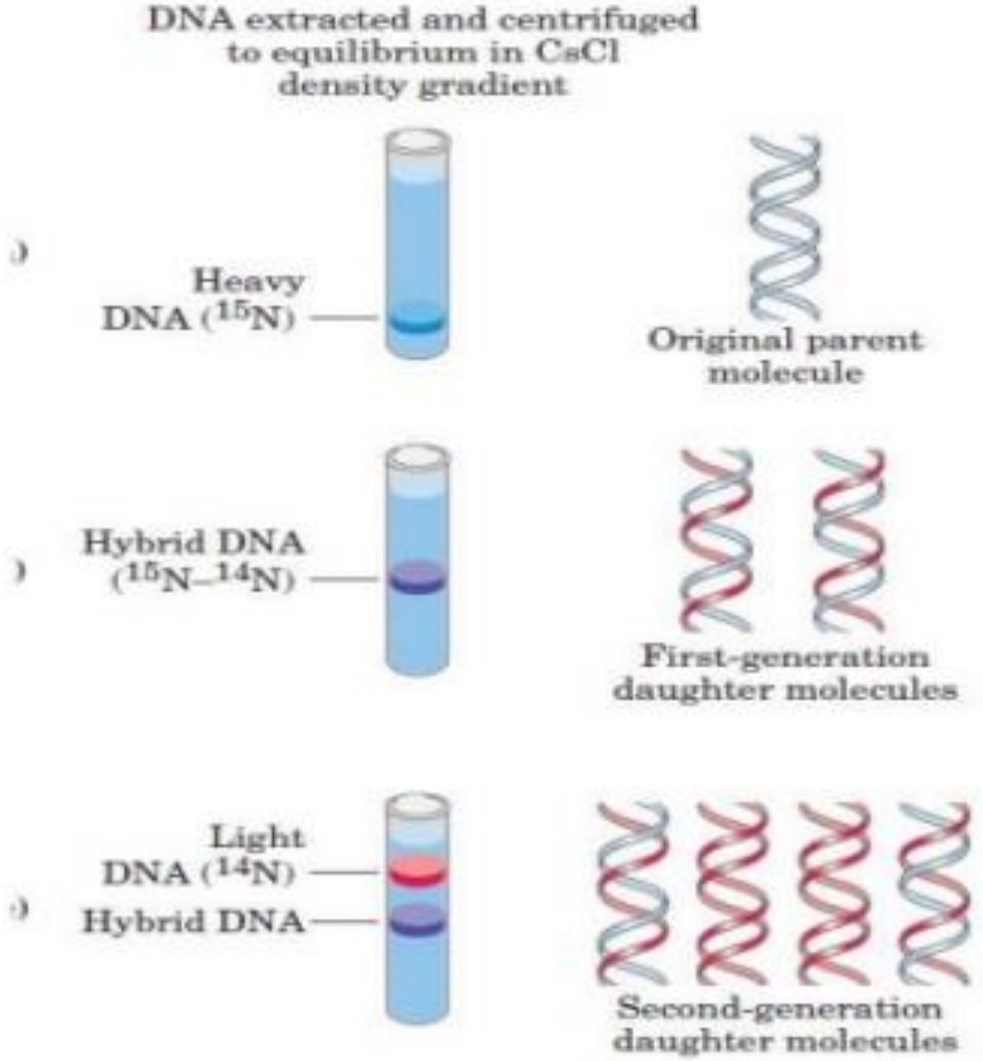
HYPOTHESIS: DNA replicates semiconservatively.



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.

- 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

MESELSON AND STAHL EXPERIMENT



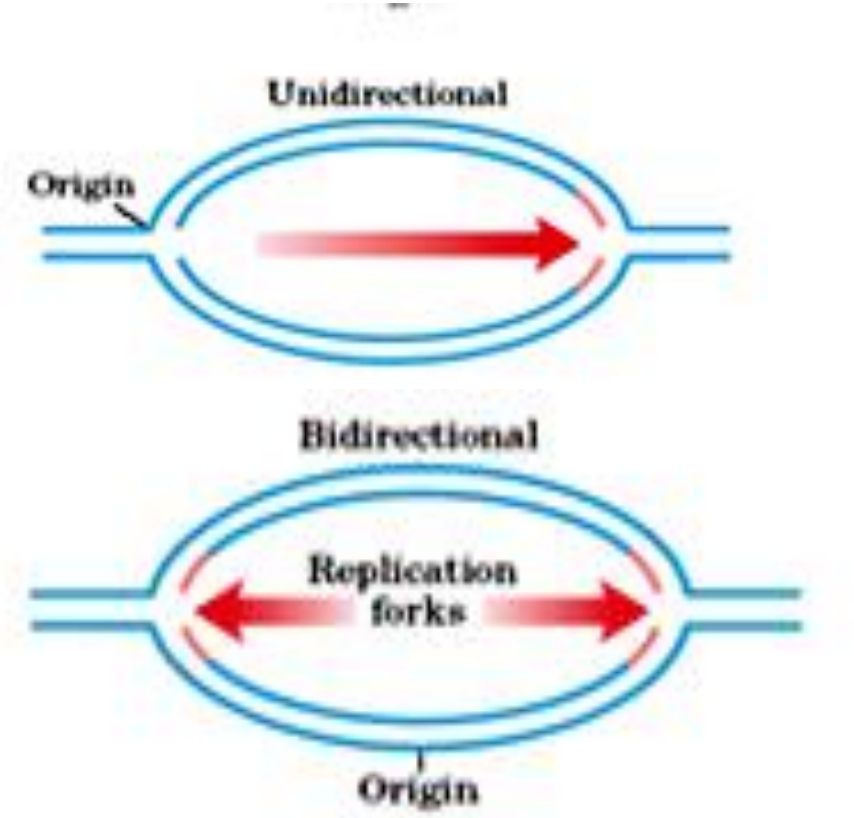
conclusion:
Semiconservative replication

What is unidirectional and bidirectional replication

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

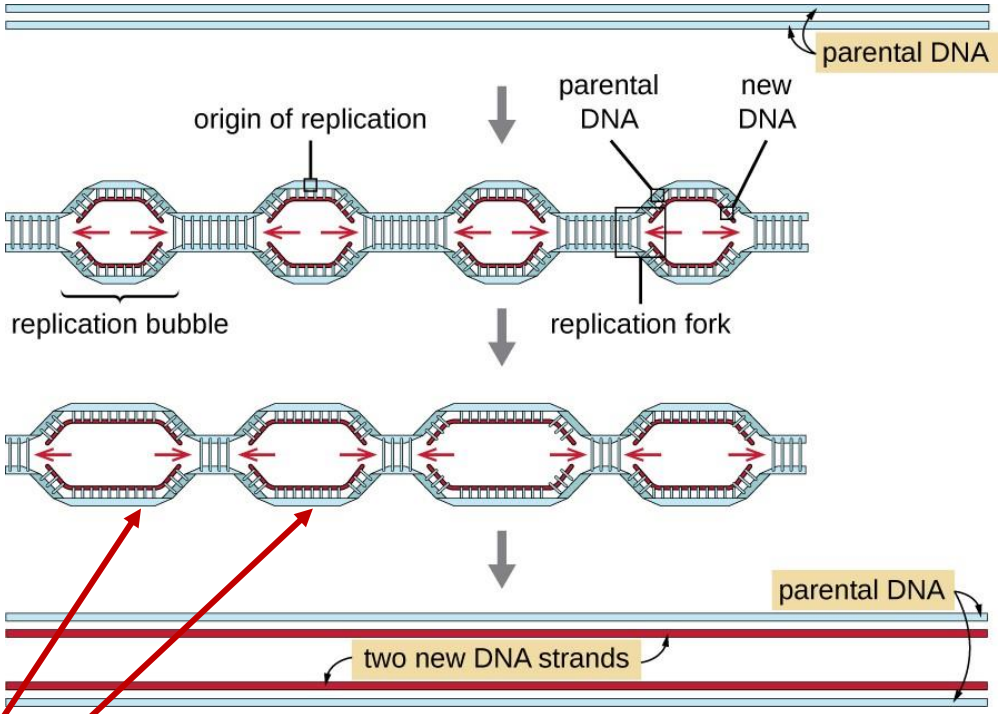
- In **unidirectional replication**, a single growing point moves around the circular **DNA** until **replication** is complete.

- 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.

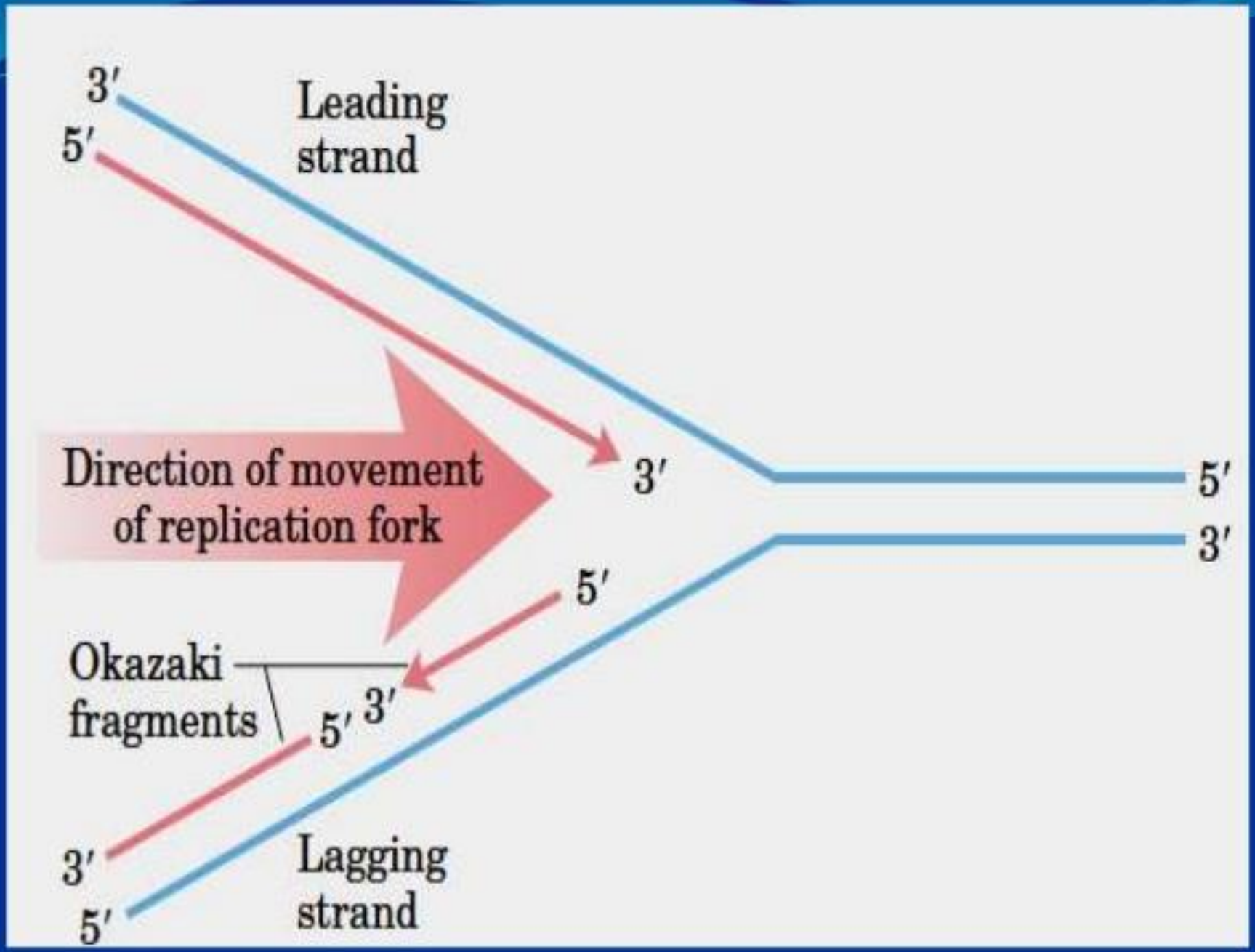


Bidirectional replication

- 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**


3′
5′
Leading strand
Direction of movement of replication fork
3′
5′
3′
5′
Okazaki fragments
5′
3′
3′
5′
Lagging strand

Key points in the process of DNA replication

- **Direction of replication**- 5’ to 3’ direction
- **Leading strand-** synthesized continuously in 5’ to 3’ direction by DNA polymerase.
- **Lagging strands**-synthesized discontinuously. The short discontinuous fragments are called **“okazaki fragments”.**
- **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’.
- **DNA synthesis requires the four deoxy nucleoside triphosphates-** dATP, dGTP, dCTP, dTTP. Nucleoside triphosphates (NTPs) have three phosphoryl groups which are attached via the **5’-hydroxyl of the 2’-deoxyribose sugar**. The innermost phosphoryl group is called alpha-phosphate, the middle one is beta & the outermost one is gamma phosphate.
- **DNA is synthesized by extending the 3’ end of the primer**. The 3’-end contains the –OH group to which new nucleotides are added. The 3’-OH group attacks the alpha phosphoryl group of incoming Nucleoside triphosphate. The leaving group is pyrophosphate which arises due to release of beta and gamma phosphates of the substrate.

The reaction is indicated as NTP + (NMP)n→(NMP)n+1 + PPi (pyrophosphate)

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

Key enzymes that are involved in DNA replication

- **DNA helicase**: breaks the hydrogen bonds between the DNA strands. Thus, unzipping the DNA double strands
- **Single strand binding proteins** (SSBs)-keep the parental strands apart.
- **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.
- **Primase**- RNA polymerase which synthesizes the primer by adding ribonucleotides that are complementary to the DNA template.
- **DNA polymerase**-synthesizes a daughter strand of DNA by adding deoxyribonucleotides.
- **DNA polymerase I in bacteria and DNA polymerase epsilon**-excises the RNA primers and fills in with DNA.
- **DNA ligase-** covalently links the Okazaki fragments. They join the fragments by phosphodiester linkage.

Steps of DNA replication

1. DNA helicase and topoisomerase unwinds the DNA double helix

↓

2. SSBs stabilizes the unwound parental strands

↓

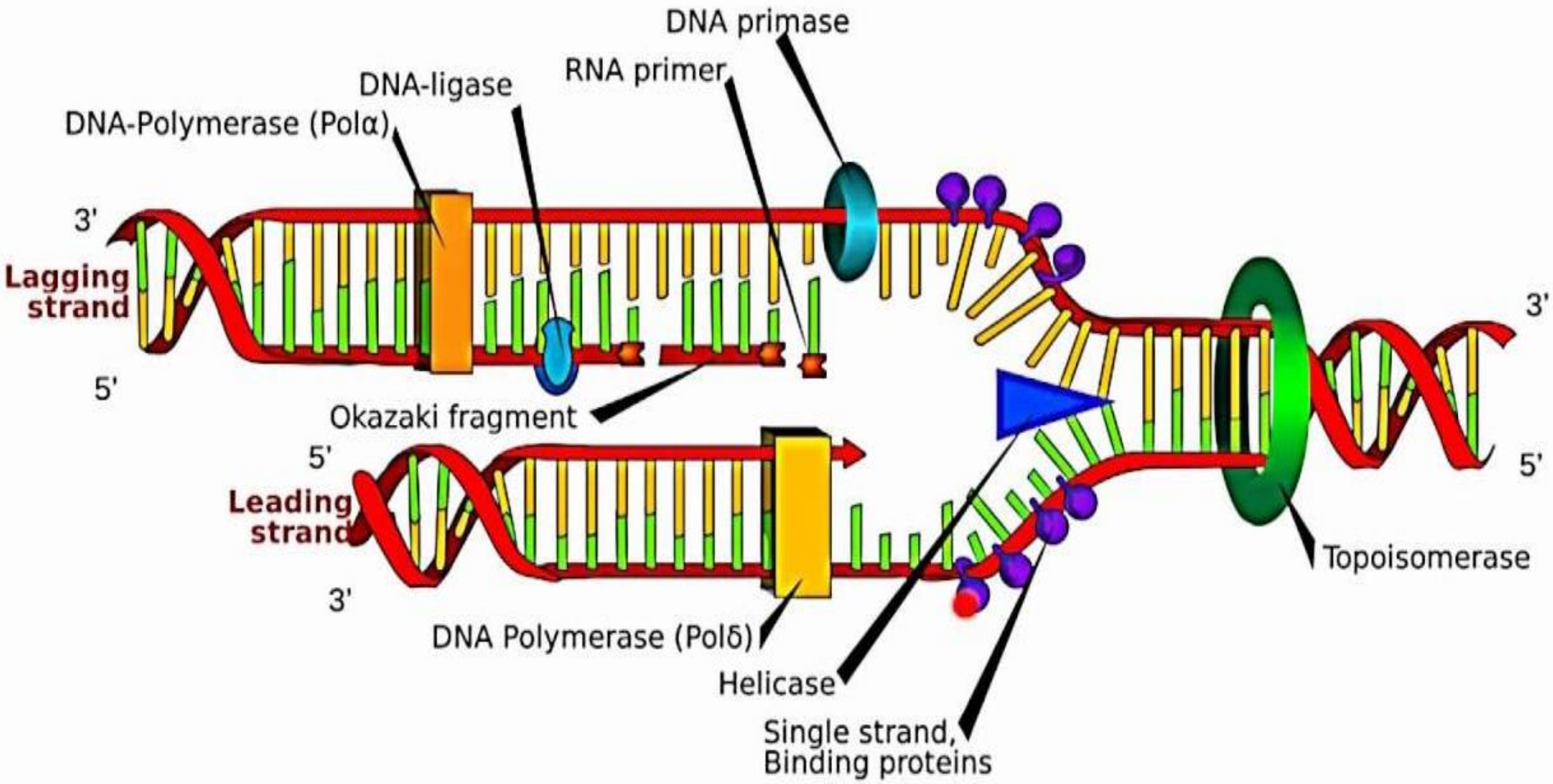
3. The leading strand is synthesized continuously in 5’ to 3’ direction by DNA polymerase delta.

↓

4. The lagging strand is synthesized discontinuously in 5’ to 3’ direction by DNA polymerase alpha. Primase synthesizes short starting fragment called as RNA primer which is extended by DNA polymerase to form Okazaki fragments.

↓

5. RNA primers are removed from the okazaki fragments by DNA polymerase epsilon. The fragments are joined by DNA ligase through phosphodiester bond



The first step in DNA replication is to separate the strands of DNA using helicase
Then DNA primase comes in to create a RNA primer
Then DNA polymerase comes in and synthesizes the new strand of DNA
Single strand binding proteins make sure the two strands don't come back together
Topoisomerase helps relieve the tension created by unwinding the DNA
Finally, DNA ligase sticks the okazaki fragments together in the lagging strand