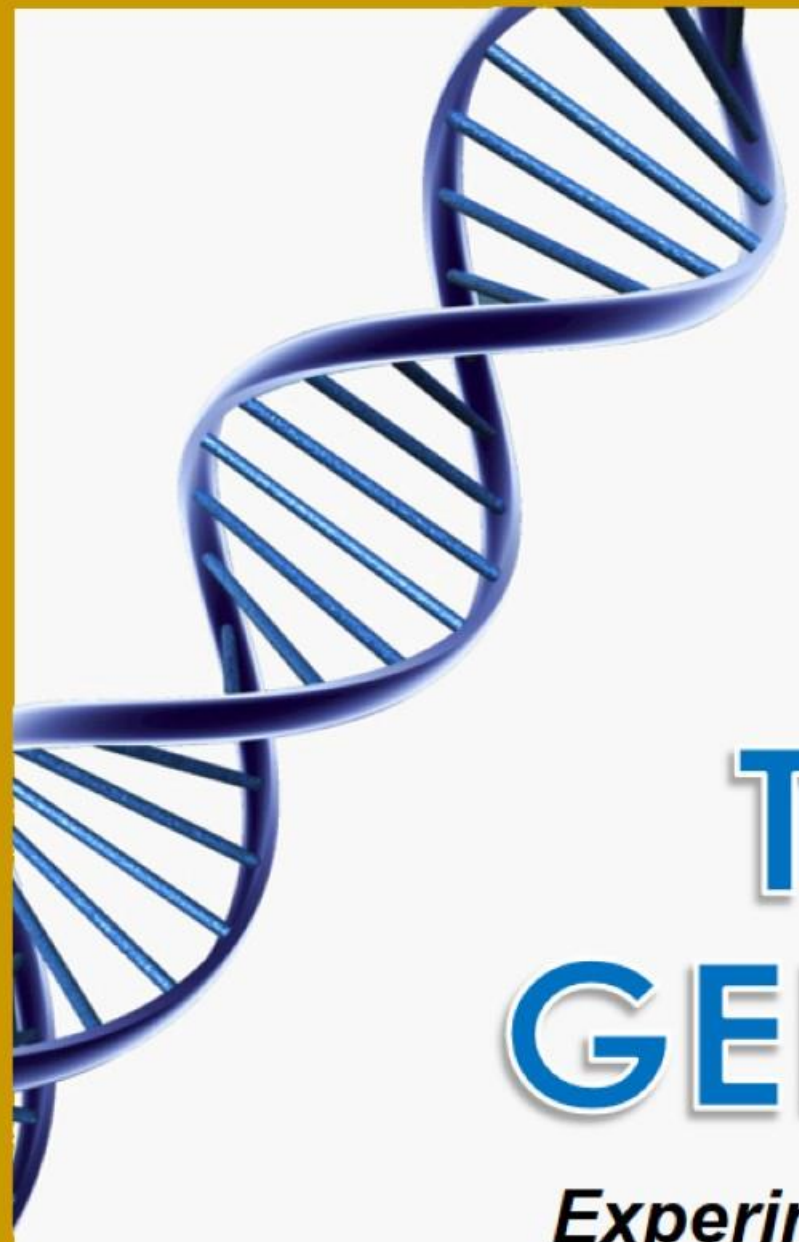
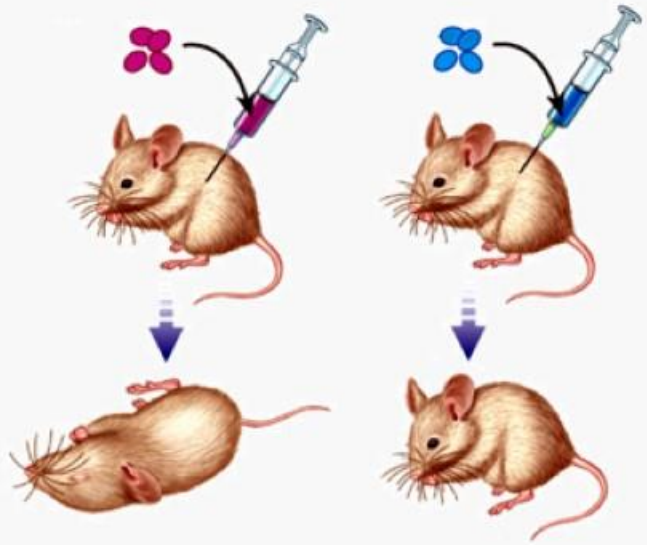
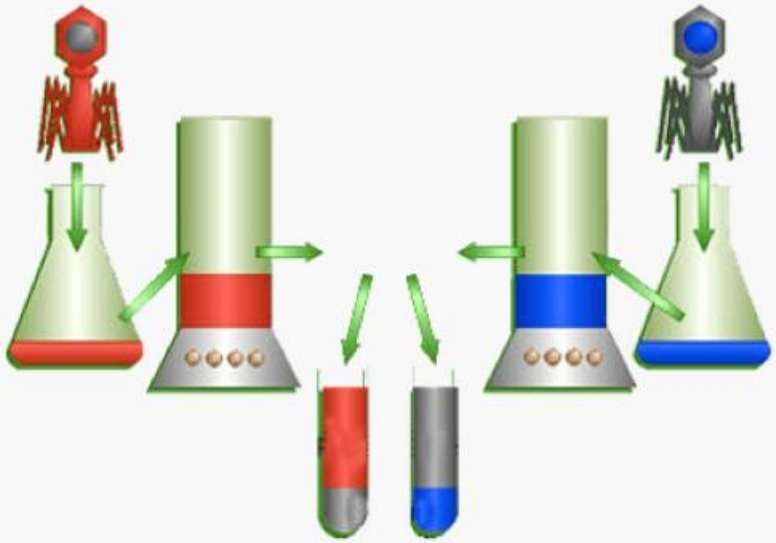






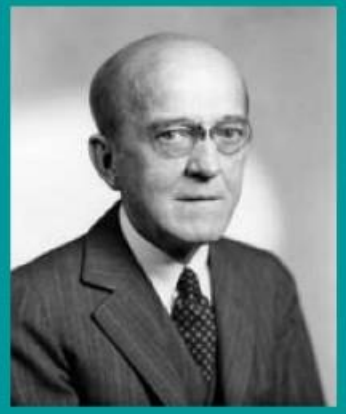
THE SEARCH FOR GENETIC MATERIAL

Experiments to prove which is the genetic material…

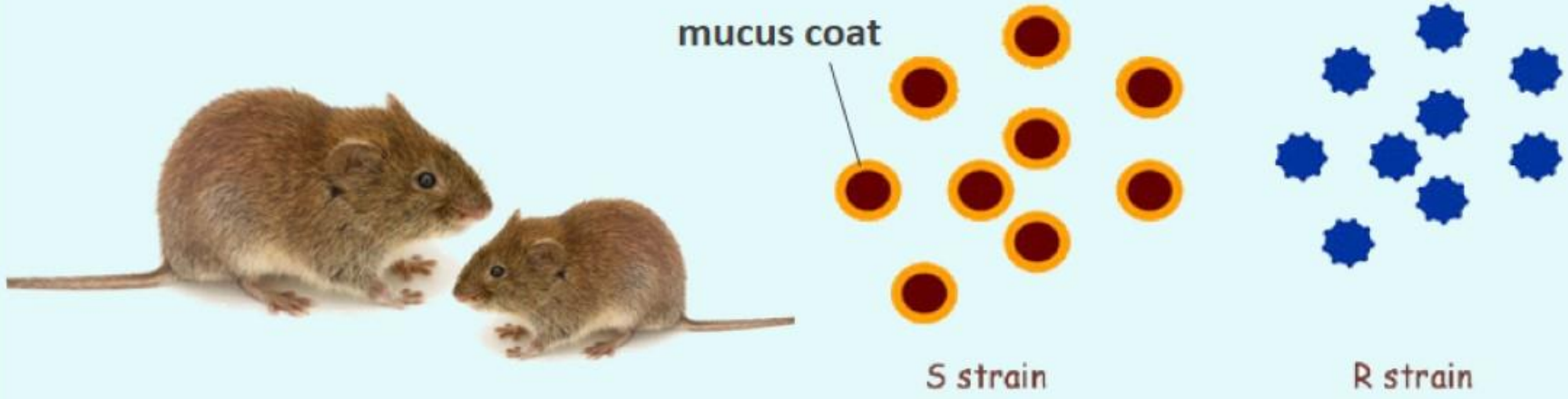
THE SEARCH FOR GENETIC MATERIAL

1

GRIFFITH'S TRANSFORMING PRINCIPLE EXPERIMENT



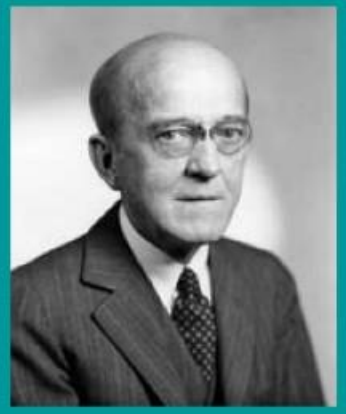
- **Frederick Griffith (1928)** used **mice & *Streptococcus pneumoniae.***
- ***Streptococcus pneumoniae*** has 2 strains:
  - **Smooth (S) strain (Virulent):** Has polysaccharide mucus coat. Cause pneumonia.
  - **Rough (R) strain (Non-virulent):** No mucus coat. Do not cause Pneumonia.

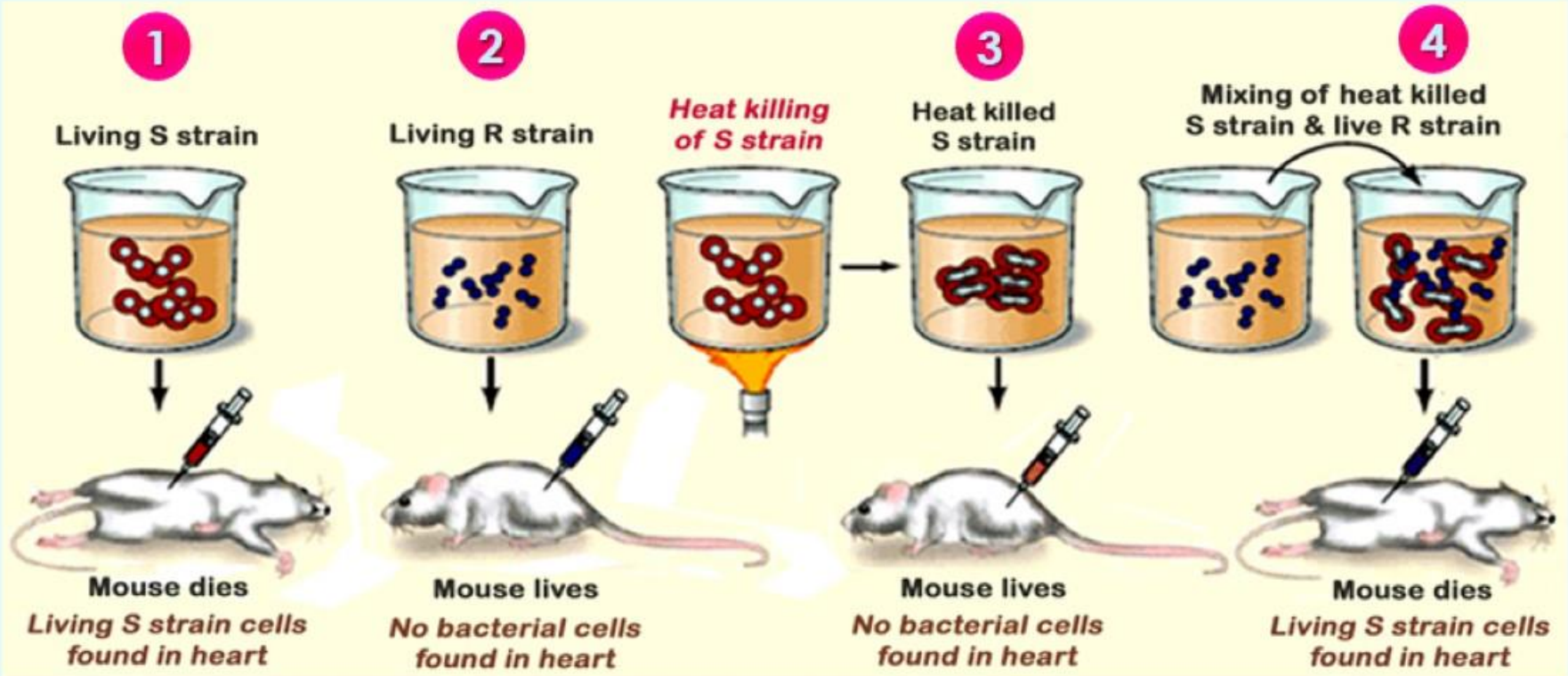


THE SEARCH FOR GENETIC MATERIAL

1

GRIFFITH'S TRANSFORMING PRINCIPLE EXPERIMENT



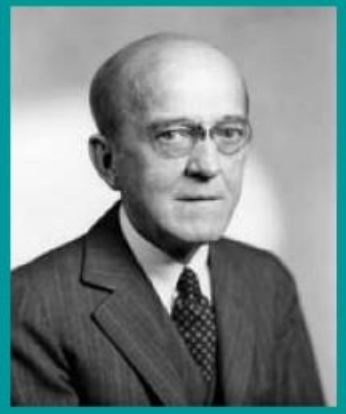


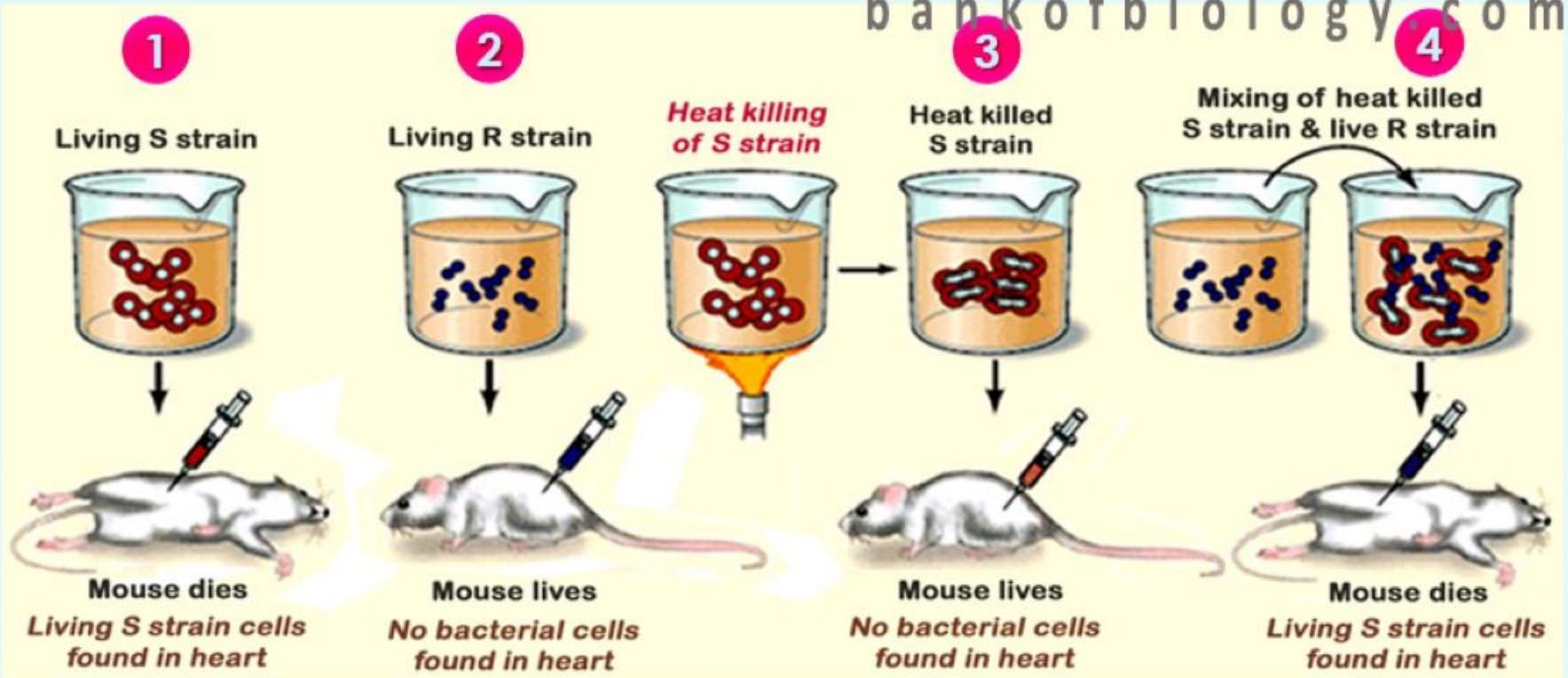
1. S-strain → Inject into mice → Mice die
2. R-strain → Inject into mice → Mice live
3. S-strain (Heat killed) → Inject into mice → Mice live
4. S-strain (Heat killed) + R-strain (live) → Inject into mice → Mice die

THE SEARCH FOR GENETIC MATERIAL

1

GRIFFITH'S TRANSFORMING PRINCIPLE EXPERIMENT





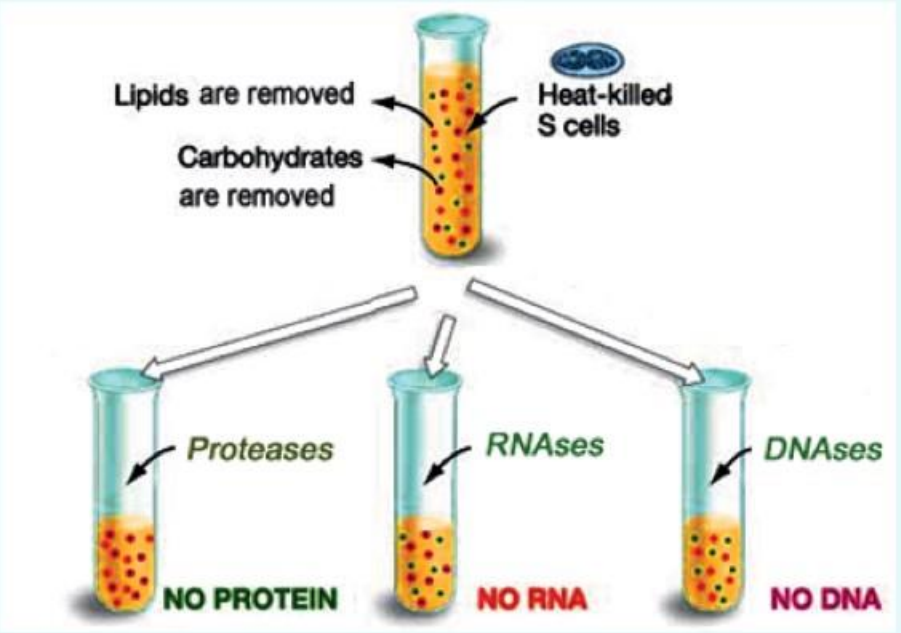
He concluded that some **'transforming principle'** transferred from heat-killed S-strain to R-strain. It enabled R-strain to synthesize smooth polysaccharide coat and become virulent. This is due to the transfer of genetic material.

THE SEARCH FOR GENETIC MATERIAL

2

BIOCHEMICAL CHARACTERIZATION OF TRANSFORMING PRINCIPLE

- **Oswald Avery, Colin MacLeod & Maclyn McCarty worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.**
- **They purified biochemicals (proteins, DNA, RNA etc.) from heat killed S cells using suitable enzymes.**



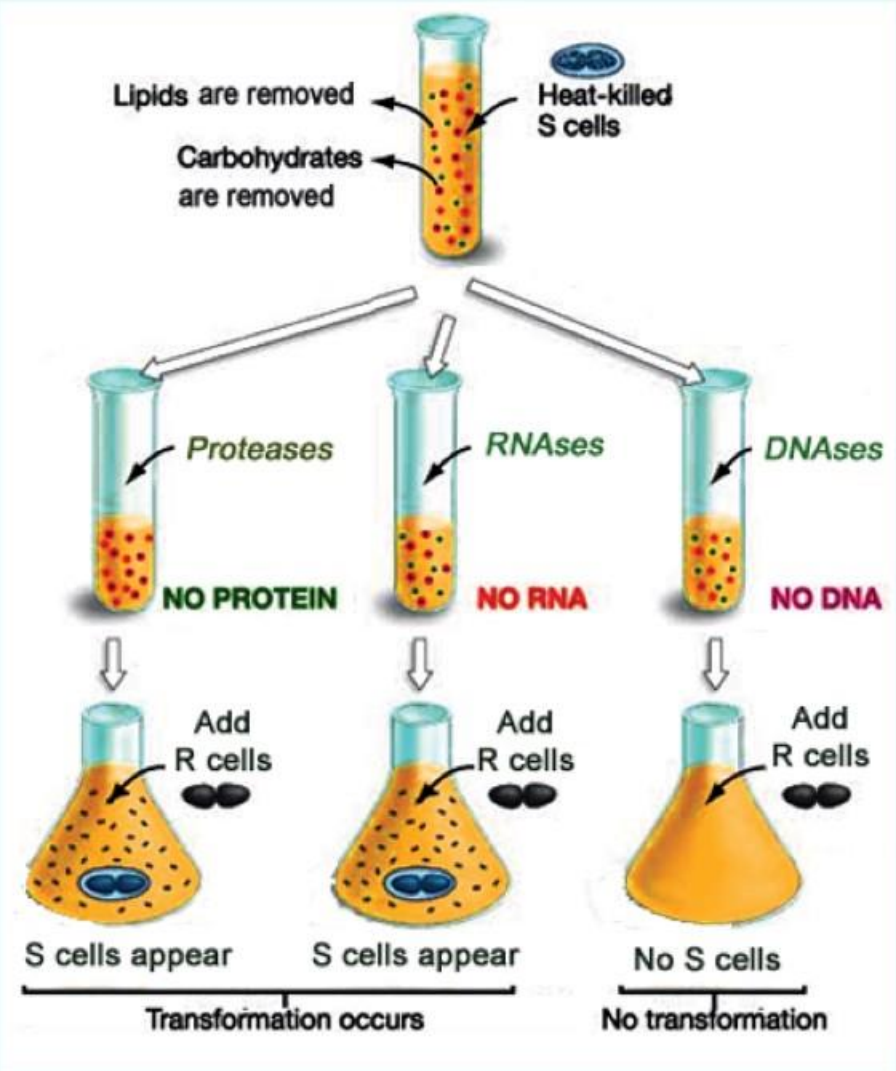
THE SEARCH FOR GENETIC MATERIAL

2

BIOCHEMICAL CHARACTERIZATION OF TRANSFORMING PRINCIPLE

They discovered that

- ✓ **Digestion of protein & RNA** (using *Proteases* & *RNases*) **did not affect transformation.** So, transforming substance was not a protein or RNA.
- ✓ **Digestion of DNA** with ***DNase*** **inhibited transformation.** It means that DNA caused transformation of R cells to S cells, i.e. **DNA was the transforming principle.**

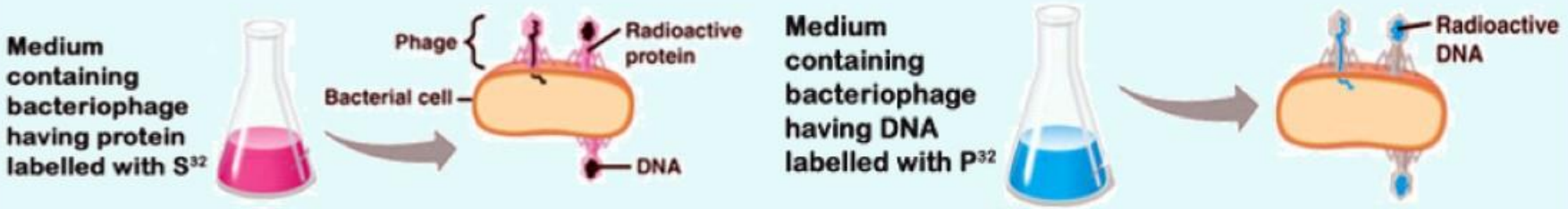


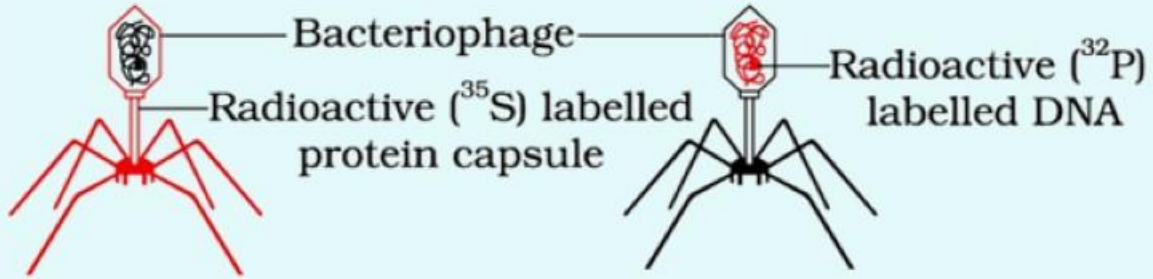
THE SEARCH FOR GENETIC MATERIAL

3

HERSHEY-CHASE EXPERIMENT (BLENDER EXPERIMENT)

- Hershey & Chase grew some bacteriophage viruses on a medium containing radioactive phosphorus (P^{32}) and some others on medium containing radioactive sulphur (S^{35}).
- Viruses grown in P^{32} got radioactive DNA because only DNA contains phosphorus. Viruses grown in S^{35} got radioactive protein because protein contains sulphur.



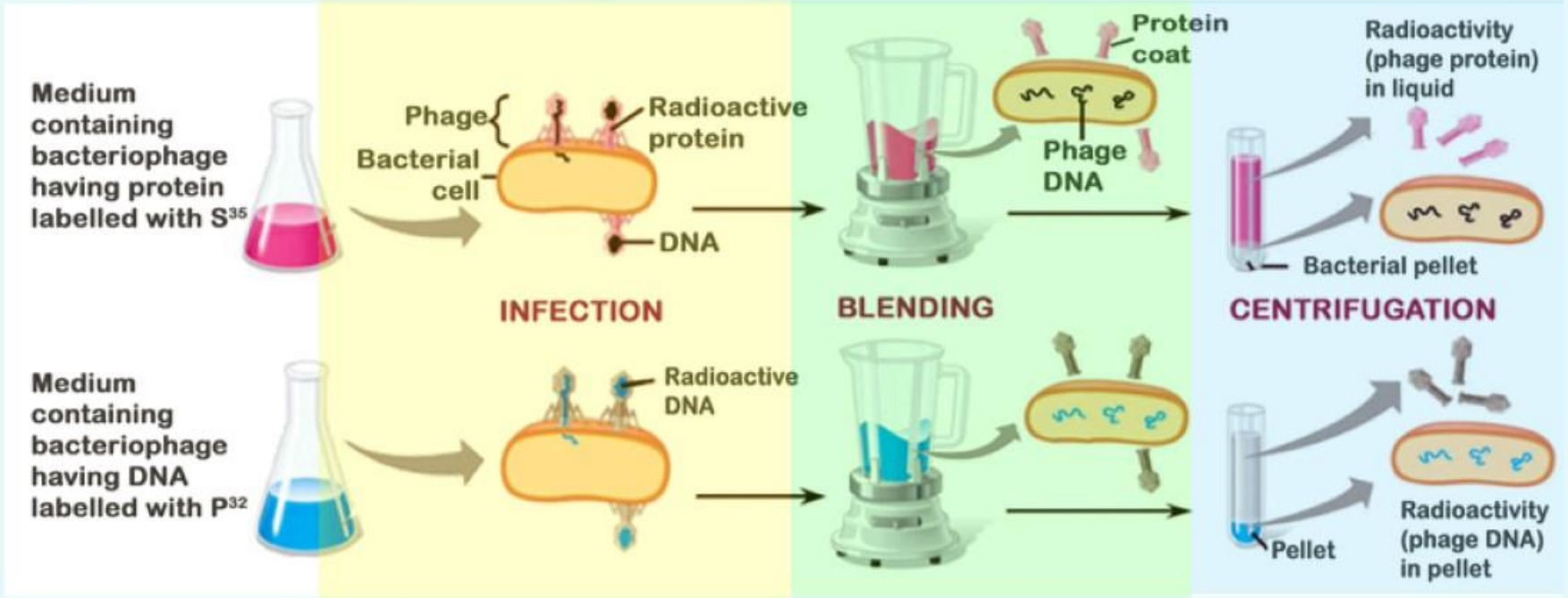


THE SEARCH FOR GENETIC MATERIAL

3

HERSHEY-CHASE EXPERIMENT (BLENDER EXPERIMENT)

- These preparations were used separately to infect *E. coli.*
- After infection, the *E. coli* cells were gently agitated in a blender to remove the virus particles from the bacteria.
- Then the culture was centrifuged to separate lighter virus particles from heavier bacterial cells.

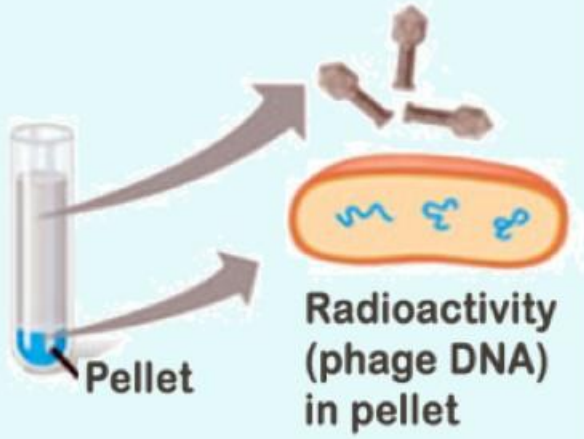


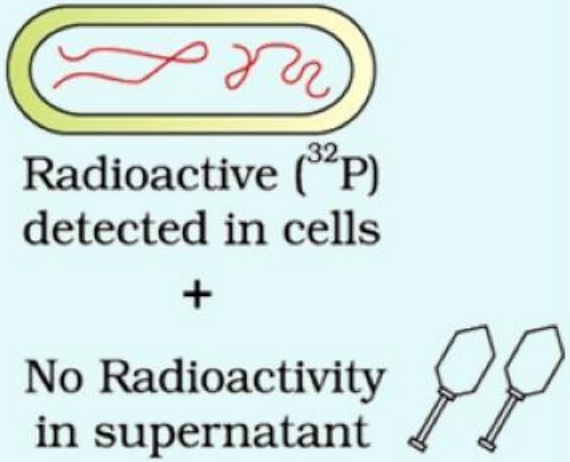
THE SEARCH FOR GENETIC MATERIAL

3

HERSHEY-CHASE EXPERIMENT (BLENDER EXPERIMENT)

- Bacteria infected with viruses having radioactive DNA were radioactive. i.e., DNA had passed from virus to bacteria.
- Bacteria infected with viruses having radioactive proteins were not radioactive. i.e., proteins did not enter the bacteria from the viruses.



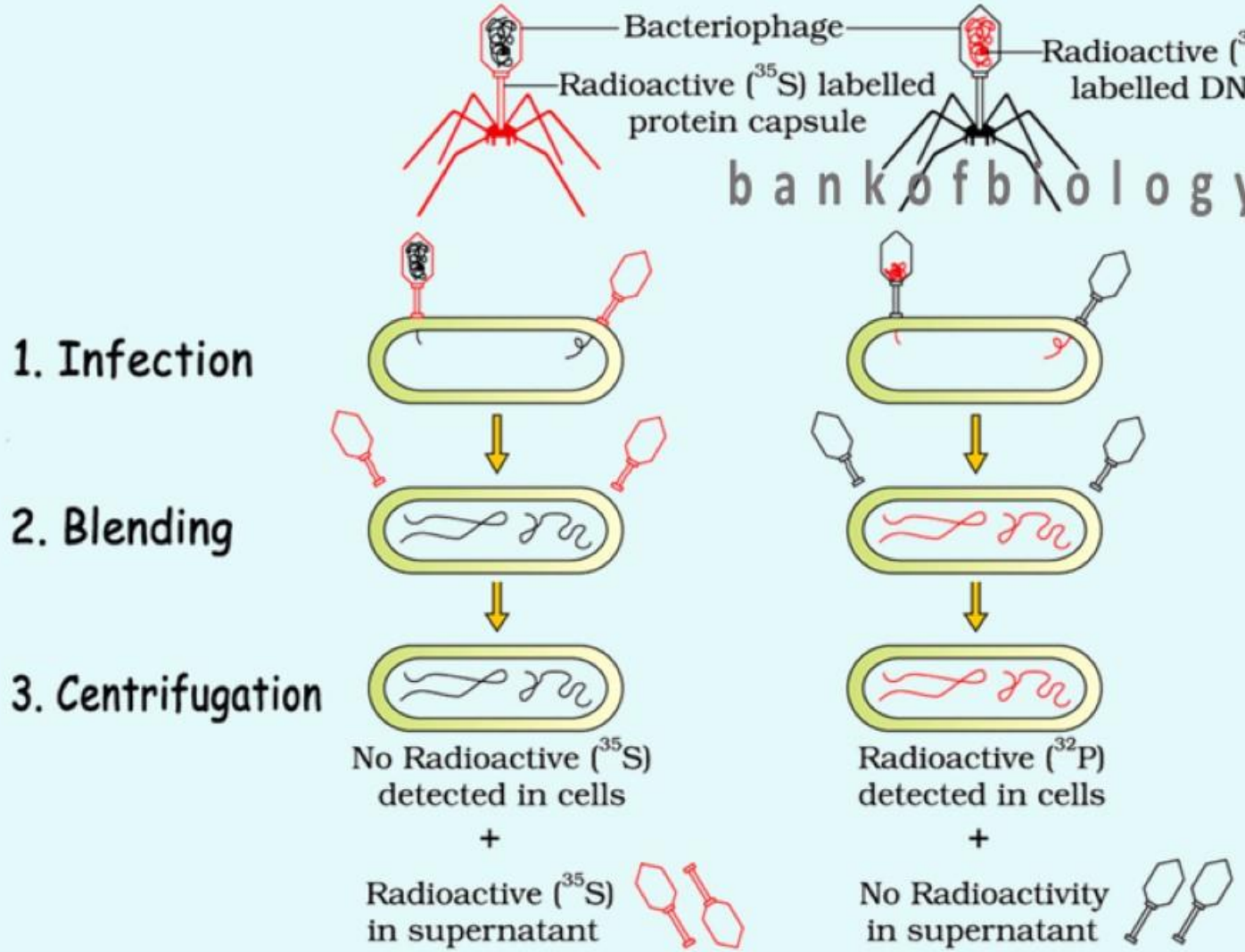


This proves that *DNA is the genetic material.*

THE SEARCH FOR GENETIC MATERIAL

3

HERSHEY-CHASE EXPERIMENT (BLENDER EXPERIMENT)



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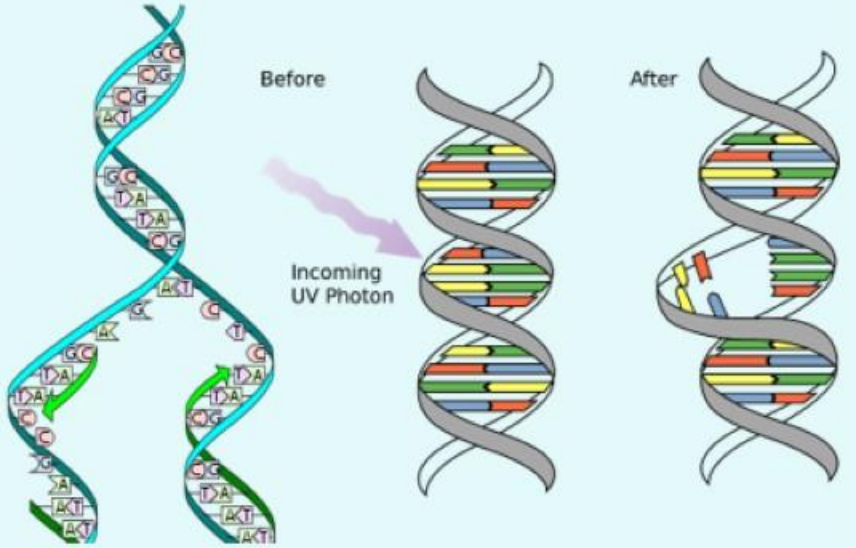
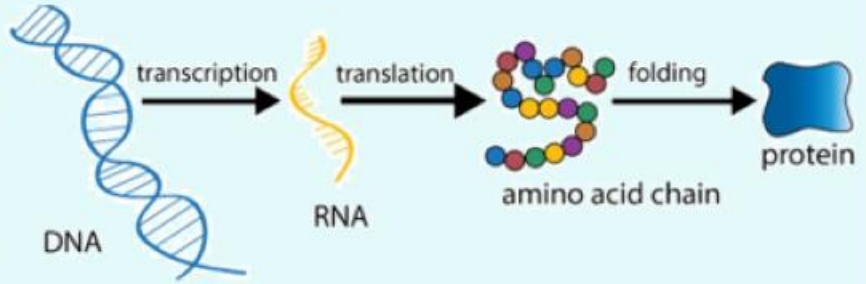
PROPERTIES OF GENETIC MATERIAL

A genetic material must have the following properties:

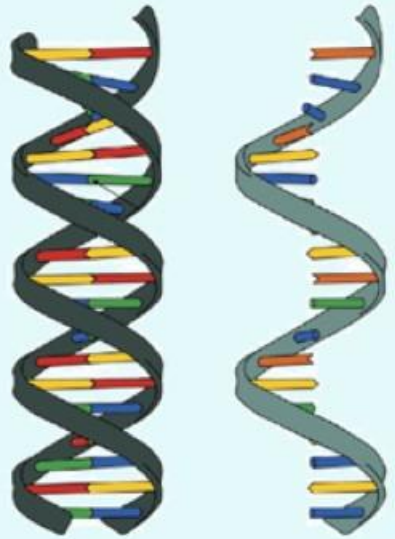
- ✓ **Ability to generate its replica (Replication).**
- ✓ **Chemical and structural stability.**
- ✓ **Provide the mutations needed for evolution.**
- ✓ **Ability to express as 'Mendelian Characters'.**

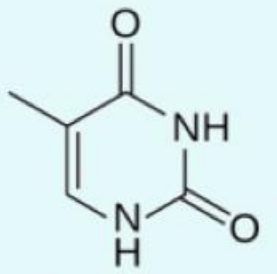


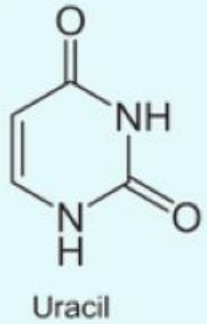


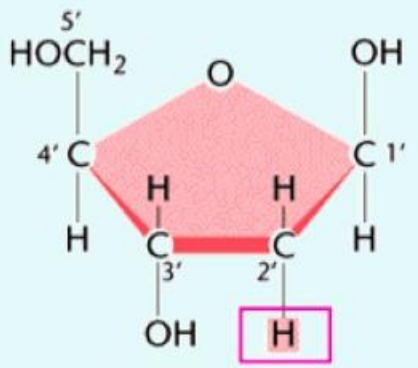
PROPERTIES OF GENETIC MATERIAL

Reasons for stability (less reactivity) of DNA	Reasons for mutability (high reactivity) of RNA
• Double stranded	• Single stranded
• Presence of thymine	• Presence of Uracil
• Absence of 2'-OH in sugar	• Presence of 2'-OH in sugar

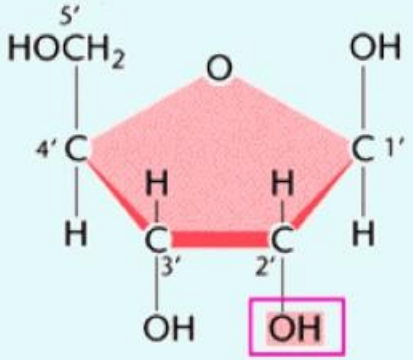








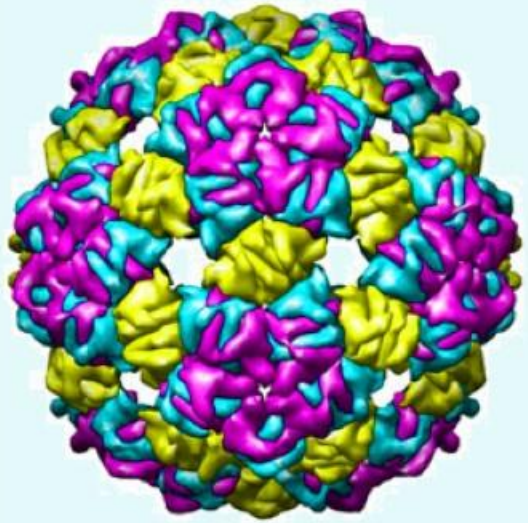
2-Deoxyribose



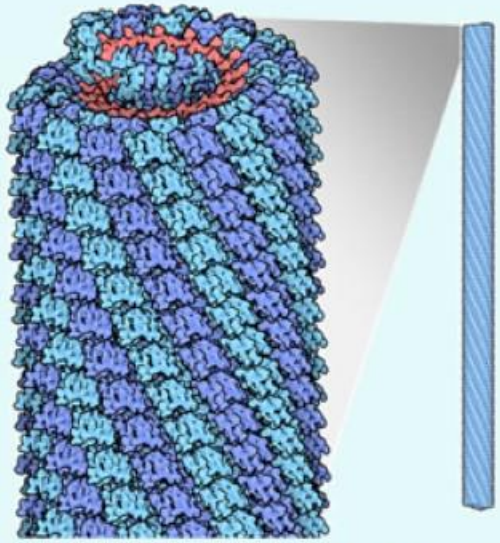
Ribose

PROPERTIES OF GENETIC MATERIAL

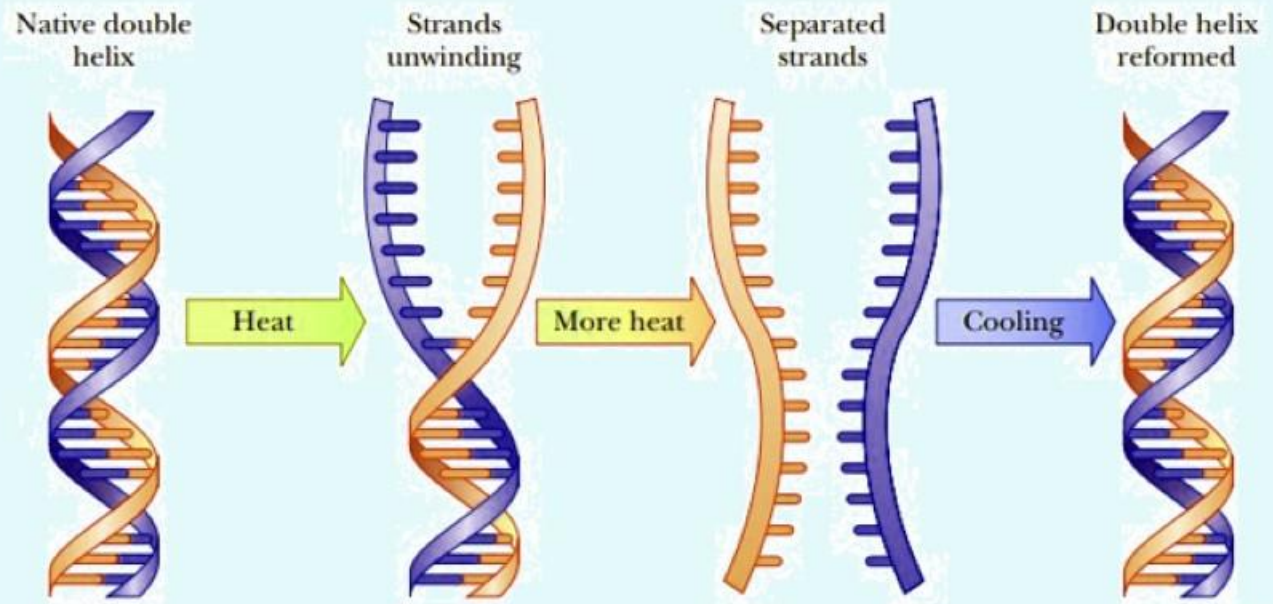
- Thus RNA is unstable. So, RNA viruses (E.g. Q.B bacteriophage, Tobacco Mosaic Virus etc.) mutate and evolve faster.
- DNA strands are complementary. On heating, they separate. In appropriate conditions, they come together. In Griffith's experiment, some properties of DNA of the heat killed bacteria did not destroy. It indicates the stability of DNA.



Q.B bacteriophage

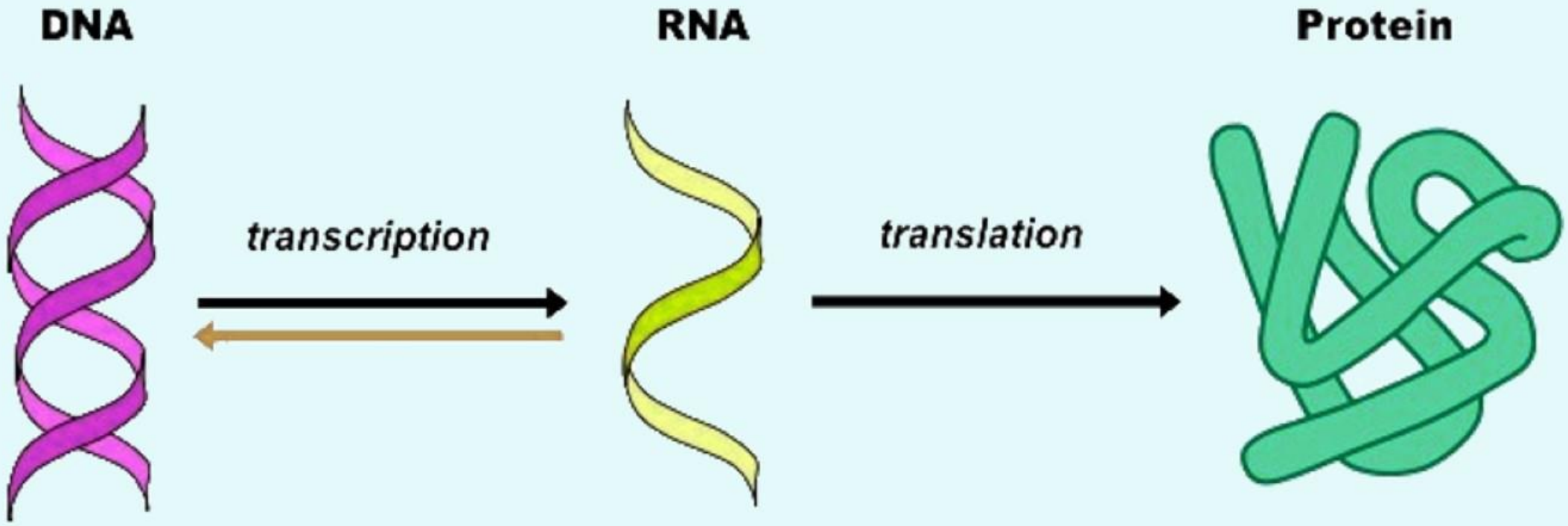


Tobacco Mosaic Virus

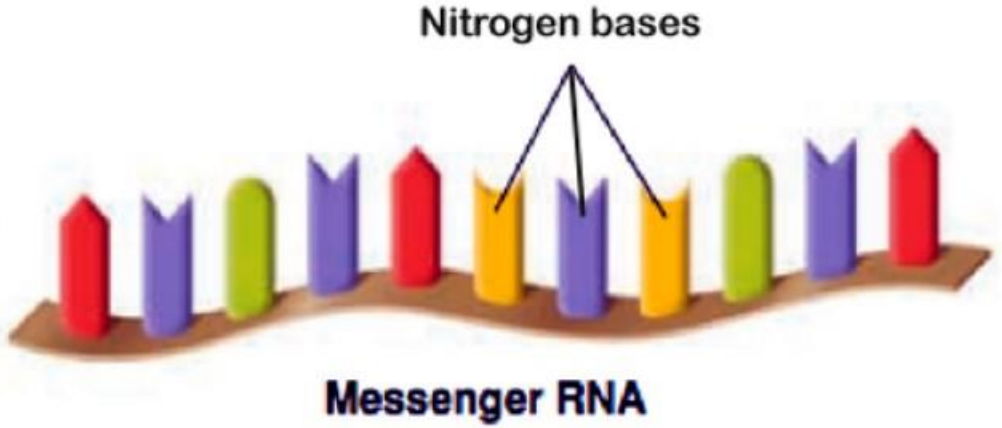


PROPERTIES OF GENETIC MATERIAL

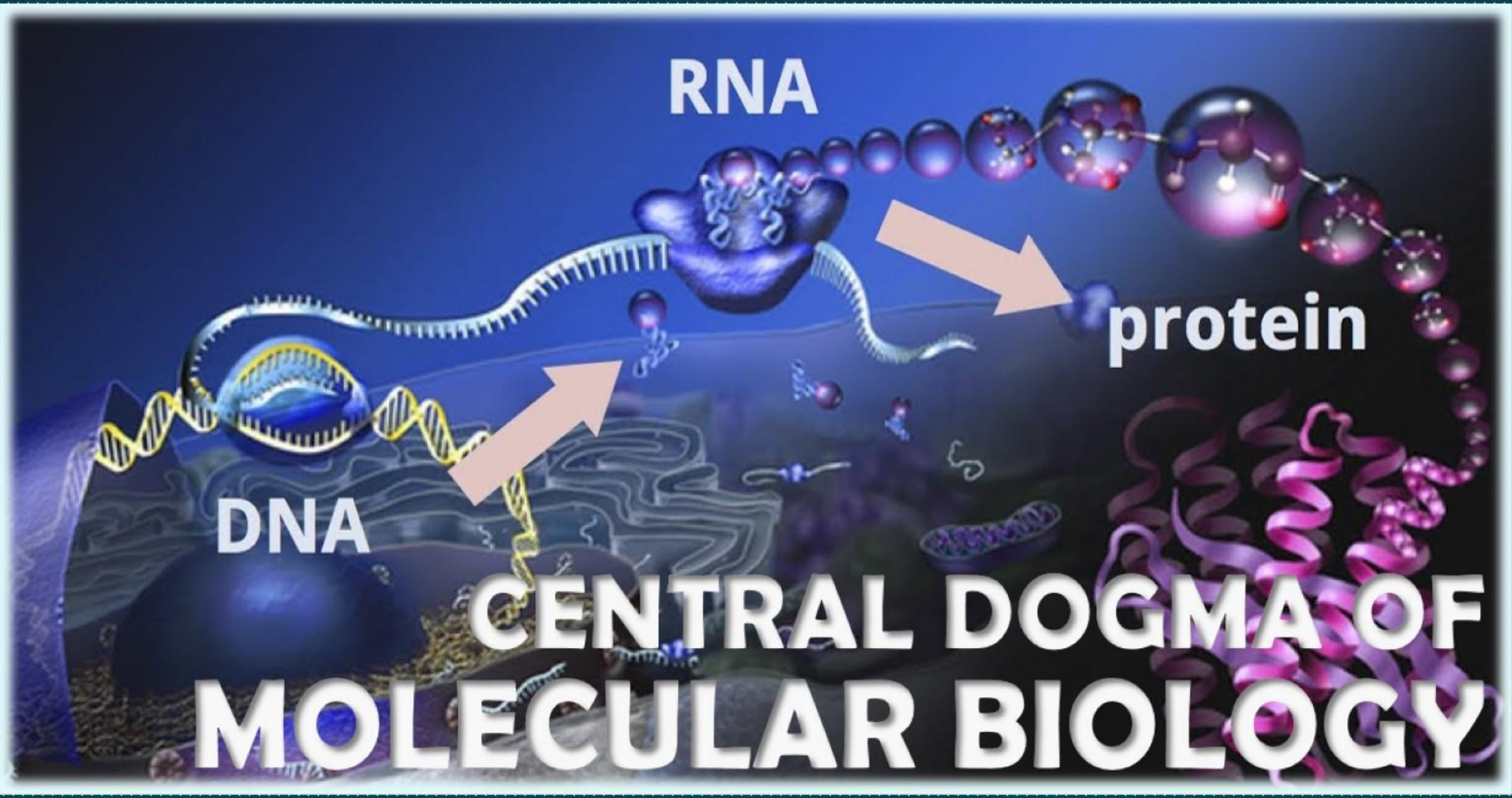
- For the storage of genetic information, DNA is better due to its stability. But for the transmission of genetic information, RNA is better.
- RNA can directly code for the protein synthesis, hence can easily express the characters. DNA is dependent on RNA for protein synthesis.



RNA WORLD

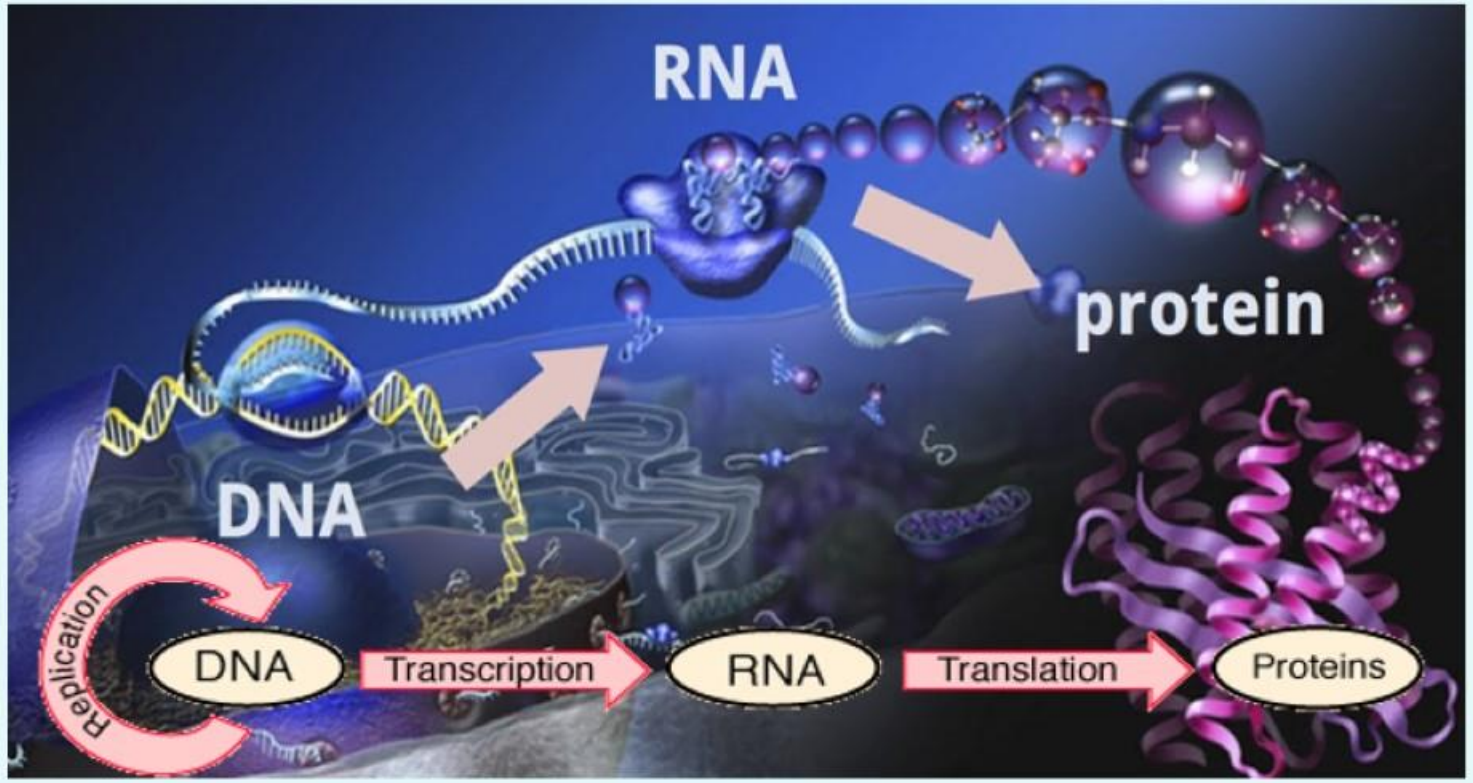


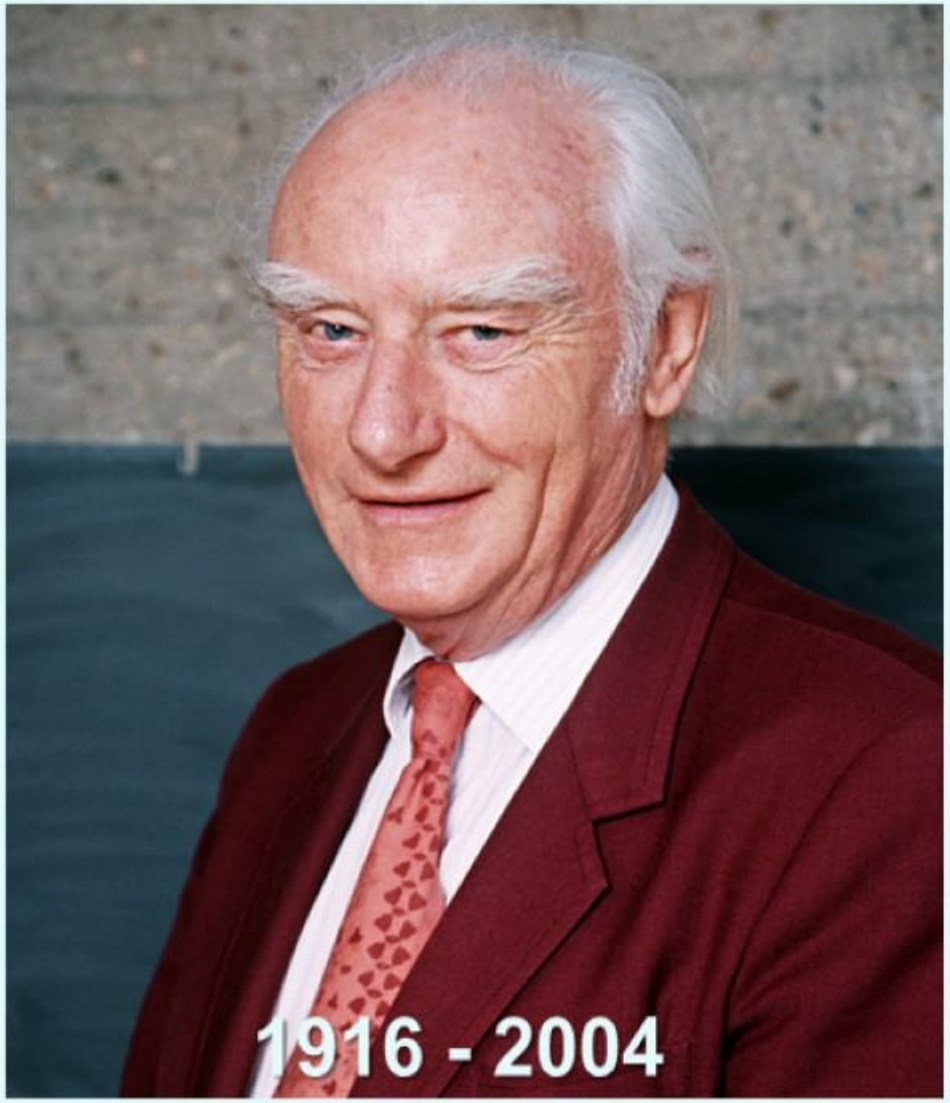
- RNA was the first genetic material.
- It acts as genetic material & catalyst.
- Essential life processes (metabolism, translation, splicing etc.) evolved around RNA.
- DNA evolved from RNA for stability.



CENTRAL DOGMA OF MOLECULAR BIOLOGY

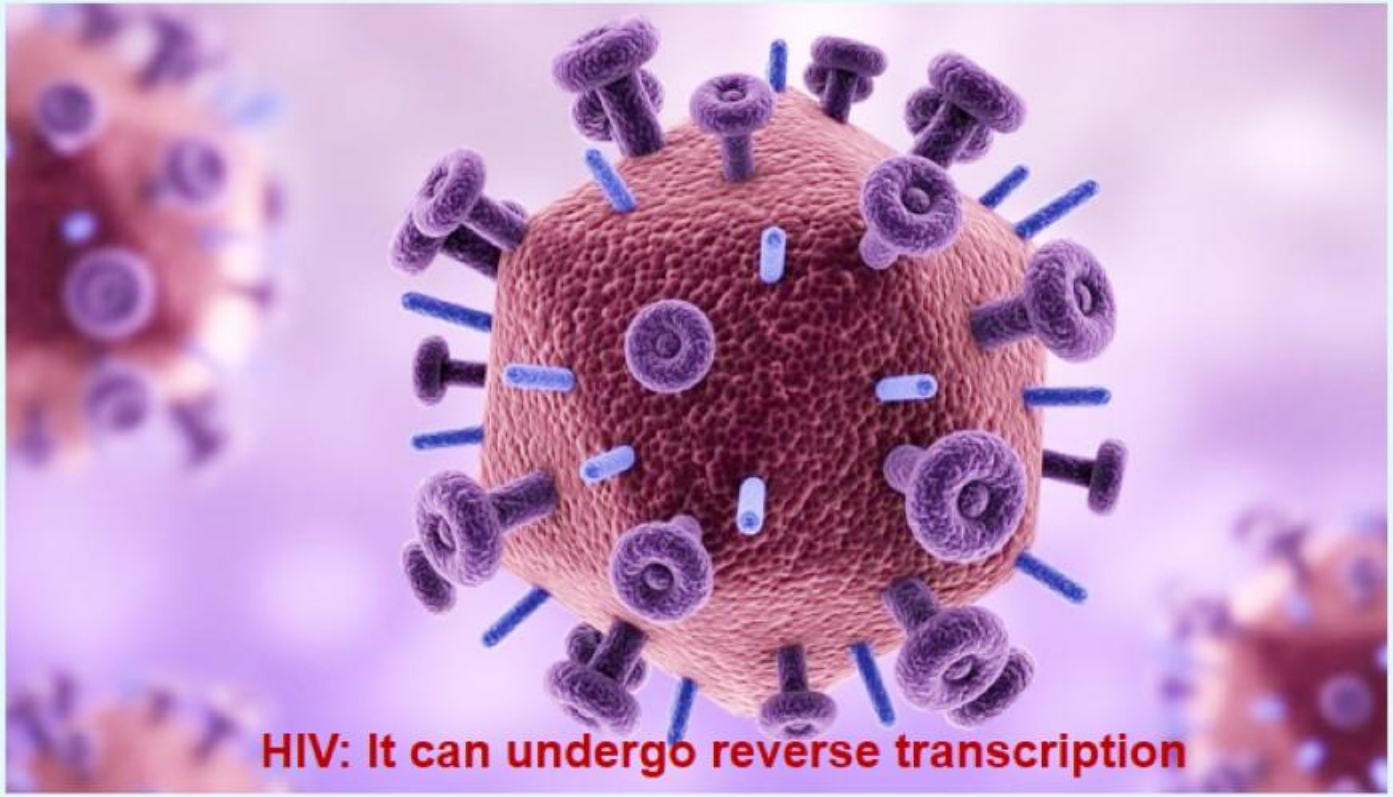
- It is proposed by **Francis Crick.**
- It states that the genetic information flows from **DNA → RNA → Protein.**

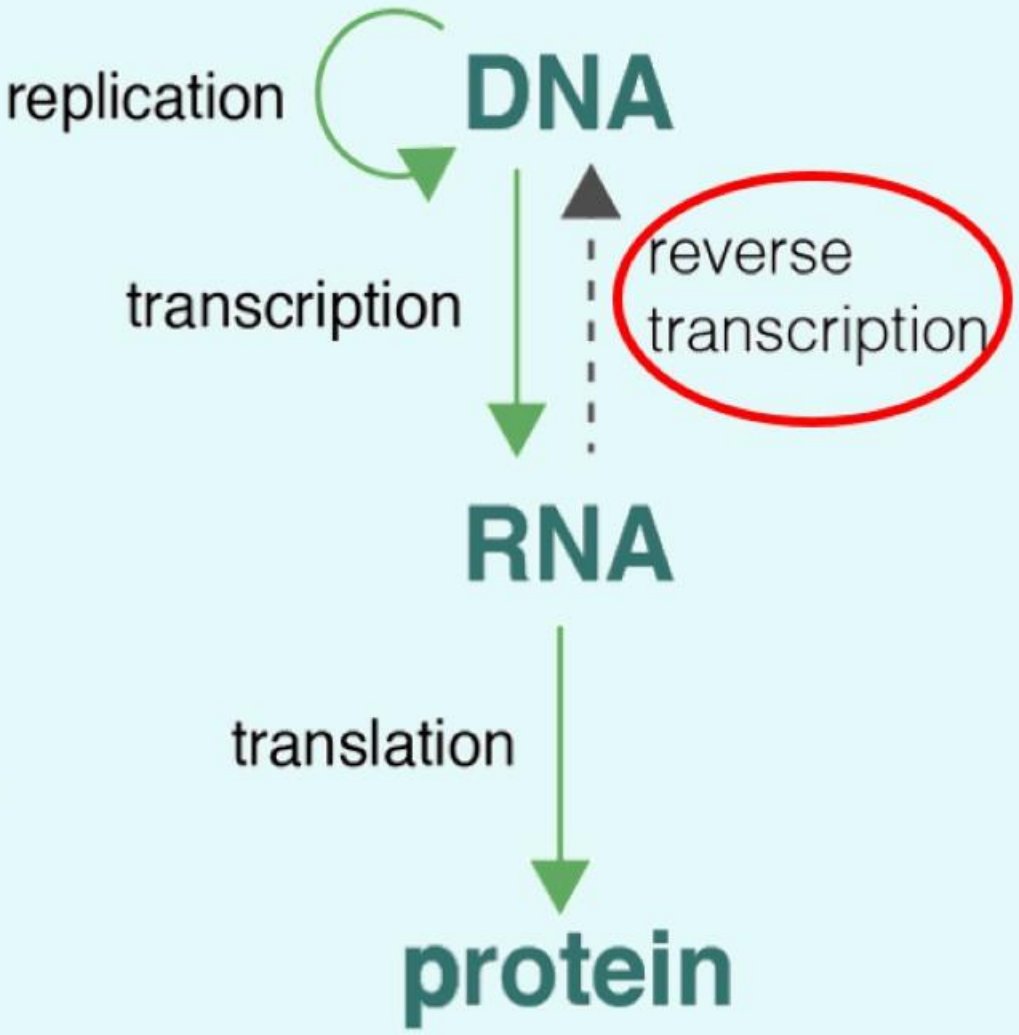




CENTRAL DOGMA OF MOLECULAR BIOLOGY

- In some viruses, flow of information is in reverse direction (from RNA to DNA). It is called **reverse transcription.**

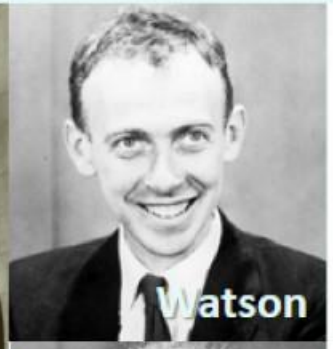


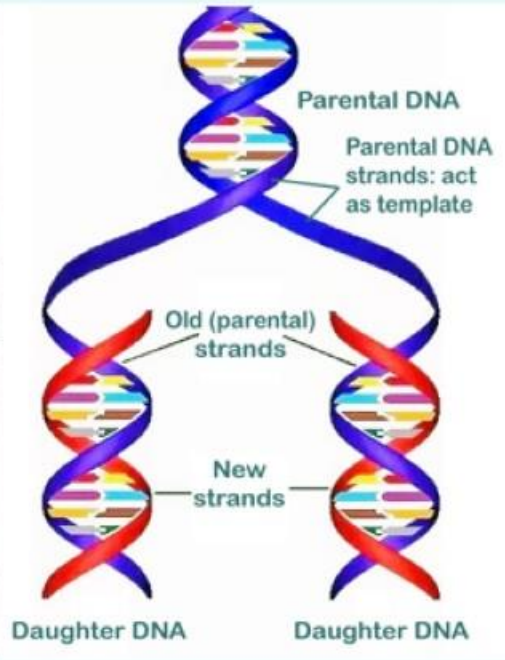


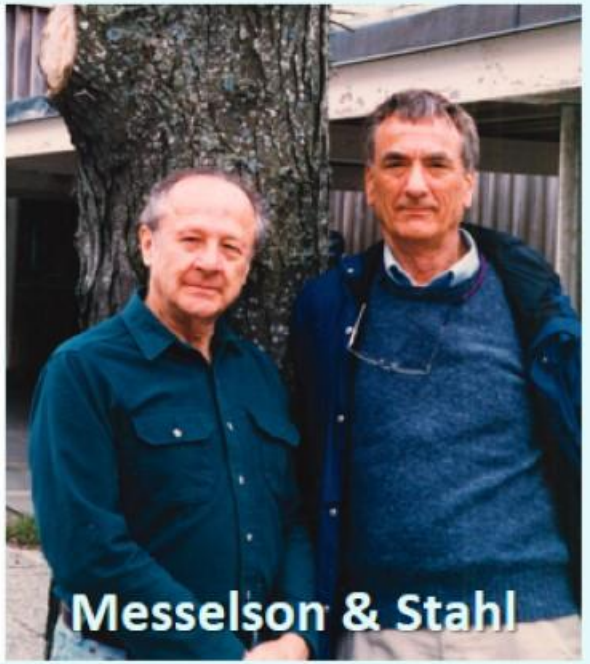
DNA REPLICATION

- Replication is the copying of DNA from parental DNA.
- Watson & Crick proposed Semi-conservative model of replication. It suggests that the parental DNA strands act as template for the synthesis of new complementary strands. After replication, each DNA molecule would have one parental and one new strand.
- Matthew Messelson & Franklin Stahl (1958) proved Semi-conservative model.

James Watson Francis Crick



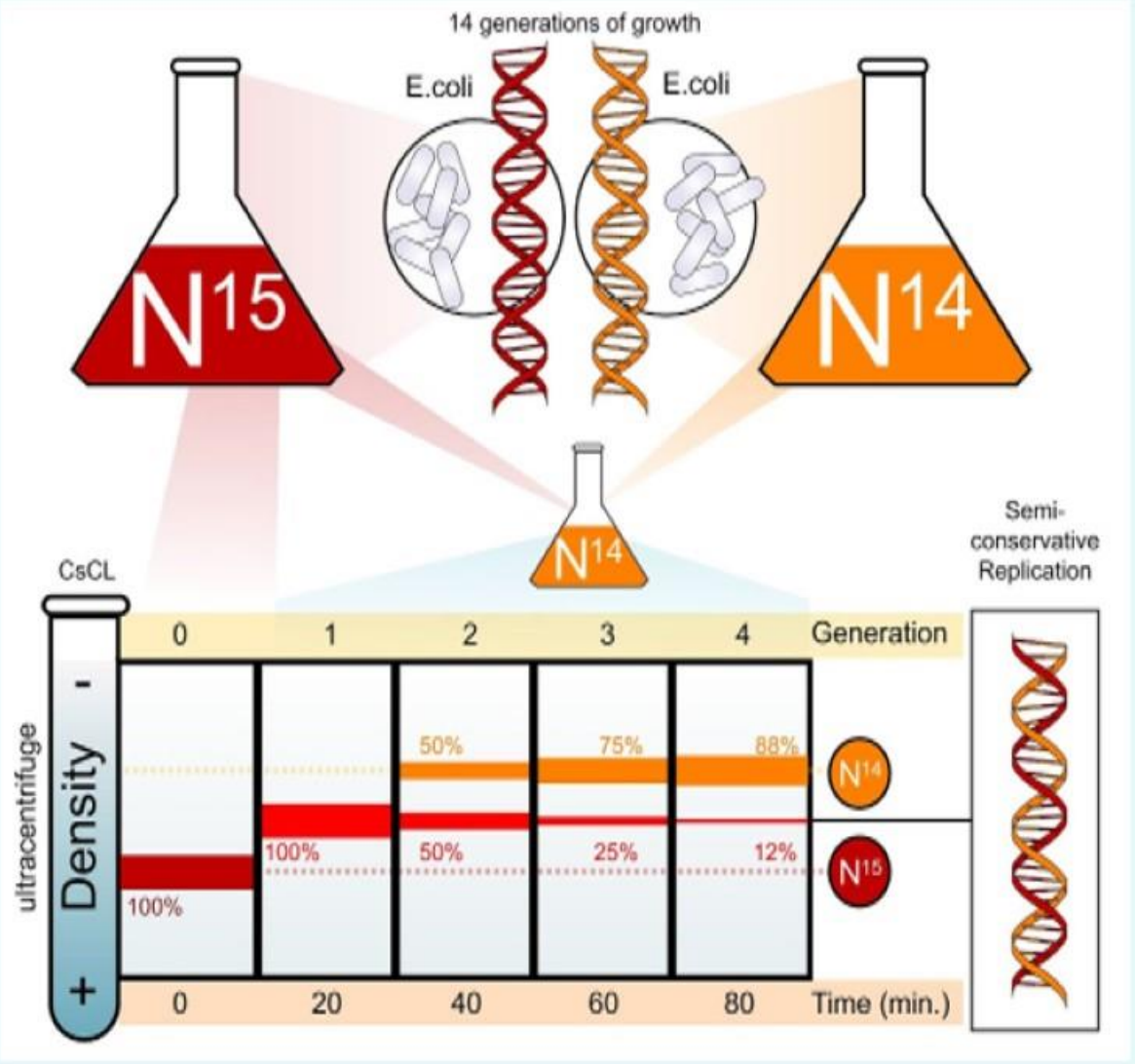




DNA REPLICATION

MESSELSON & STAHL'S EXPERIMENT

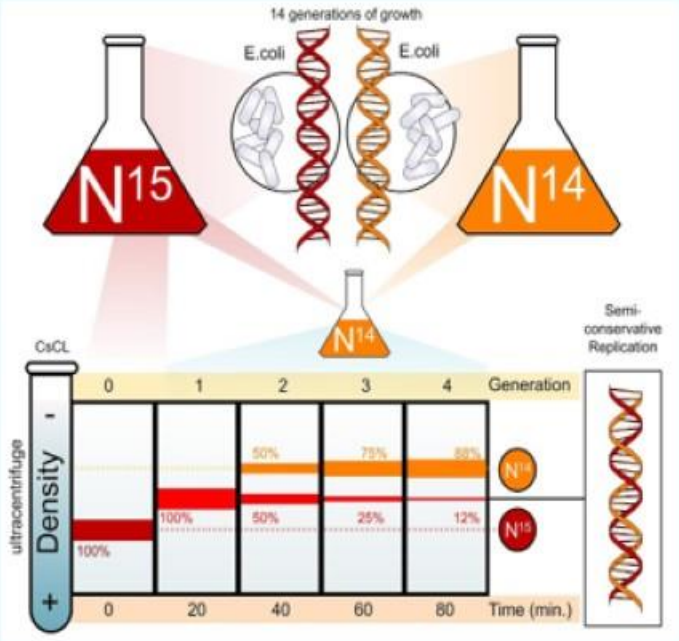
- They prepared 2 culture media of *E. coli.*
- One preparation contains $^{15}NH_4Cl$ salt (^{15}N: heavy isotope of N). So ^{15}N was incorporated into both strands of bacterial DNA and the **DNA became heavier.**
- Other preparation contains ^{14}N salts. So ^{14}N was incorporated in both strands of DNA and became **lighter.**
- These 2 types of DNA can be separated by centrifugation in a **CsCl density gradient.**

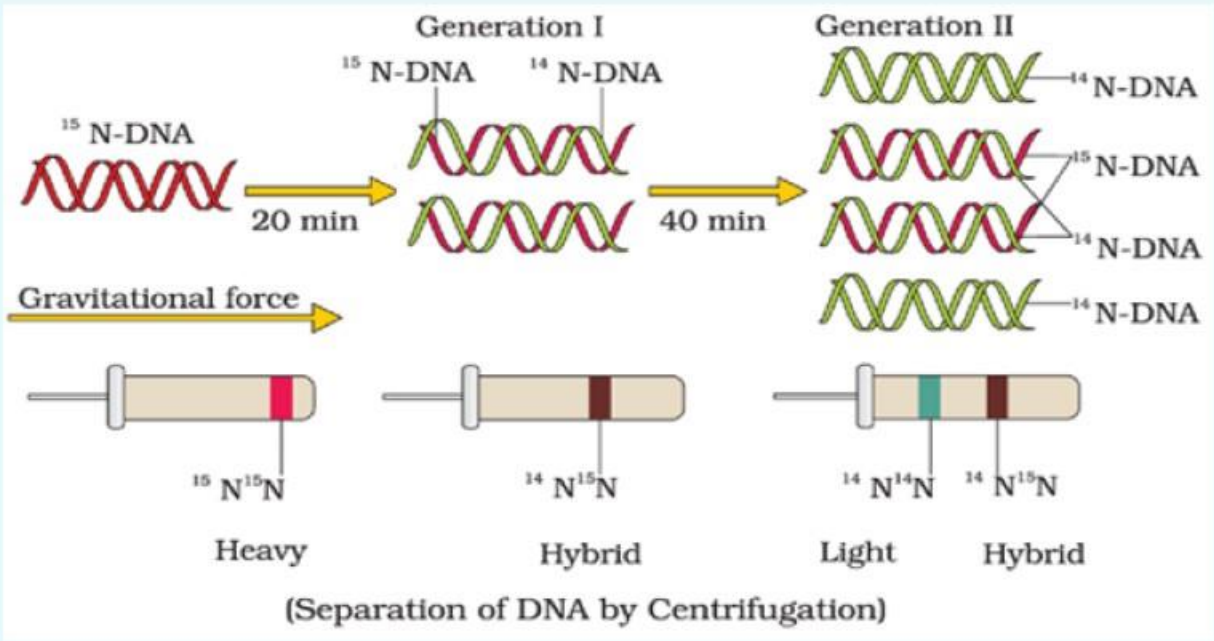


DNA REPLICATION

MESSELSON & STAHL'S EXPERIMENT

- They took *E. coli* cells from ^{15}N medium and transferred to ^{14}N medium.
- After one generation (i.e. after 20 minutes), they isolated and centrifuged the DNA. Its density was intermediate (hybrid) between ^{15}N DNA and ^{14}N DNA.
- This shows that in newly formed DNA, one strand is old (^{15}N type) and one strand is new (^{14}N type). This confirms semi-conservative replication.





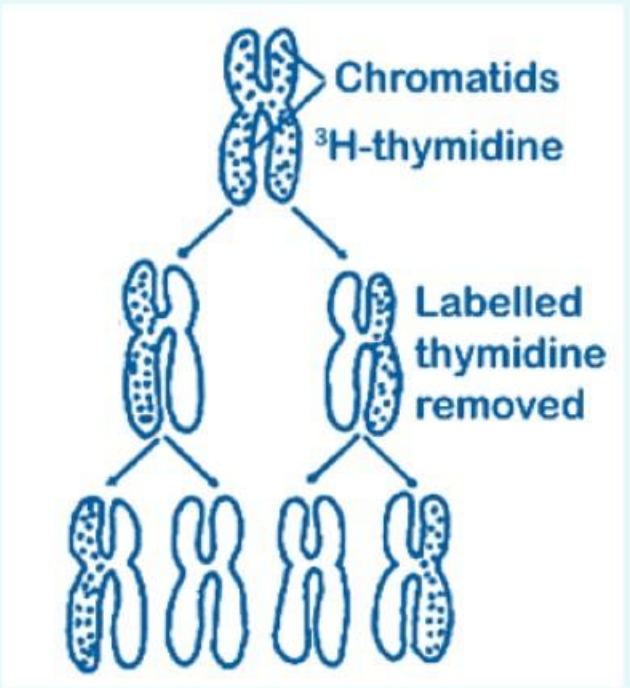
After II generation (i.e. after 40 min), there was equal amounts of hybrid DNA and light DNA.

DNA REPLICATION MESSELSON & STAHL'S EXPERIMENT

- Taylor & colleagues (1958) performed similar experiments on *Vicia faba (faba beans)* using radioactive thymidine to detect distribution of newly synthesized DNA in the chromosomes.
- It proved that the DNA in chromosomes also replicate semi-conservatively.


Dr. J. Herbert Taylor

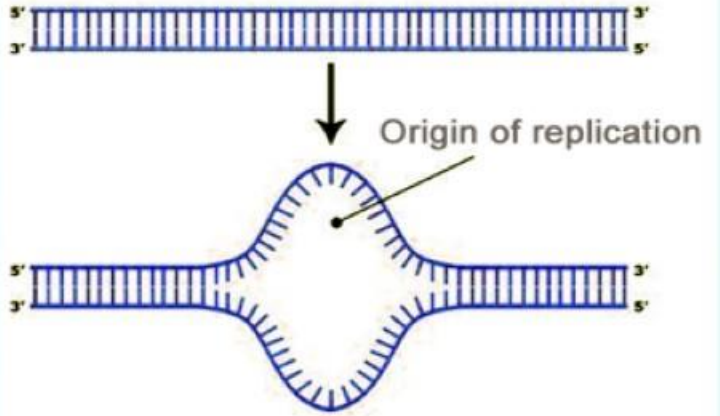

Vicia faba (faba beans)

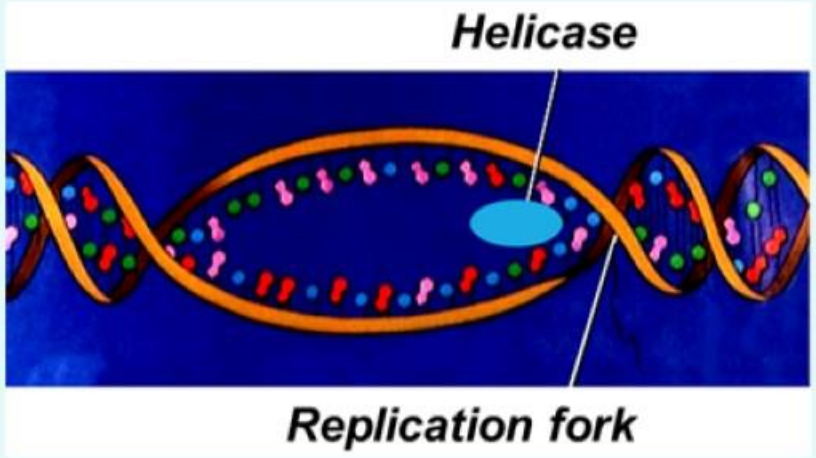


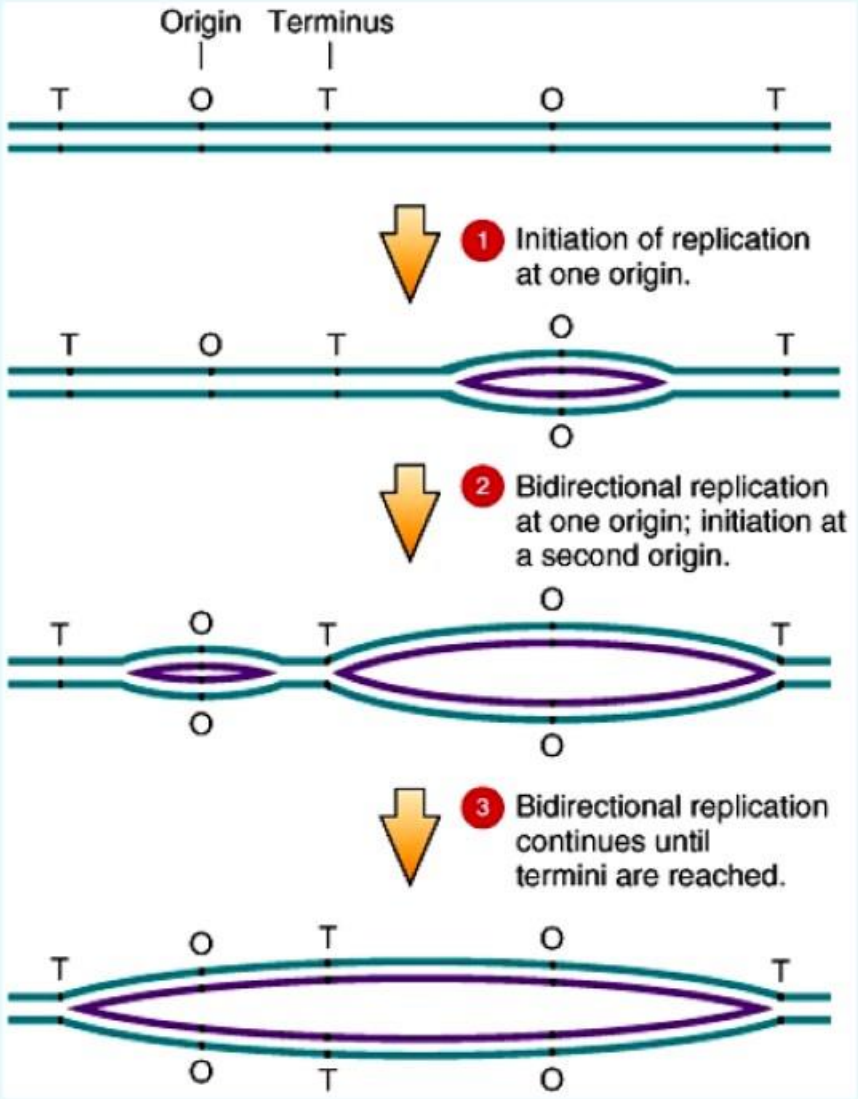
DNA REPLICATION

THE MACHINERY & ENZYMES

- DNA replication starts at a point called **origin *(ori).***
- A unit of replication with one origin is called a **replicon.**
- During replication, the 2 strands unwind and separate by breaking H-bonds in presence of an enzyme, ***Helicase.***
- Unwinding of the DNA molecule at a point forms a 'Y'-shaped structure called **replication fork.**



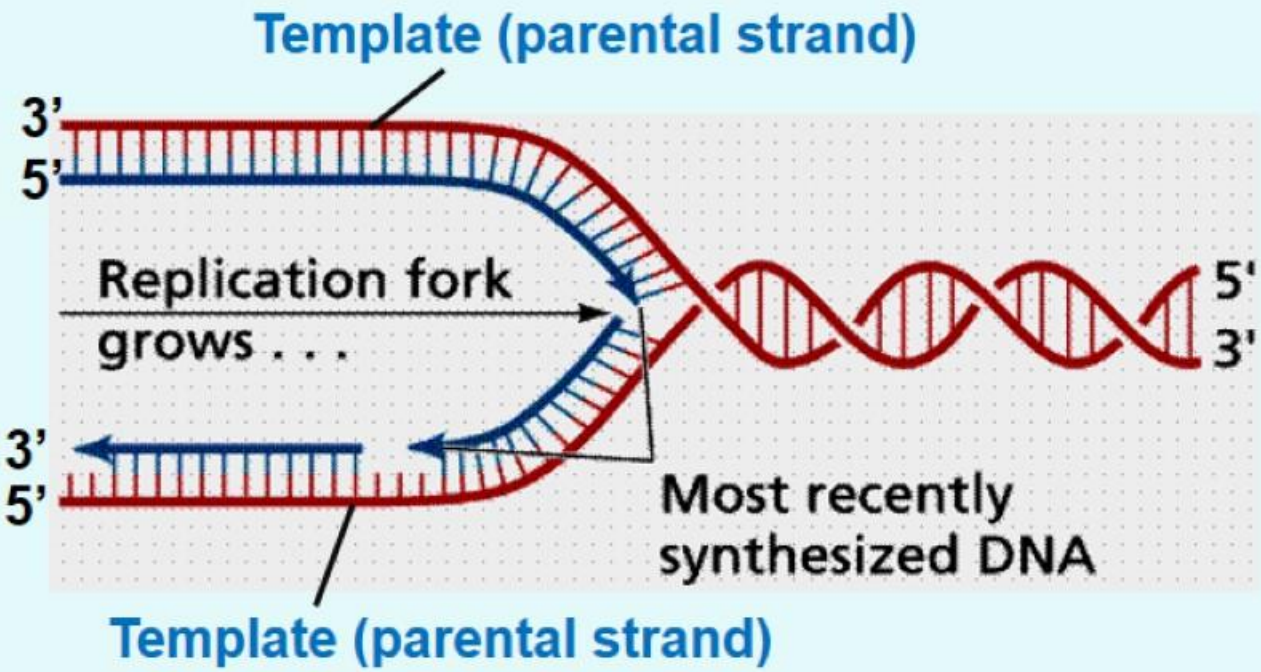


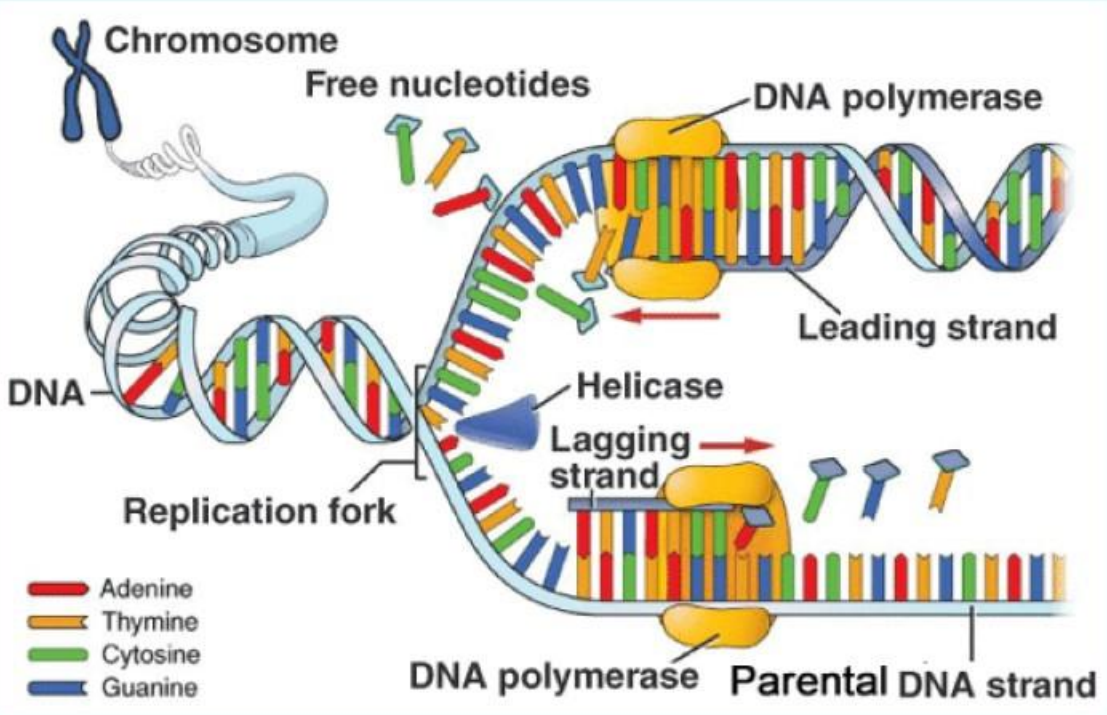


DNA REPLICATION

THE MACHINERY & ENZYMES

- The separated strands act as templates for the synthesis of new strands.
- DNA replicates in the 5'→3' direction.
- Deoxyribonucleoside triphosphates (dATP, dGTP, dCTP & dTTP) act as substrate and provide energy for polymerization.

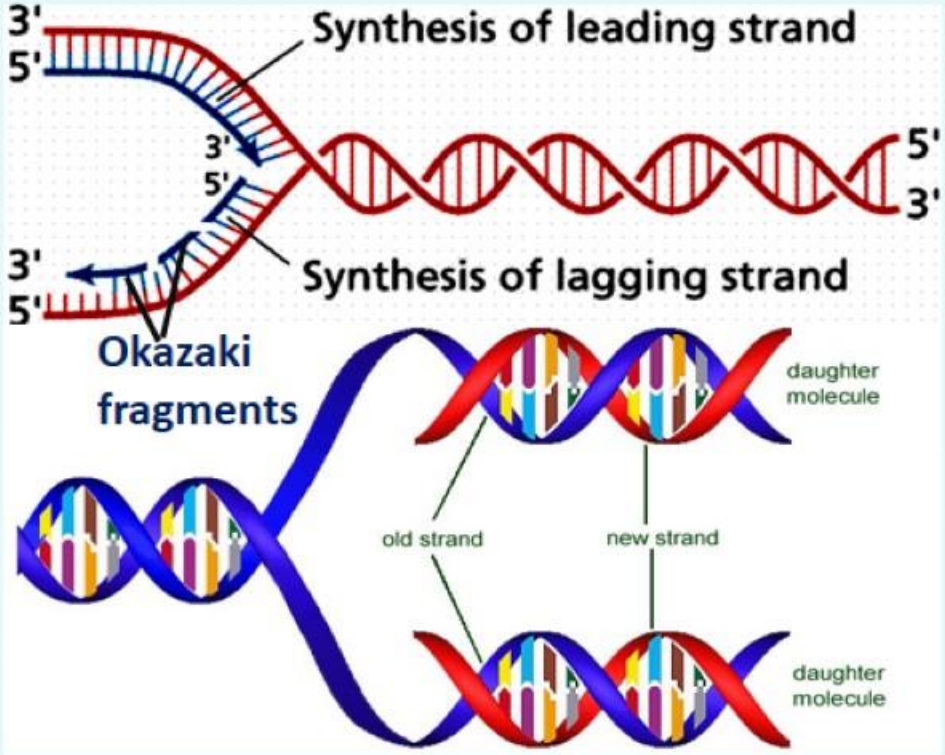


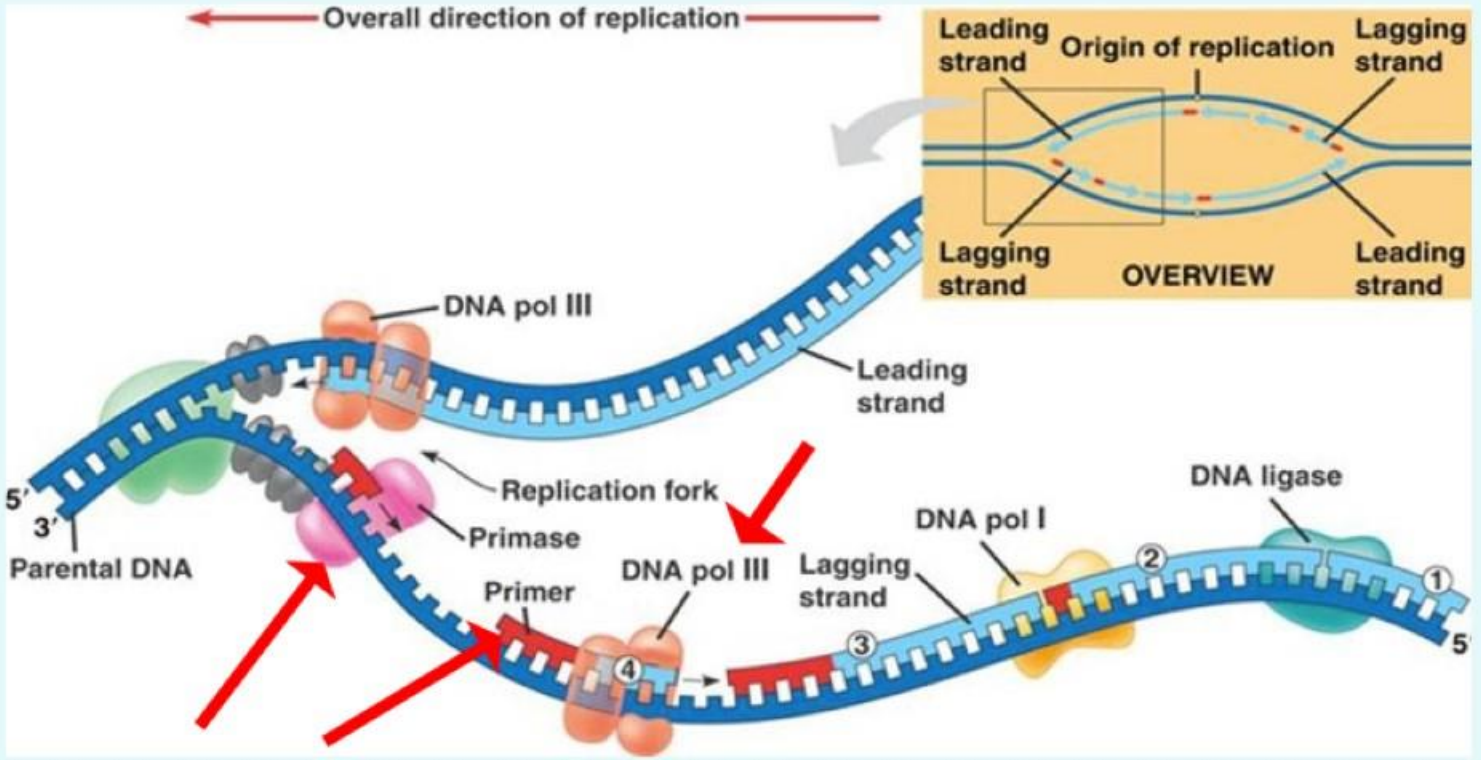


DNA REPLICATION

THE MACHINERY & ENZYMES

- Firstly, a small **RNA primer** is synthesized in presence of an enzyme, ***primase.***
- In presence of an enzyme, DNA dependent ***DNA polymerase,*** many nucleotides join with one another to primer strand and form a polynucleotide chain (new strand).

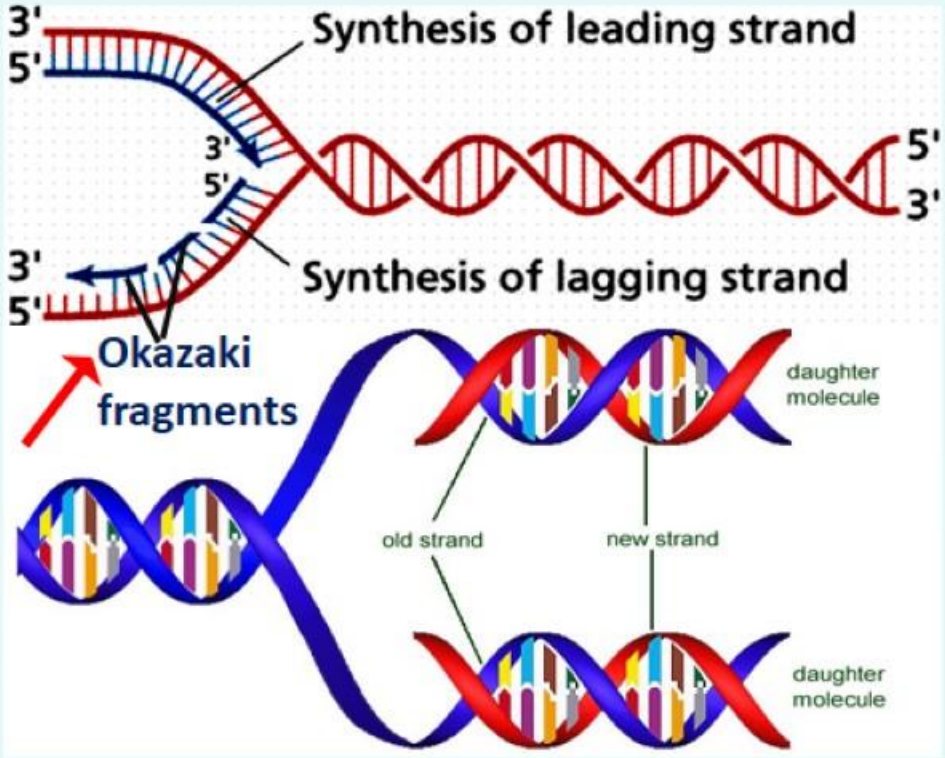


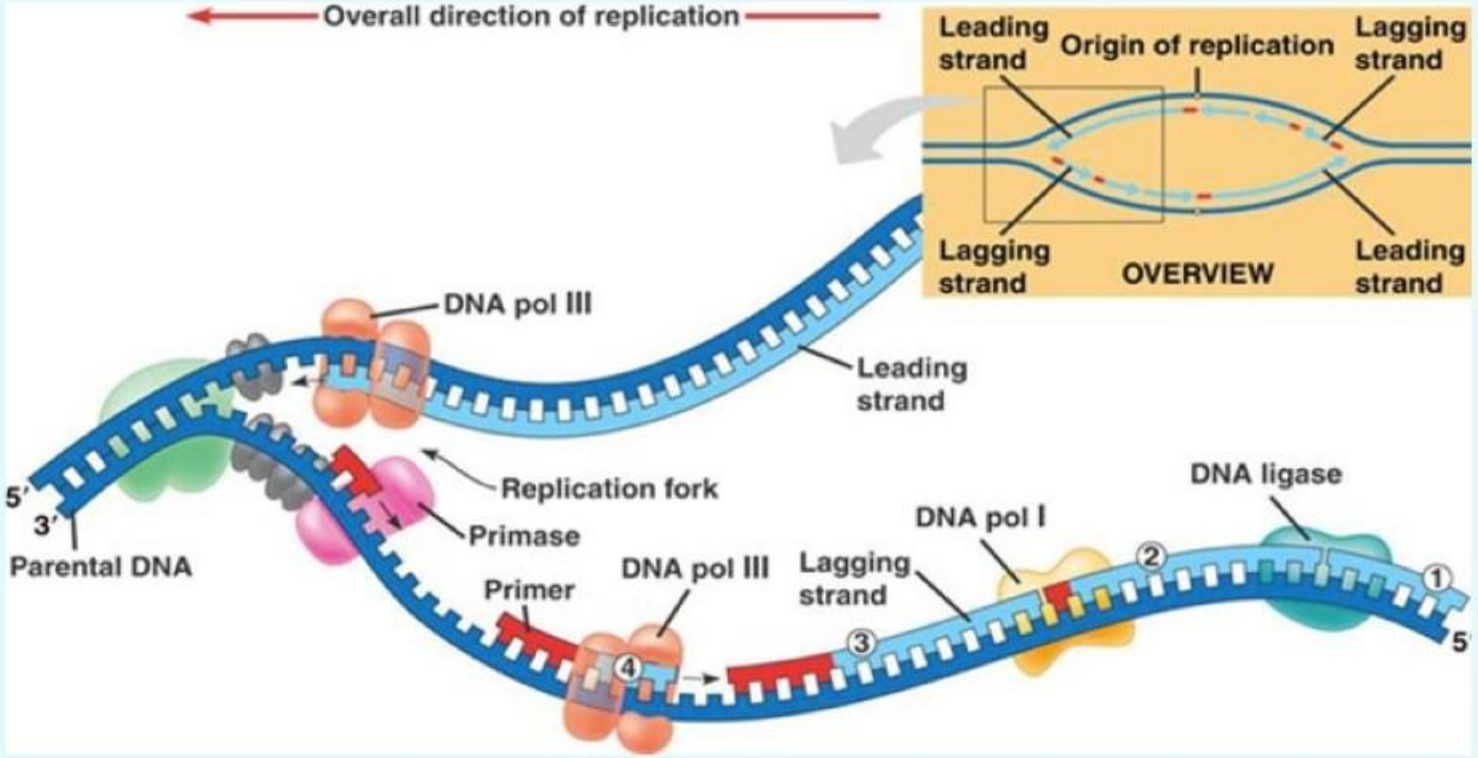


DNA REPLICATION

THE MACHINERY & ENZYMES

- During replication, one strand is formed as a continuous stretch in 5'→ 3' direction (Continuous synthesis). This strand is called leading strand.
- The other strand is formed in small stretches (Okazaki fragments) in 5'→ 3' direction (Discontinuous synthesis).

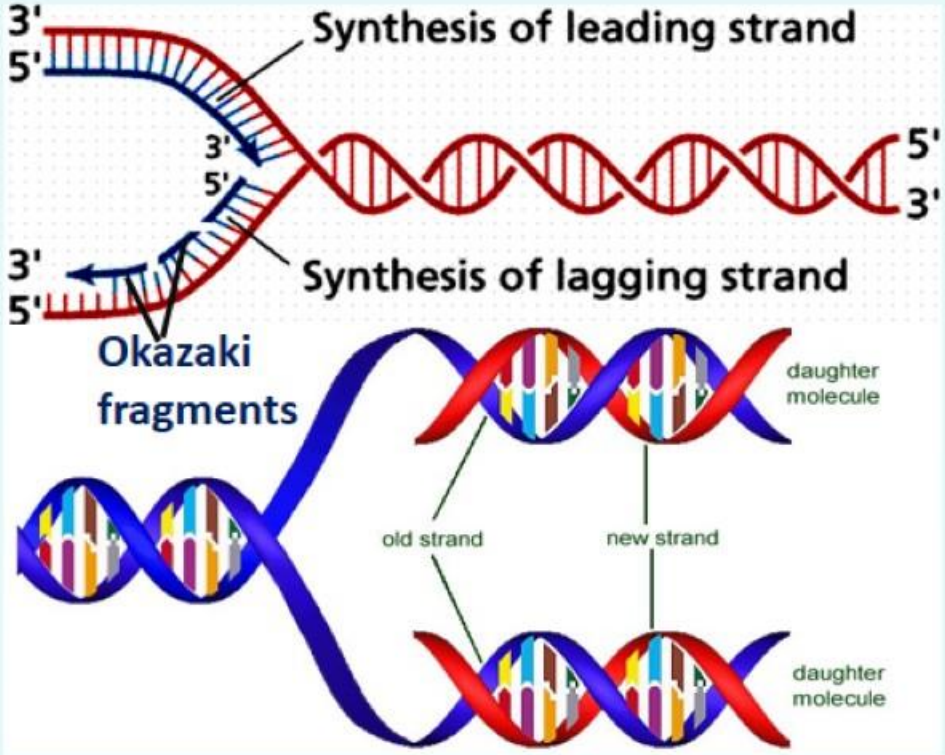


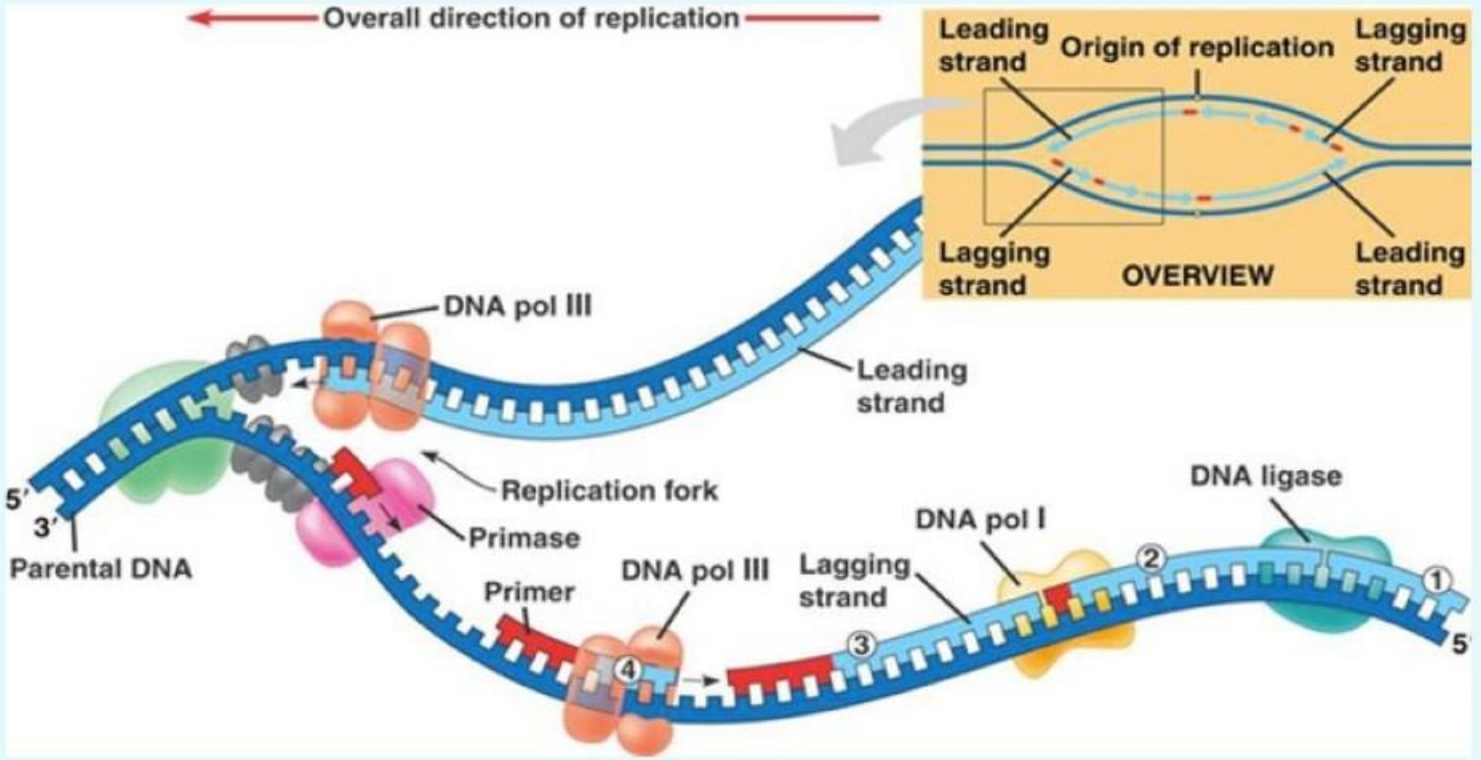


DNA REPLICATION

THE MACHINERY & ENZYMES

- The Okazaki fragments are then joined together to form a new strand by an enzyme, *DNA ligase.* This new strand is called lagging strand.
- If a wrong base is introduced in the new strand, *DNA polymerase* can do proof reading.

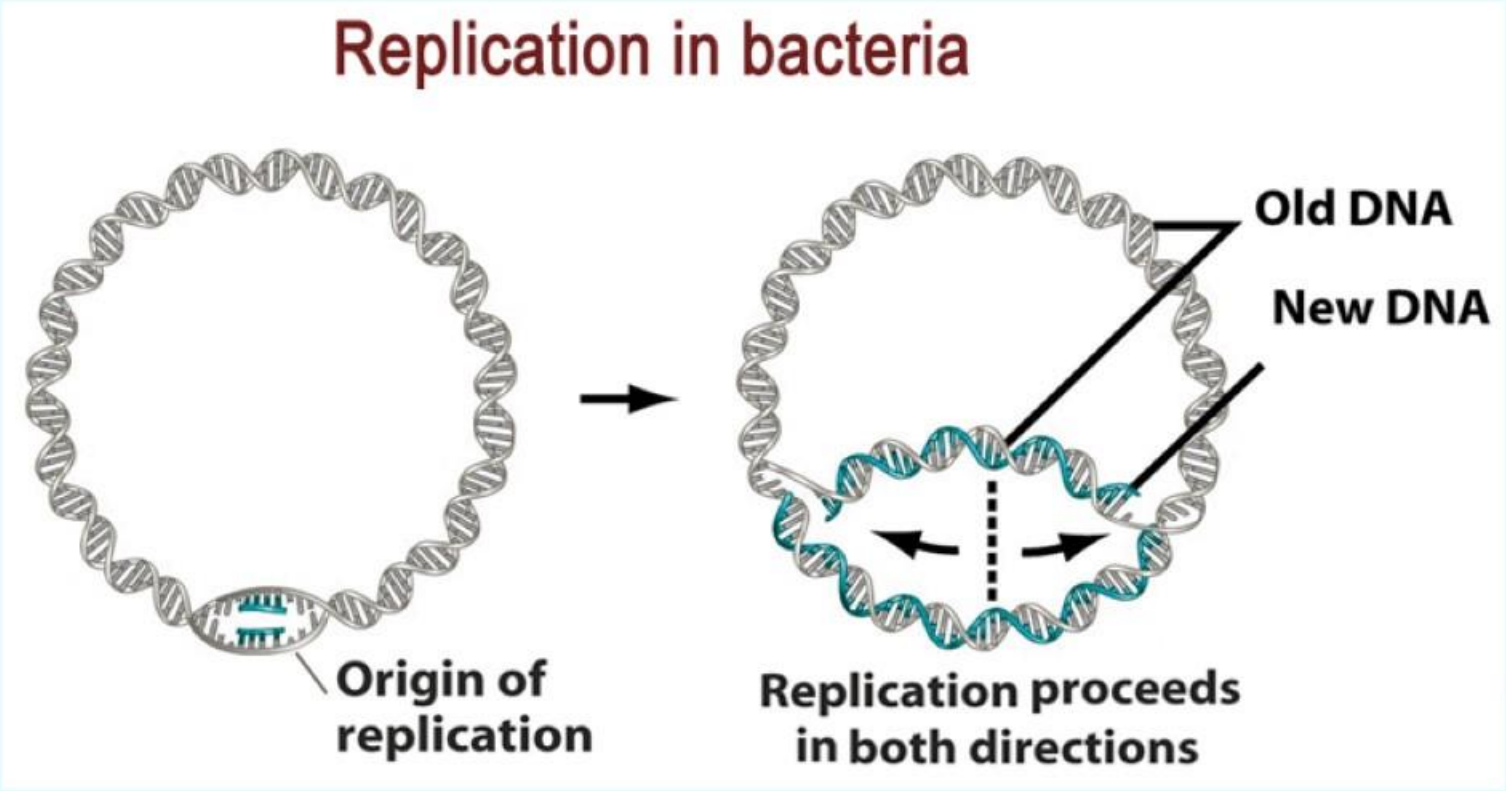


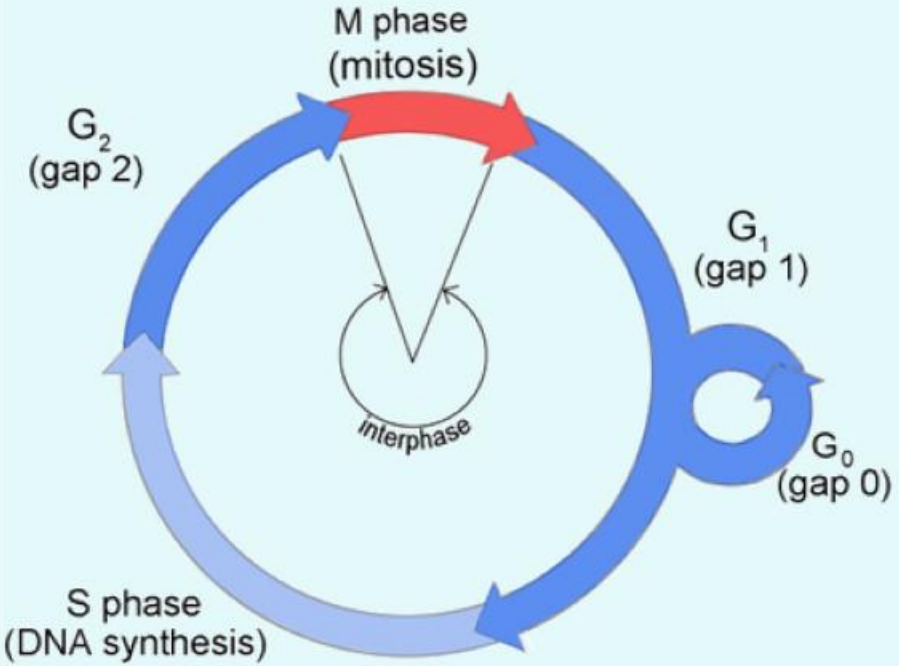


DNA REPLICATION

THE MACHINERY & ENZYMES

- *E. coli* completes replication within 18 minutes. i.e. 2000 bp per second.
- In eukaryotes, the replication of DNA takes place at S-phase of the cell cycle.
- Failure in cell division after DNA replication results in polyploidy.





DNA REPLICATION

THE MACHINERY & ENZYMES

- Nuclear Chromosomes
- "Unzipping" DNA
- Complementary Base Pairs
- Simultaneous Replication
- Two New DNA Strands