Aakash + BYJU'S NOTES

# Molecular Basis of Inheritance

# Key Takeaways

1. **Search for genetic material**
   - Griffith's experiment
   - Avery, MacLeod and McCarty experiment
   - Hershey and Chase experiment
2. **Nucleic acids**
   - Double helix model
   - DNA
3. **DNA packaging**
4. **Properties of genetic material (DNA vs RNA)**

# Key Takeaways

5. RNA world and replication
6. Semi- conservative replication
7. Process of replication
8. Transcription
   - Prokaryotes
   - Eukaryotes
   - Post-transcriptional modifications
9. Genetic code
10. tRNA

# Key Takeaways

11 Translation

12 Regulation of gene expression

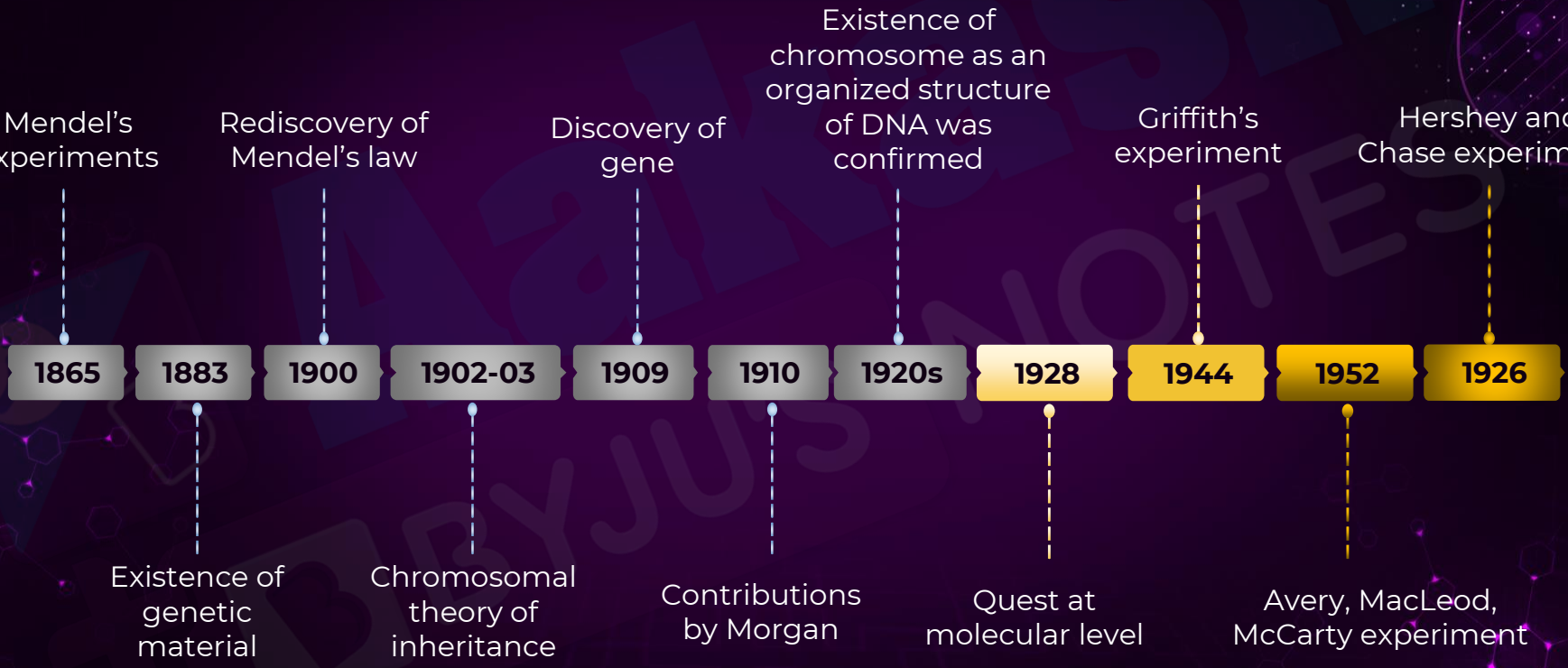
13 *Lac* operon

14 Human genome project

15 DNA fingerprinting

## Summary

# Search for Genetic Material



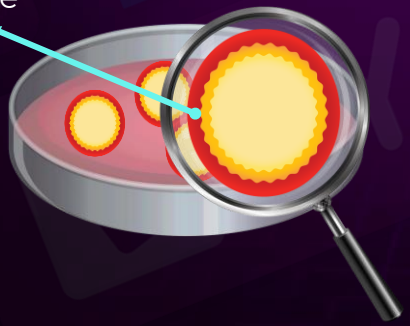
# Griffith's Experiments

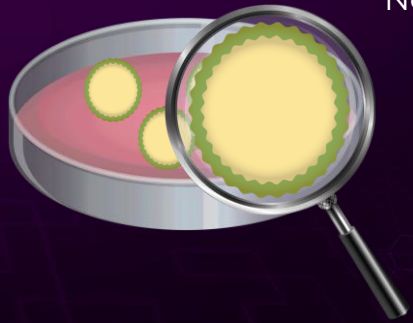
***Streptococcus pneumoniae***
Is a bacteria that causes pneumonia

**Smooth colonies**
S strain
Virulent

**Rough colonies**
R strain
Non-virulent

Polysaccharide coat

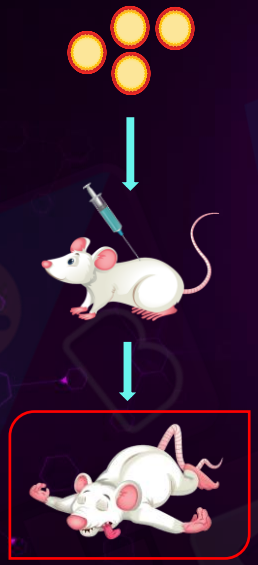




No Polysaccharide coat

# Griffith's Experiments

**Live S strain**



S strain bacteria isolated from dead mice

**Live R strain**

Mice did not die

**Heat-killed S strain**

No living bacteria isolated from live mice

**Heat-killed S strain + Live R strain**

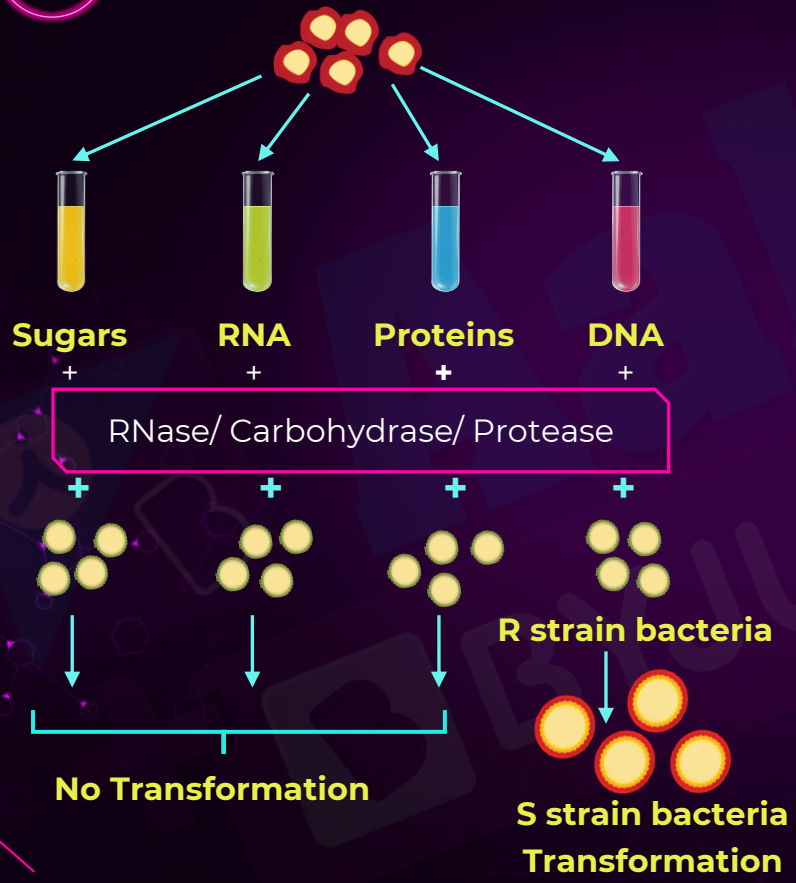
Living S- strain bacteria isolated from dead mice

# Griffith's Experiments

## Conclusion

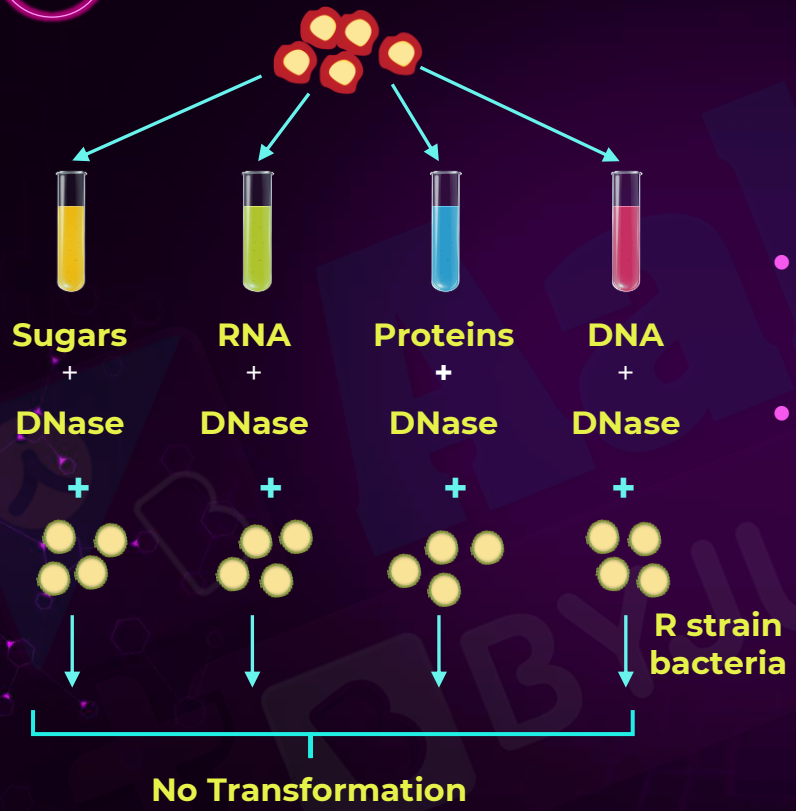

- When S strain and R strain bacteria were mixed, the **non-virulent R strain** of bacteria got transformed into the virulent **S strain bacteria**.
- This process is called **transformation**, and through which it happened is called **transforming principle**.
- This **'transforming principle'** got transferred from the heat-killed S strain.
- This had enabled the R strain to **synthesise** a smooth polysaccharide coat and become **virulent**.
- Griffith concluded that this must be due to the **transfer of the genetic material.**
- The biochemical nature of genetic material was still not defined from his experiments.

# Avery, MacLeod and McCarty Experiment




- Avery, MacLeod and McCarty purified biochemicals, carbohydrates, proteins, DNA and RNA, from the **heat-killed S cells**.
- They added live R strain bacteria to it.
- Sugars/carbohydrates, RNA and proteins showed **no transformation**.
- The **one with DNA in it transformed** the **R strain bacteria** into the S strain.
- Hence, DNA is the **transforming principle**.
- To confirm, they added either:
  - **Carbohydrase** (the enzyme which breaks down carbohydrates in all the solutions)
  - **RNase** (the enzyme which degrades RNA molecules)
  - **Protease** (the enzyme that breaks down proteins)
- The solution with DNA caused **transformation of R strain to the virulent S strain**

# Avery, MacLeod and McCarty Experiment




- However, when they added **DNase** in all the solutions, none of the solutions showed transformation.
- This proved that **DNA is the genetic material**.

# Hershey and Chase's Experiment

**Bacteriophage:** A virus that infects bacteria

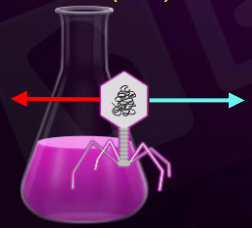
**Transduction:** Process by which foreign DNA is introduced into a cell by a virus vector.


- They worked with virus **($T_2$ Bacteriophage)** which infects E.coli and multiplies inside it.

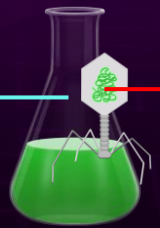
Medium with radioactive sulfur ($S^{35}$)

Medium with radioactive phosphorus ($P^{32}$)

**Radioactive protein capsid**



**Bacteriophage**



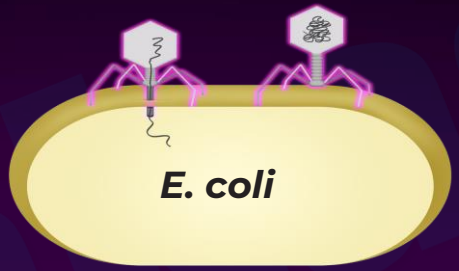
**Radioactive DNA**

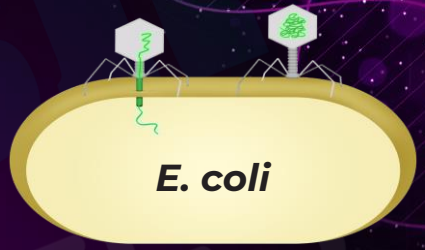
- They **grew some viruses** on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulfur.

# Hershey and Chase's Experiment

## Step 1: Infection

Both types were **allowed to infect** normally cultured bacteria separately.



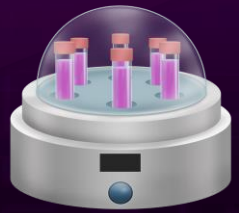



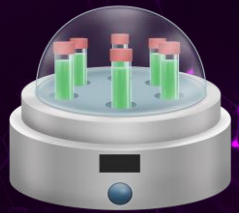

## Step 2: Blending

They were then agitated, to **break the contact between virus and bacteria**.

## Step 3: Configuration

This separated bacterial cells and viruses into two different levels as **bacterial cells are heavier**, therefore they settle down.

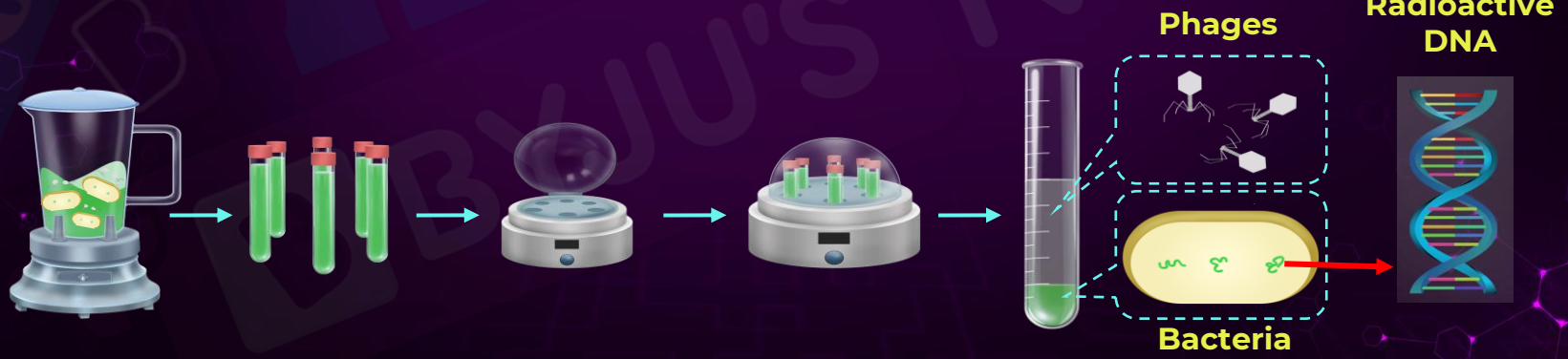




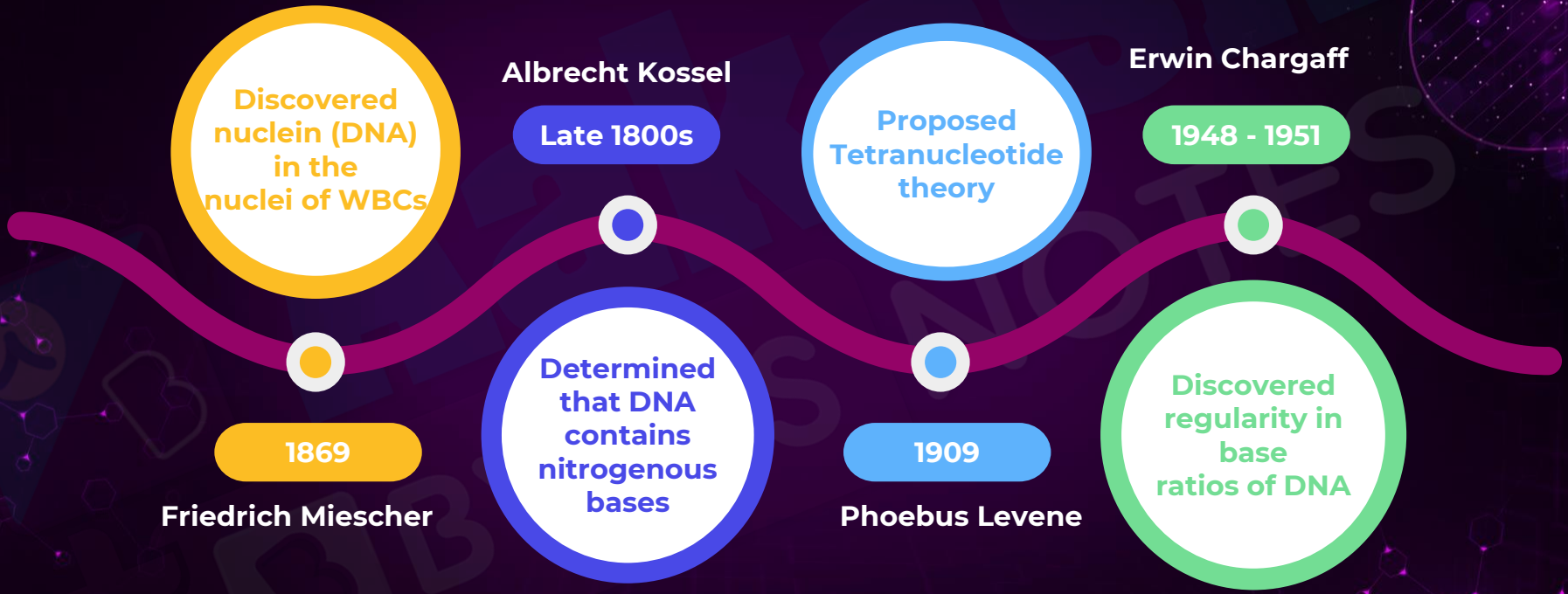
# Hershey and Chase's Experiment

**Results**

- The bacteria which were infected with **radioactive DNA viruses were radioactive**, indicating that **DNA was the material that passed** from the virus to the bacteria.
- However, **bacteria** that were **infected with viruses containing radioactive proteins** were **not radioactive**.
- This showed that **proteins did not enter** the **bacteria** from the viruses.
- Hence, it was proved that **DNA** is the genetic material.



# Nucleic Acids : Discovery



# Nucleic Acids

**Nucleic acids**

- **DNA** — Deoxyribonucleic acid

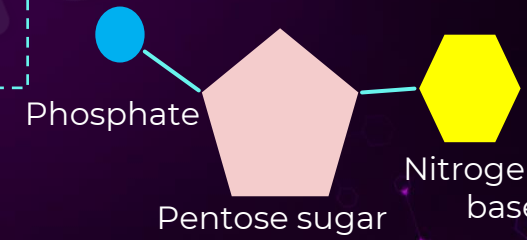
- **RNA** — Ribonucleic acid

**Nucleotide** — Monomers of nucleic acid

- **Pentose sugar**
- **Nitrogenous base**
- **Phosphate group**

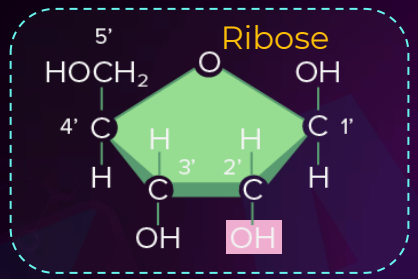
Pentose sugar + Nitrogenous base → Nucleoside

Nucleoside + Phosphate → **Nucleotide**

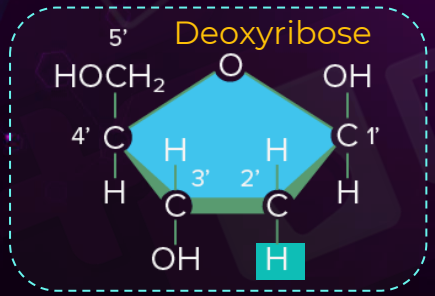


# Pentose sugar and Phosphate Group

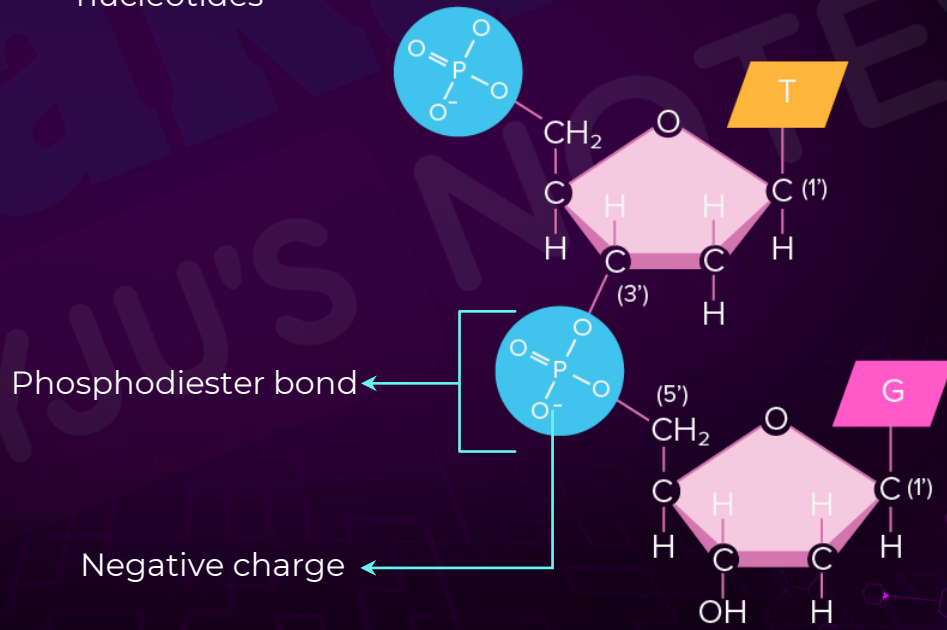
**RNA has ribose sugar, with** -OH group at 2’ position




**DNA has deoxyribose sugar, with** -H group at 2’ position




- Phosphate group links the **3’-carbon** of one sugar of one nucleotide to the **5’-carbon** of the sugar of the succeeding nucleotide through an **ester bond**.
- Phosphodiester bond is a connecting link between two consecutive nucleotides



# Nitrogenous Base

**Nitrogenous bases**

Heterocyclic Nitrogen-containing compounds

## Purines

9 membered double ringed structure

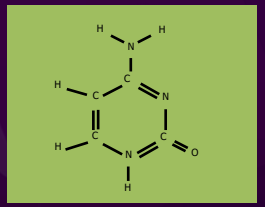
**Adenine (A)**

**Guanine (G)**

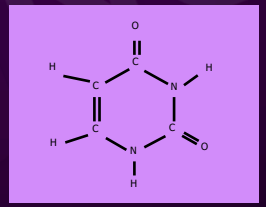
- In both DNA and RNA

## Pyrimidines

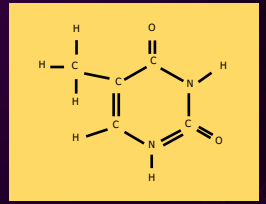
6 membered single ringed structure



**Cytosine (C)**



**Uracil (U)**



**Thymine (T)**

- In DNA, cytosine and thymine are found
- In RNA, cytosine and uracil are found

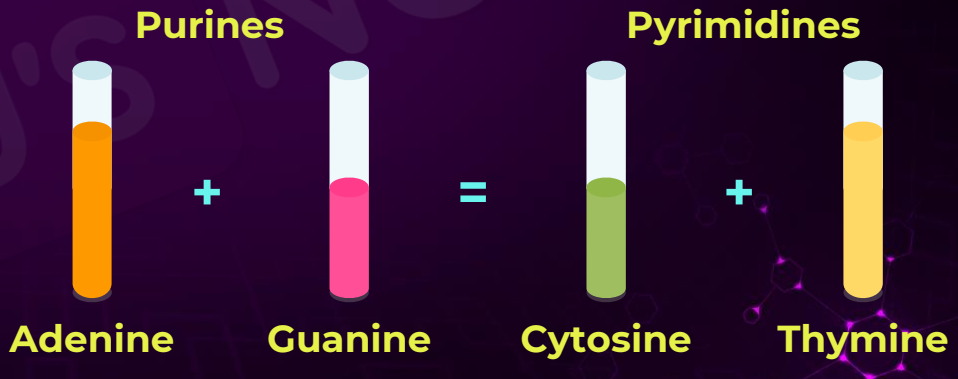
# Derivation of DNA structure

## X-ray crystallography :

- **Maurice Wilkins** and **Rosalind Franklin** obtained very fine X-ray diffraction pictures of DNA
- Suggested that structure of **DNA was sort of helix with 3.4 Å periodicity**
- However, did not propose a definitive model for DNA

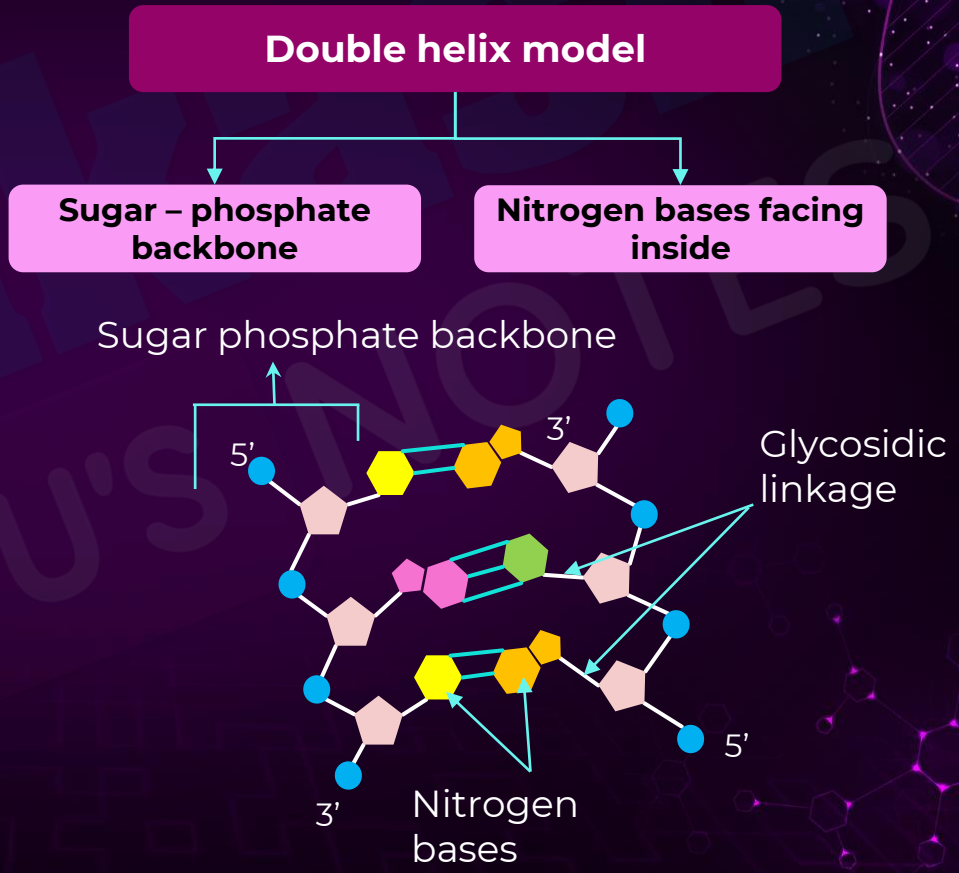
## Chargaff's rule :

- In DNA, Adenine = Thymine; Cytosine = Guanine
- A + G = C + T
- Total number of Purines = Total number of Pyrimidines
- Not applicable for single stranded DNA

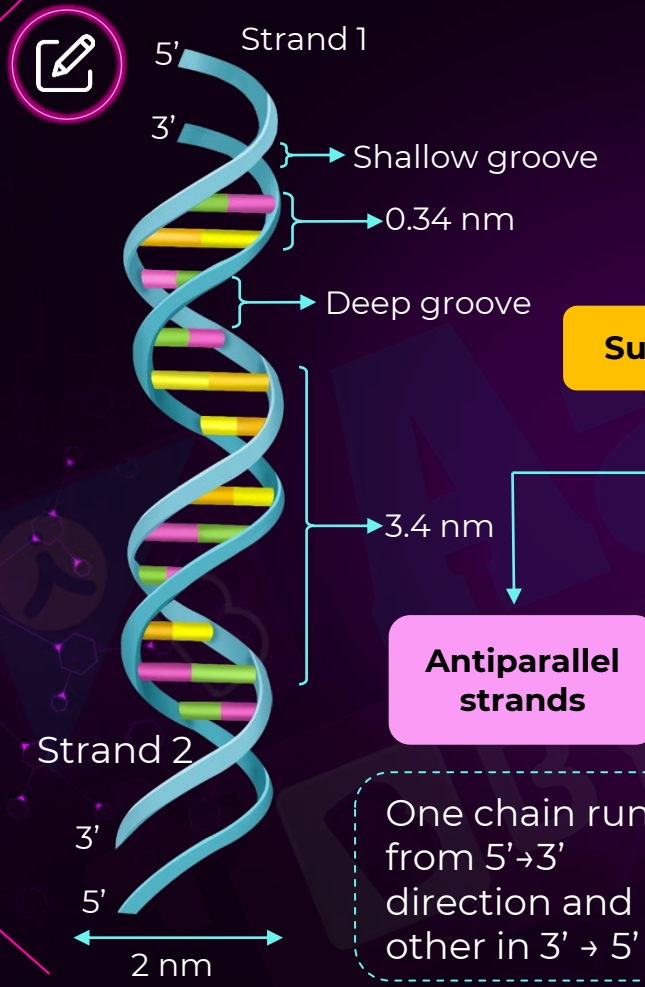


# Double Helix Model

- **James Watson** and **Francis Crick** proposed double helix model
- Made up of **two polynucleotide chains**, existing as a double helix
- Two polynucleotide strands are joined together by **hydrogen bonds between purines and pyrimidines**



# Double Helix Model




**Double helix model**

- **Sugar – phosphate backbone**
  - **Antiparallel strands**
  - **Right-handed coiling**
  - **Helical pitch 3.4 nm**
  - **Helix diameter 2 nm**
  - **Helical rise 0.34 nm**
- **Nitrogen bases facing inside**

One chain runs from 5'→3' direction and other in 3' → 5'

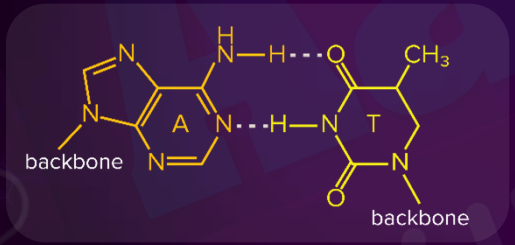
Helix pitch can be defined as the height of one complete helix turn

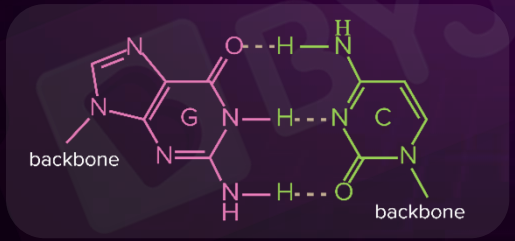
# Double Helix Model

**Double helix model**

**Sugar – phosphate backbone**

**Nitrogen bases facing inside**




**Purines**

**Pyrimidines**

**Complementary base pairing**

A = T

2 Hydrogen bonds

C ≡ G

3 Hydrogen bonds

# DNA

## Forms of DNA

### B - form

- Usual DNA
- 10 base pairs per turn
- Right-handed coiling

### A - form

- 11 base pairs per turn
- Not perpendicular to the axis but slightly tilted
- Right-handed coiling

### C - form

- Like B-form
- 9.33 base pairs per turn
- Right-handed coiling

### Z - form

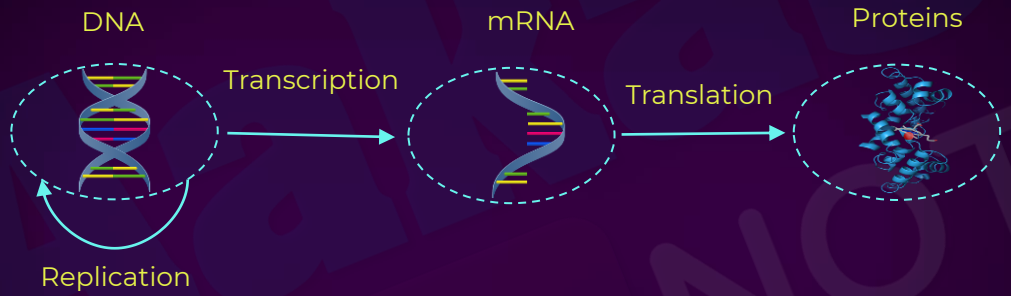
- 12 base pairs per turn
- Left-handed coiling

- **Linear double stranded DNA :** found in eukaryotes and PPLO
- **Repetitive DNA :** part of DNA with long sequence of short repetitive DNA called satellite DNA
- **Palindromic DNA :** base sequences which reads the same from either of the strands

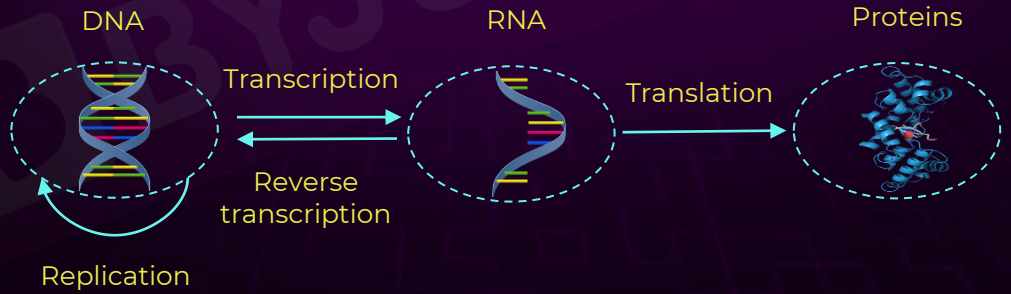
- **Denaturation/ Melting :** Separation of two strands of DNA from each other due to breakage of H-bonds when it is exposed to high temperature, acid or alkali
- **Renaturation/ Annealing :** Reassociation of separated DNA by H-bonds formation
  - DNA with more A = T, low melting areas
  - DNA with more G = C than A = T has high melting areas
- **C- value :** Total amount of DNA per genome. Expressed in picogram

# DNA

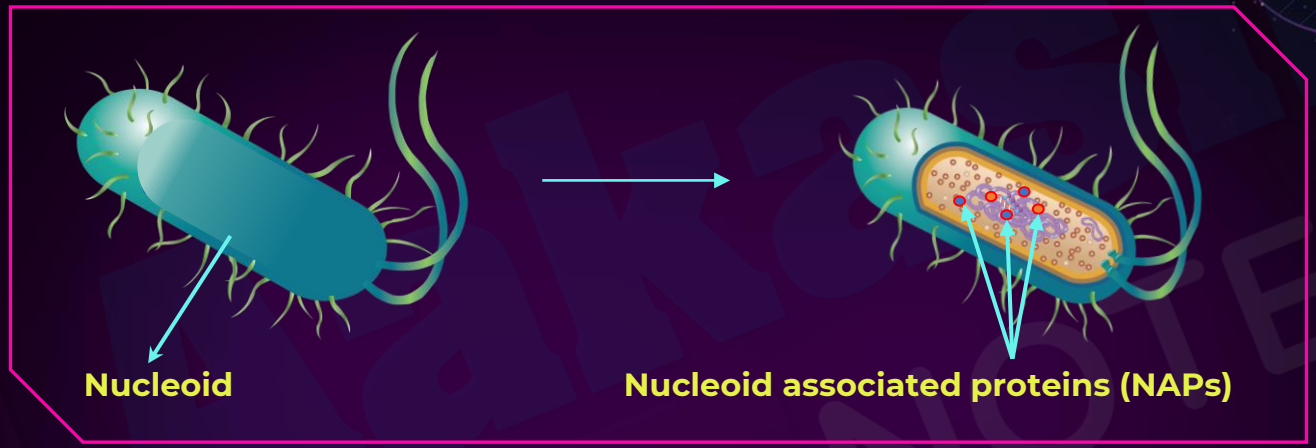
- **Central dogma of Molecular Biology :** Unidirectional flow of information from master copy DNA to working copy RNA and from RNA to building molecule or trait expressing molecule polypeptide; Proposed by **Francis Crick**




- **Reverse Central Dogma or Teminism :** Reported in 1970 by **H. Temin and D. Baltimore**
  - Independently discovered reverse transcription in some viruses
  - Viruses produce an enzyme **reverse transcriptase** which synthesizes DNA from RNA template



# DNA Packaging : Prokaryotes



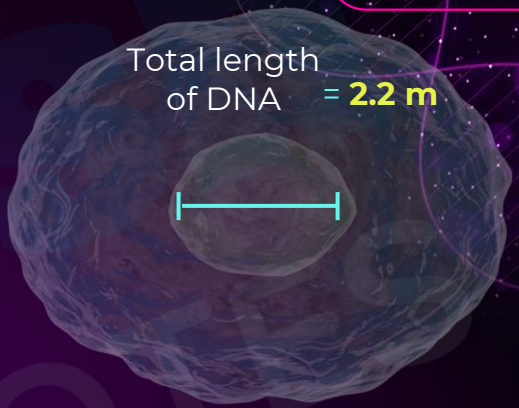

- DNA is not scattered throughout. It does **not have a defined nucleus.**
- DNA is found in cytoplasm in **super coiled stage**
  - The coils are maintained by **non-histone basic protein polyamines** which have **positive charge**
  - Packaged structure of DNA is called **nucleoid** or **genophore**
- Genomic DNA in prokaryotes is organized in **large loops held by special proteins** called **NAPs**

# DNA Packaging : Eukaryotes

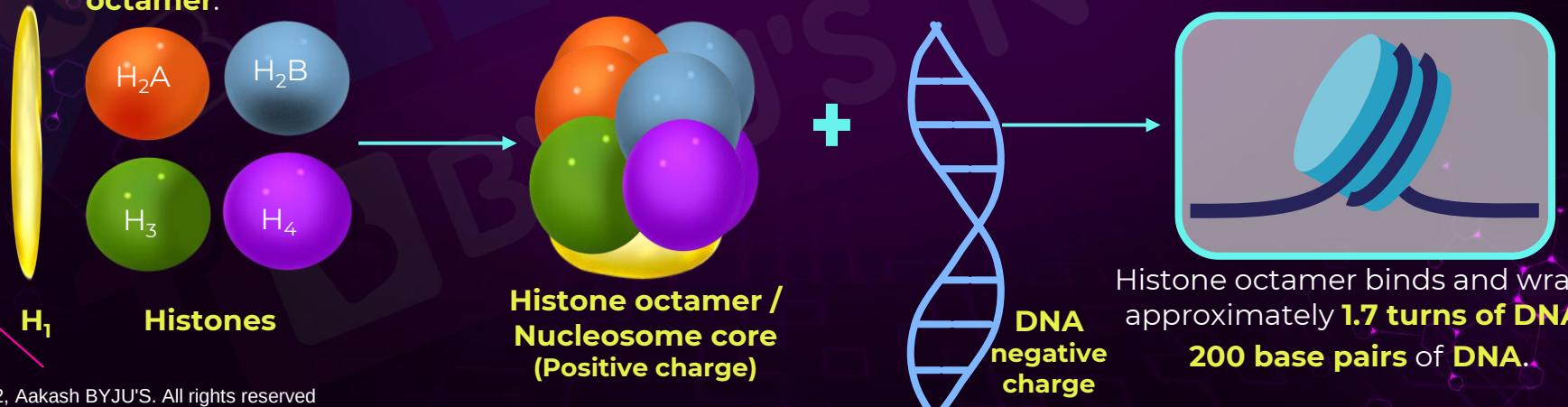
Base pairs in a cell = **$6.6 \times 10^9$**

Distance between adjacent base pairs = **0.34 nm**

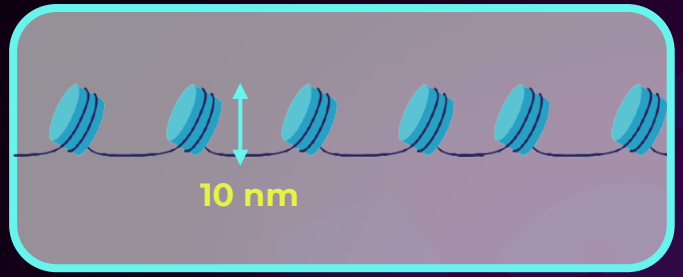
Total length of DNA = **$6.6 \times 10^9 \times 0.34 \times 10^{-9}$ m = 2.24 m**




- **In** eukaryotes, the positively charged basic **proteins involved in packaging** are called **histones**. Histones are rich in lysine and arginine amino acids.
- **Histones** are organised to form a **unit of eight molecules** called **histone octamer**.



# DNA Packaging : Eukaryotes

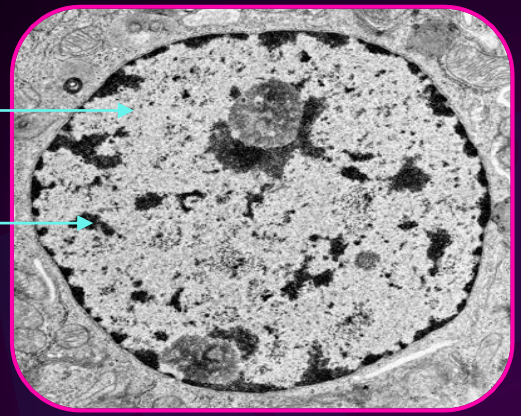



Chromatin

Chromosome

- DNA present between two adjacent nucleosome is called **linker DNA**.
- Nucleosomes are seen as beads on **thread-like structures** in the nucleus under electron microscope.
- These structures are known as **chromatin** because they are seen as **coloured bodies** when stained. Chromatin fibres are approximately 10 nm in diameter.
- The nucleosomes further coils to form **solenoid/ chromatin fibre**. It has a diameter of 30 nm.
- Chromatin fibre further condenses at metaphase stage to form **chromosome**.
    - This process requires an additional set of proteins that are collectively called **non-histone chromosomal protein (NHC).**

# DNA Packaging : Eukaryotes



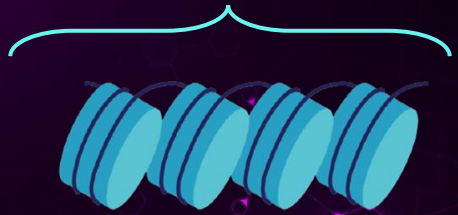

- When nucleus is stained some parts of the chromatin are **lightly stained** whereas others are stained darker.
- **Lightly stained** regions are called **euchromatin**.
- **Dark stained** regions are called **heterochromatin**.

**Euchromatin**

- Loosely packed region
- Stains light
- Transcriptionally active

- Densely packed region
- Stains dark
- Transcriptionally inactive

**Heterochromatin**



# Properties of Genetic Material (DNA or RNA)

**Criteria for genetic material :**

- Chemical and structural **stability**
- Able to generate its replica (**replication**)
- Provide the scope for **slow mutation** that is required **for evolution**
- Able to **express** itself in the form of **Mendelian characters**

**DNA being more stable is preferred for storing genetic material, as**

- Free 2'OH of RNA makes it **more labile** and **easily degradable**. Therefore, DNA in comparison is **more stable**.
- Presence of **thymine** (5-Methyl uracil) at the place of uracil, which provides **additional stability to DNA**
- RNA being unstable, mutates at a faster rate
- Viruses having RNA genome can directly code for the synthesis of proteins, hence can easily express the characters

# RNA World and Replication

- **RNA = first genetic material**
- RNA = adapter, structural molecule and catalytic
- Due to stability :
- DNA (more stable) - preferred for **storage of genetic material**
  - RNA (less stable) - preferred for the **transmission of genetic information**
- **Replication :** A process of copying and duplicating of the genetic material (DNA)
- Watson and Crick - Believed in **semi-conservative** DNA replication


- **Semi-conservative DNA replication**: Two strands of DNA unwind from each other and each act as a **template** for synthesis of a new, **complementary strand**

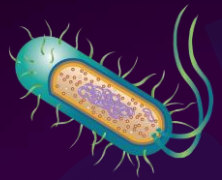
# Meselson and Stahl's Experiment - Setup

**Step 1**

**Grow *E. coli* in $^{15}NH_4Cl$ Media**

$^{15}NH_4Cl$ Media

+



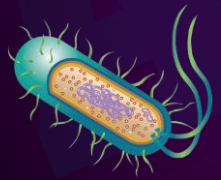
*E. coli*

$^{15}N$ is a heavier isotope of Nitrogen and not a radioactive isotope.

**Step 2**

**Transfer of *E.coli* with heavier DNA ($^{15}N$) into regular $^{14}NH_4Cl$ media**

*E. coli* **with heavier DNA**



$^{14}NH_4Cl$ Media

Heavier DNA : settle down as heavier bands
Lighter DNA : get suspended in the middle

**Step 3**

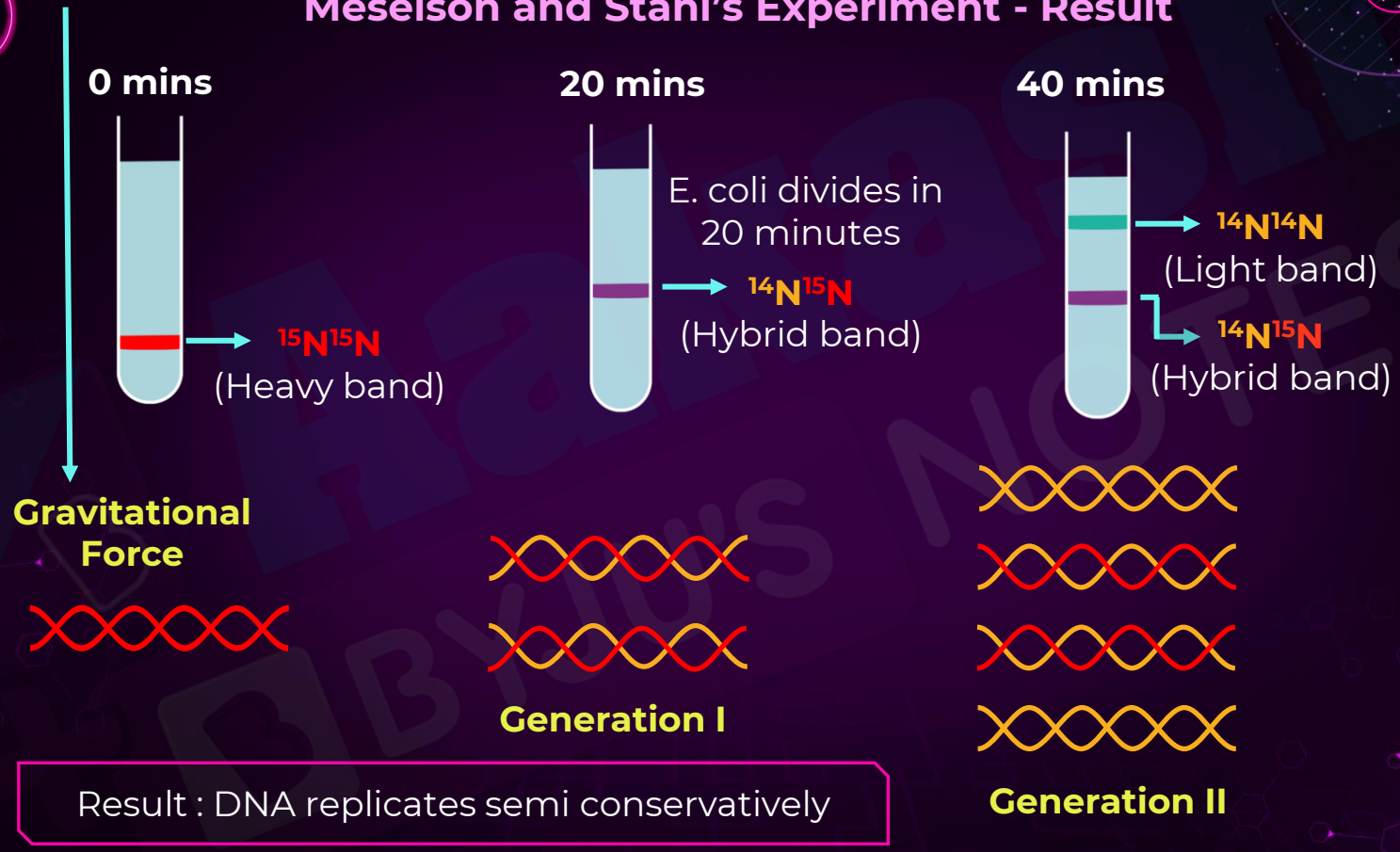
**CsCl centrifugation of the DNA samples**

**DNA isolated + CsCl**

Centrifuge

Bands as result of CsCl centrifugation

# Meselson and Stahl's Experiment - Result




Result : DNA replicates semi conservatively

# Semi-Conservative Replication

**Herbert Taylor** (on eukaryotes) performed similar experiment as Meselson & Stahl (on prokaryotes)

**Steps**

Used radioactive thymidine in root of *Vicia faba* to detect distribution of newly synthesised DNA in the chromosomes

**Results**

**Proved DNA replicates semi-conservatively**

**Faba bean plant**

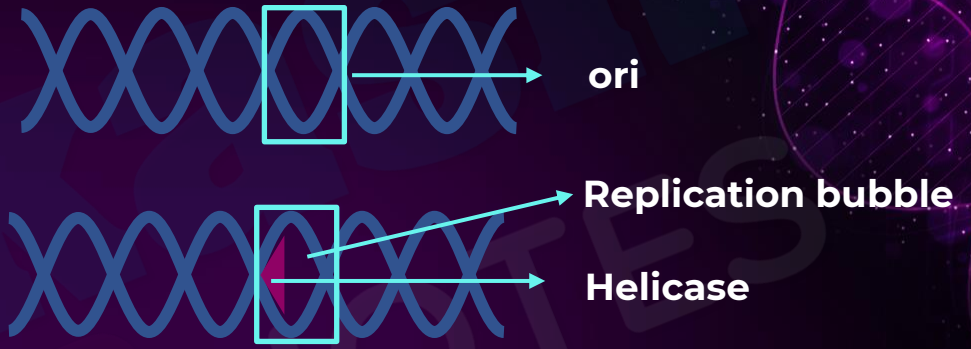
# Process of Replication

## Steps

1. Starts at the **origin of replication** (ori)

2. Activation of **deoxyribonucleotides**

3. **DNA Helicase** separates the two strands forming replication fork




**Origin of replication:** Specific regions of DNA where replication starts

**Deoxyribonucleotides :**

$$\left\{\begin{matrix}\text{dAMP}\\ \text{dGMP}\\ \text{dTMP}\\ \text{dCMP}\end{matrix}\right\} + 2H_3PO_4 \xrightarrow[\text{Energy}]{\text{Phosphorylase}} \left\{\begin{matrix}\text{dATP}\\ \text{dGTP}\\ \text{dTTP}\\ \text{dCTP}\end{matrix}\right\}$$

- **DNA Polymerase** adds newer dNTPs to 3’ end with the free –OH of primer complementary to the template DNA strand.
- **dNTPs** serve as substrates and provide energy as well.

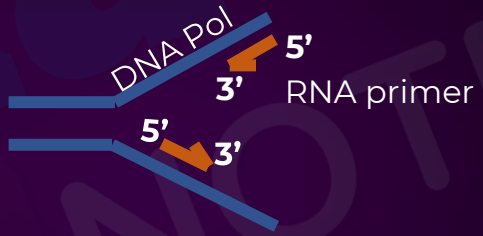
**Helicase:** Helps unwind DNA

**Replication fork:** A small opening of the DNA helix, a Y- shaped structure

# Process of Replication

Due to unwinding, a supercoiling gets developed on the end of DNA opposite to replicating fork. This tension is released by enzyme **topoisomerase**. In prokaryotes, **DNA gyrase** has topoisomerase activity.

4. **DNA dependent – DNA Polymerase** synthesise the two strands

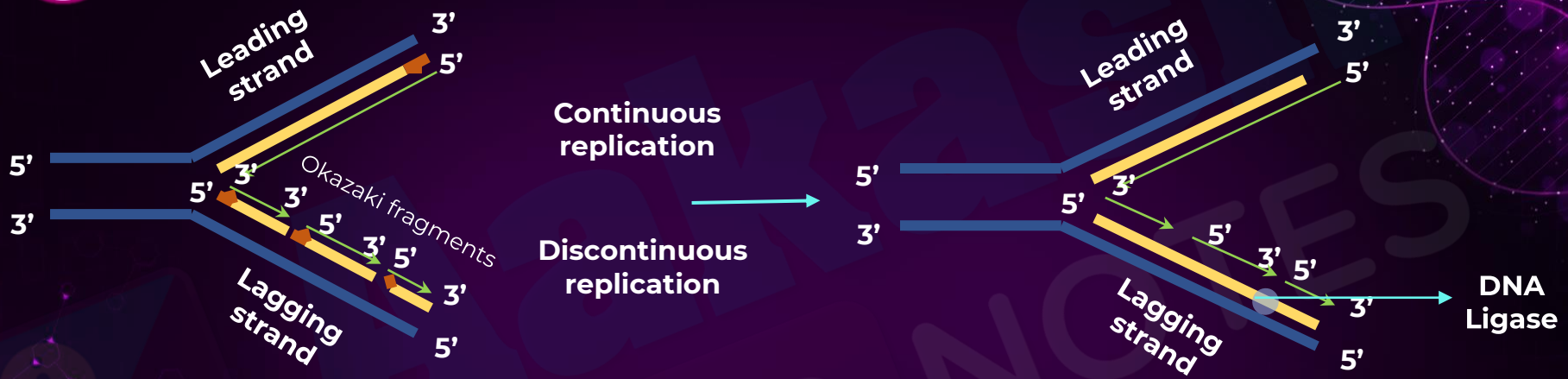



**RNA primer:** 5 – 10 nucleotide long RNA fragment that is complementary to the DNA and is synthesized by primase enzyme.

**DNA polymerase:** An enzyme that catalyzes the polymerization of deoxynucleotides.

- In prokaryotes, DNA polymerase I, II, III are the enzymes with exonuclease and polymerase are involved in the activity
- In eukaryotes, DNA polymerase α, β, ɣ,δ, ε are involved

# Process of Replication



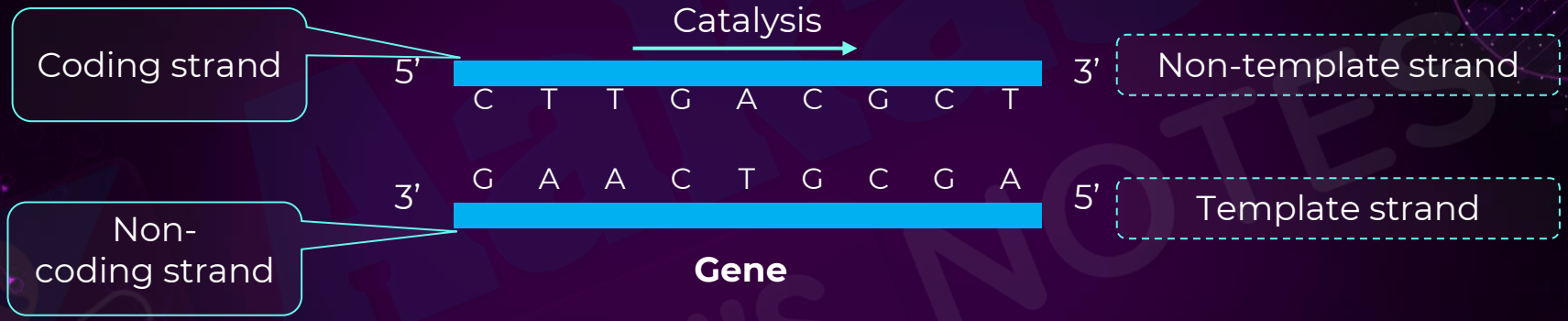

5. Using leading strand as the template, **DNA Polymerase** adds nucleotides to the new strands continuously.

6. DNA is added to lagging strands in discontinuous chunks called **Okazaki fragments**.

7. **DNA Ligase** fills small gap between the fragments.

# Transcription

Copying genetic information from one strand of the DNA into RNA is known as **transcription** (Heterocatalytic function of DNA).

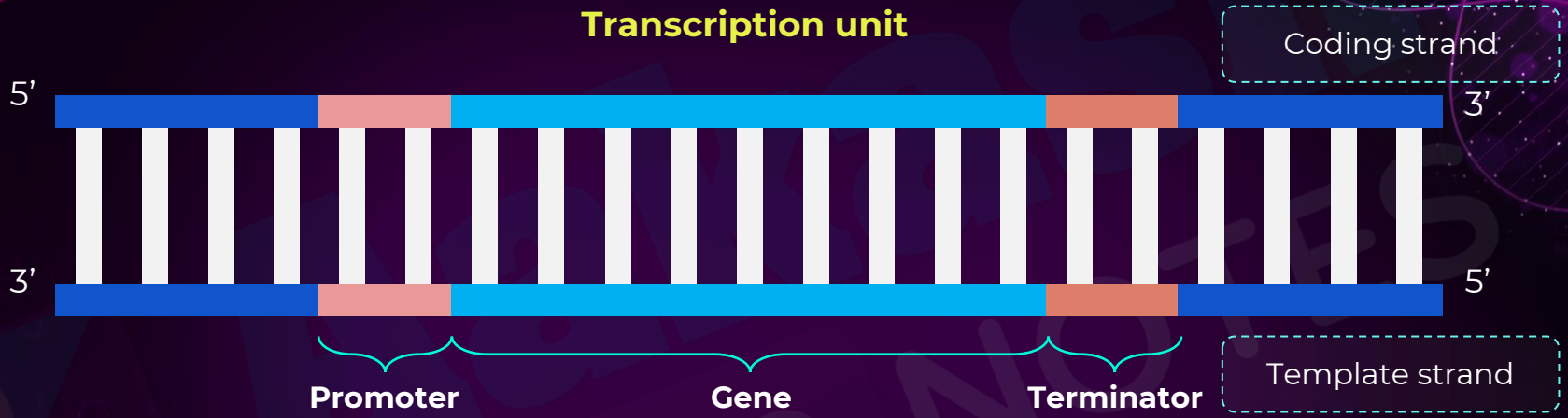



**DNA dependent RNA polymerase :**

- Uses DNA as template
- Catalyses in the direction 5' ⟶ 3'
- Does not require primer to initiate RNA synthesis
- Adds uracil instead of thymine

# Transcription

## Transcription unit




### Gene

- Codes for RNA Molecule

### Promoter

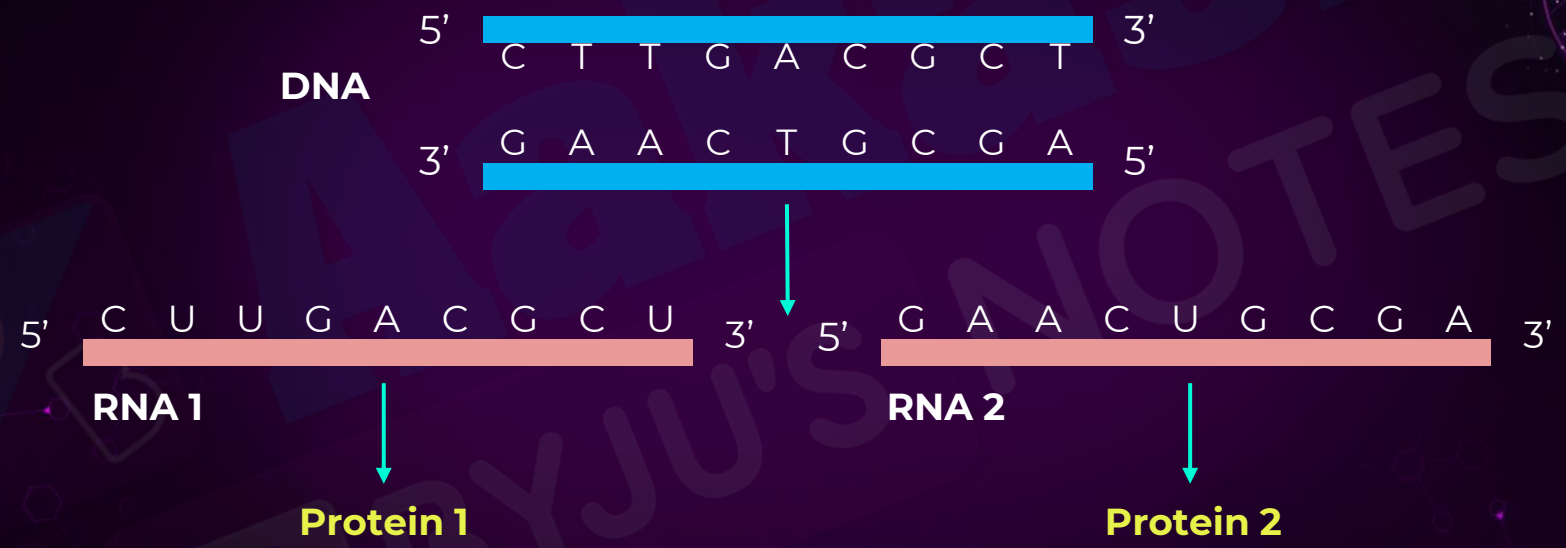
- Located towards 5' end of the coding strand of the gene
- RNA polymerase binds here to initiate transcription. Example : TATA box – has sequence TATAAT
- **Recognition sequences :** Short and conserved sequences

### Terminator

- Located towards 3' end of the coding strand of the gene
- Transcription ends at this region

# Why is Only One Strand Transcribed ?

If both strands act as a template, they would code for **RNA molecule with different sequences**




One segment of DNA would be coding for two different proteins, which would complicate the genetic information transfer machinery.

# Why is Only One Strand Transcribed ?


- Two RNA molecules produced would be **complementary** to each other, hence would form a double stranded RNA
- Would **prevent RNA from being converted into protein**

# Types of RNA

## Types of RNA

### mRNA (Messenger RNA)

- Longest
- **Carries message from DNA**
- Template for protein synthesis

### rRNA (Ribosomal RNA)

- Smaller
- Present in ribosomes
- **Helps in catalysing protein synthesis**

### tRNA (Transfer RNA)

- Smallest
- **Carries correct amino acids** to site of protein synthesis

Nucleotide sequence that codes for single protein is called **cistron**.

**Monocistronic**

- Contains single cistron
- Found in **eukaryotes**

**Polycistronic**

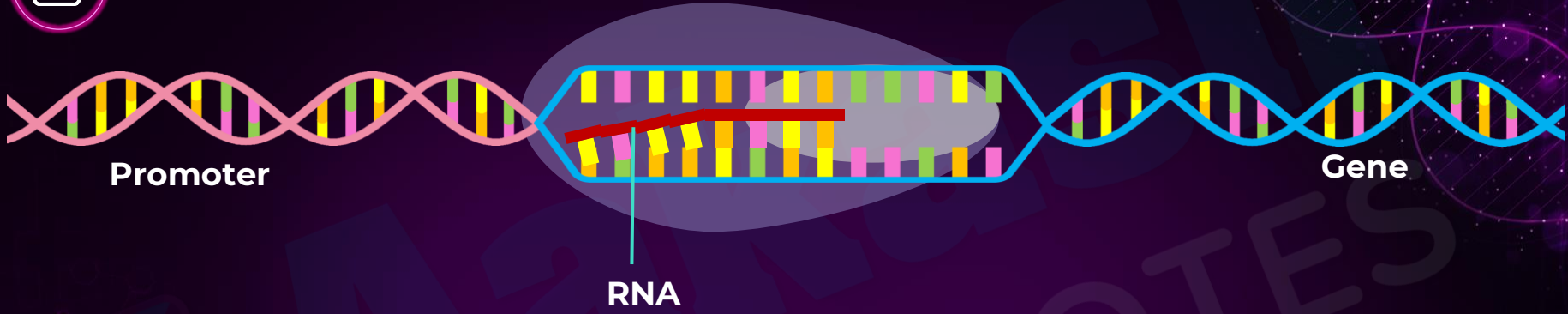
- Contains multiple cistrons
- Found in **prokaryotes**

# Transcription : Prokaryotes


**Initiation**

- Requires DNA as template and RNA polymerase
- **Sigma factor** directs RNA polymerase to **bind to promoter** and move towards gene

# Transcription : Prokaryotes




**Elongation**

- **RNA polymerase** unwinds DNA strands and starts adding nucleotides on DNA template
- Elongation continues and size of RNA grows

# Transcription : Prokaryotes


**Termination**

- Termination begins once **rho factor** is attached to RNA
- Nascent RNA along with RNA polymerase falls off

# Transcription : Eukaryotes

- Occurs in the **nucleus**
- Involves **3 RNA polymerases**
- A bit more **complex** than the prokaryotic transcription

| RNA Polymerase | Type of RNA transcribed |
|---|---|
| RNA Polymerase I | rRNA (28S, 18S and 5.8S) |
| RNA Polymerase II | hnRNA (precursor of mRNA) |
| RNA Polymerase III | tRNA, scRNA, 5S rRNA, snRNA |

*** hnRNA – Heterogeneous RNA**

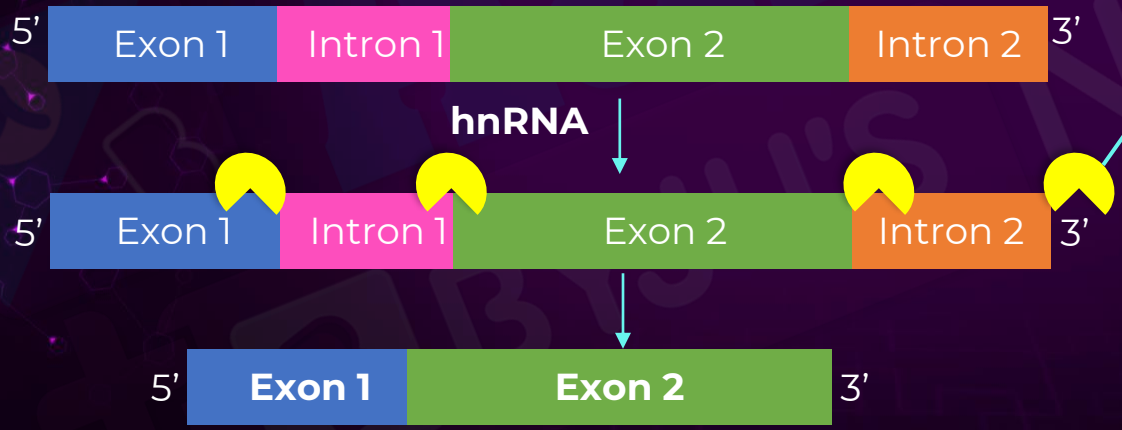
mRNA (Messenger RNA) | tRNA (Transfer RNA) | rRNA (Ribosomal RNA) | snRNA (Small nuclear RNA)

- Characteristics are same, as of prokaryotes
- Ribosome has **large (18S)** and **small (5S, 5.8S and 28S) subunit**
- Helps in **forming mRNA**

# Post – Transcriptional Modification

**Splicing** ↑ Capping ↑ Tailing

- **Involves removal of introns (non-functional)** and **joining of exons** in defined order
- Mediated by **spliceosome (snRNA + proteins)**




- **Introns :** Non-coding or intervening sequence
- **Exons :** Coding or functional sequence

# Post – Transcriptional Modification

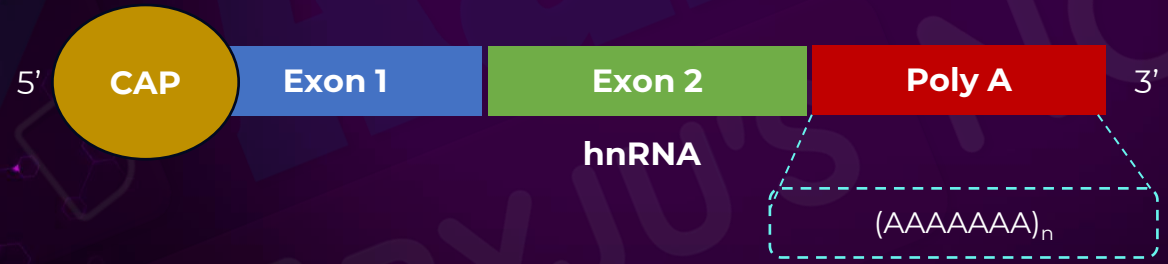
**Splicing**

**Capping**

- Addition of **unusual nucleotide** (mostly methylated guanosine triphosphate) **on 5' end**

**Tailing**

- Addition of **poly A** (200 to 300 residues) **tail at 3' end**




**Advantages:**

- Capping and tailing **protects transcript** from enzyme attack
- Modifications helps in **recognition for protein production**

# Transcription : Prokaryotic vs Eukaryotes

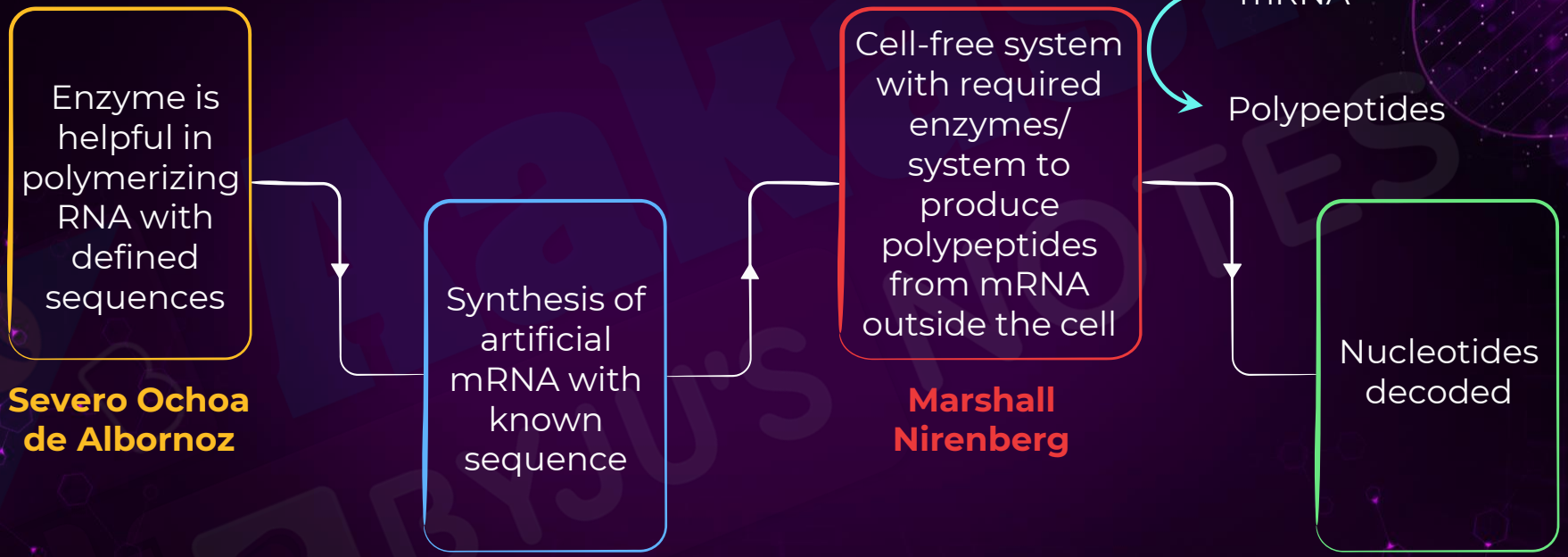
| Prokaryotic transcription | Eukaryotic transcription |
|---|---|
| Occurs in cytoplasm | Occurs in nucleus |
| 1 RNA Polymerase | 3 RNA Polymerases |
| No transcription factor required | Needs transcription factors |
| RNA formed is polycistronic | RNA formed is monocistronic |
| No modification required | Involves post-transcriptional modification |

# Genetic Code

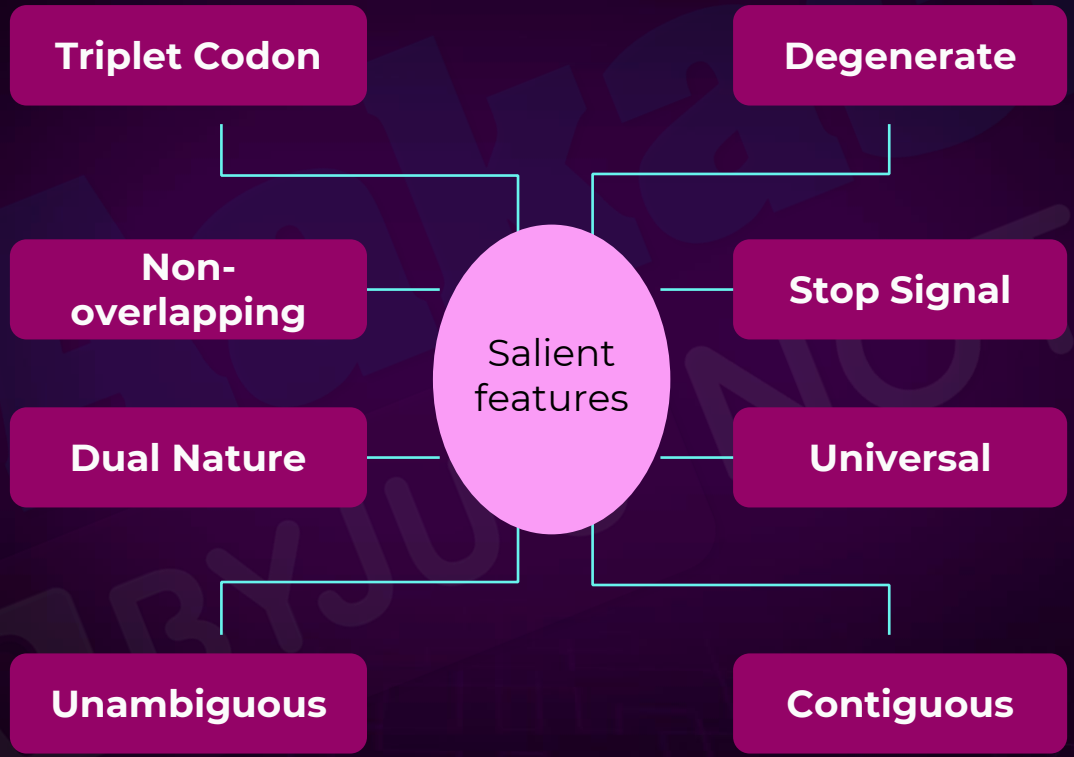
| | U | C | A | G | |
|---|---|---|---|---|---|
| U | UUU | UCU | UAU | UGU | U |
| | UUC | UCC | UAC | UGC | C |
| | UUA | UCA | UAA | UGA | A |
| | UUG | UCG | UAG | UGG | G |
| C | CUU | CCU | CAU | CGU | U |
| | CUC | CCC | CAC | CGC | C |
| | CUA | CCA | CAA | CGA | A |
| | CUG | CCG | CAG | CGG | G |
| A | AUU | ACU | AAU | AGU | U |
| | AUC | ACC | AAC | AGC | C |
| | AUA | ACA | AAA | AGA | A |
| | AUG | ACG | AAG | AGG | G |
| G | GUU | GCU | GAU | GGU | U |
| | GUC | GCC | GAC | GGC | C |
| | GUA | GCA | GAA | GGA | A |
| | GUG | GCG | GAG | GGG | G |

- **George Gamow** argued that amino acids must constitute a combination of bases as there are just **4 bases** and **20 amino acids**.
- **Three bases** would code for **1 amino acid**
- **64 combinations > 20 amino acids**
- **3 letter code (triplets)** would be **sufficient** to code for 20 amino acids

# Genetic Code



# Genetic Code




**Three nitrogenous bases** form a codon

Codons **do not overlap** with each other. They are **discrete**

**AUG** functions as an **initiator codon** as well as codes for **methionine**

Codon is **specific** to only **one amino acid**

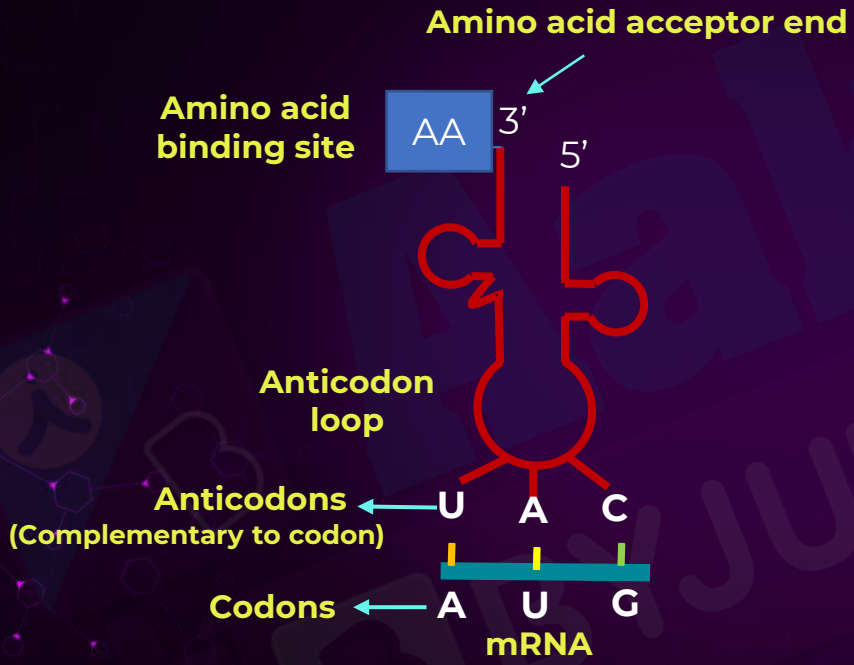
Some amino acids are coded by **more than one codon**

**Three codons** do not code for any amino acid and hence function as **stop codons**

Codon codes for the same amino acid **across all living organisms and viruses**

**No punctuations** between codons in an mRNA

# tRNA : Structure



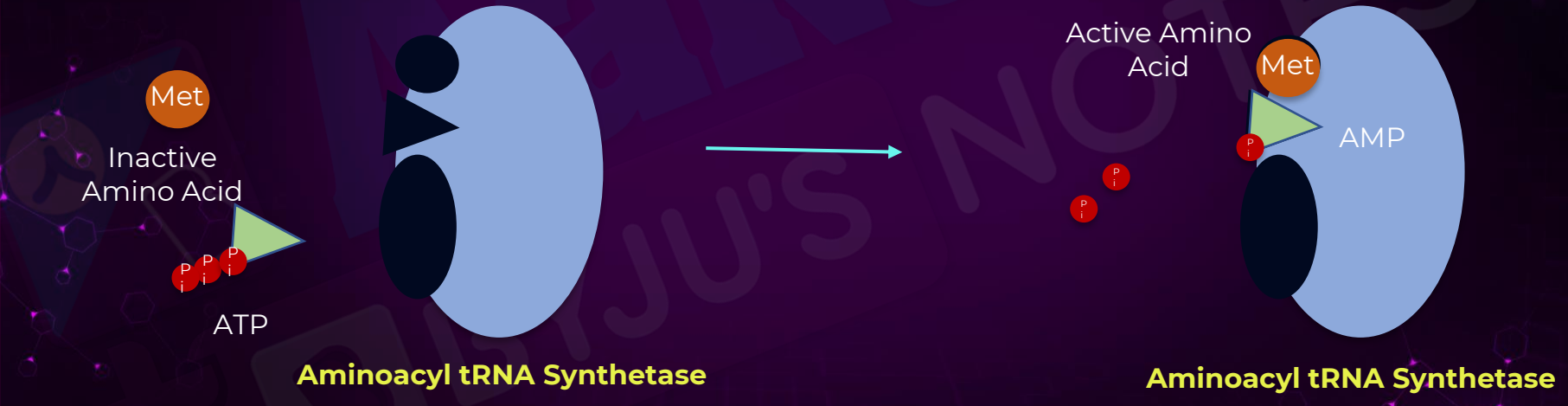

**Diagrammatic representation of structure of t-RNA**

- Has a 3' and 5' end
- Is non-linear, **clover leaf shaped** structure
- Actual 3D structure looks like an **inverted letter 'L'**
- tRNA is called an **adapter molecule** as it acts as **connecting link** between amino acids (AAs) and mRNA

# tRNA : Activation

**Activation**

- Free amino acids in cytoplasm are inactive
- Activation happens in presence of aminoacyl synthetase and ATP

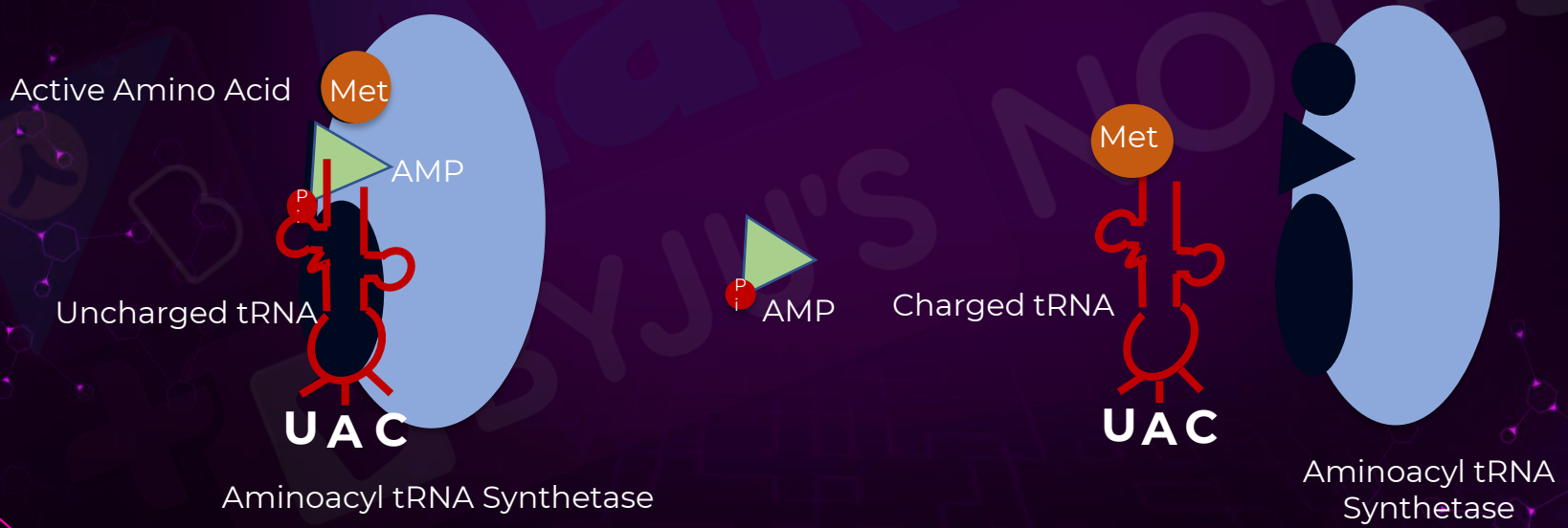



**AA + AMP + Enzyme = Aminoacyl adenylate synthetase complex**

# tRNA : Charging

**Charging**

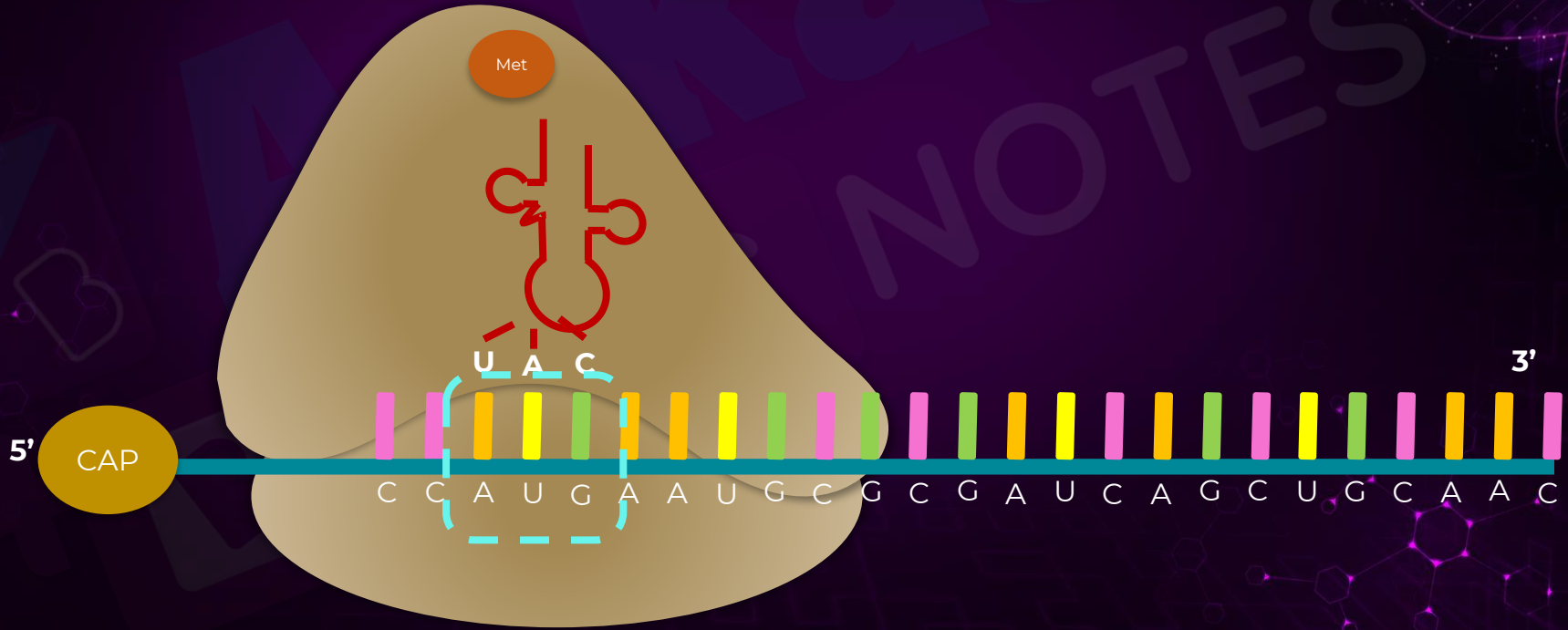
- tRNA without amino acid is called uncharged
- Addition of amino acid = Charging



# Translation

**Initiation**

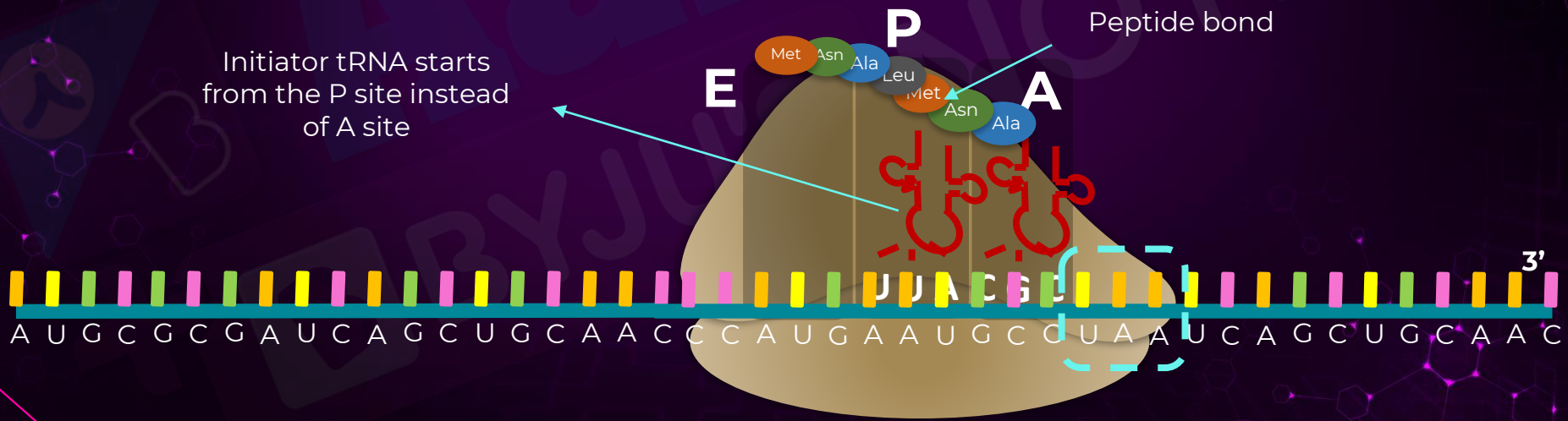
When small ribosomal subunit binds the mRNA at AUG site, translation starts



# Translation

## Elongation

- Next charged tRNA comes to the A site and peptide bond is formed between two amino acid
- Ribosome moves one whole codon hence A site becomes vacant
- tRNA at P site exits through E site and A site accepts next aminoacyl tRNA
- Polymerisation of amino acid continues



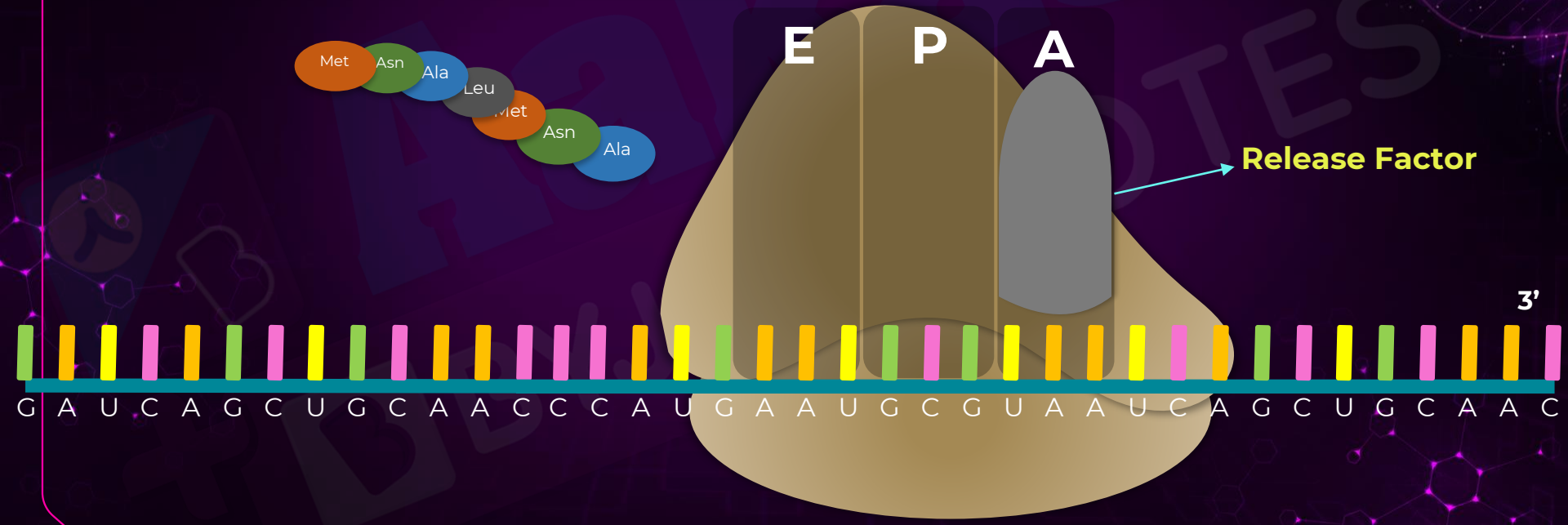
# Untranslated Regions (UTRs)

- Some additional sequences in the mRNA are present that are not translated and are known as **UTRs**.
- They are present at both 5' -end **(before the start codon)** and at 3' -end **(after the stop codon)**.
- They are important for **efficient translation**.

# Translation

**Termination**

Release factor binds to stop codon, terminating translation



# Regulation of Gene Expression

- Gene expression is the process by which **genetic information** stored in the **DNA** is **converted** into **protein** within the cell.
- Process of turning **gene expression on** or **off** is known as **gene regulation**.
- In eukaryotes, the regulation could be exerted at
  - **Transcriptional level** : Formation of primary transcript
  - **Processing level** : Regulation of splicing
  - **Transport of mRNA** from nucleus to the cytoplasm
  - **Translational level**

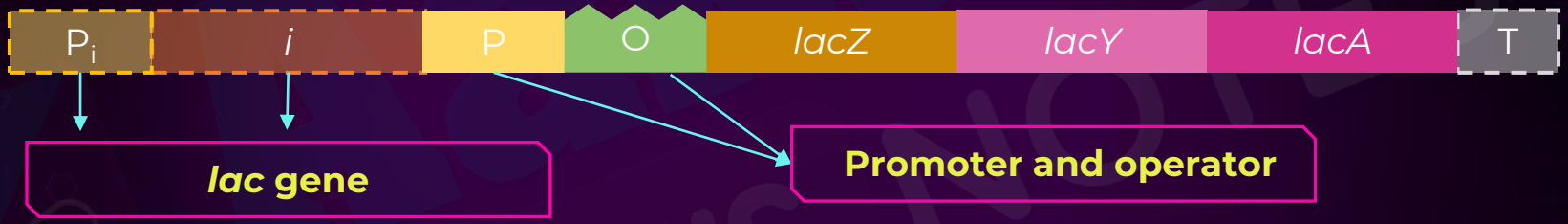
# *Lac* Operon

- **Operon** is defined as a system where the **polycistronic structural gene** is **regulated** by a common **promoter** and **regulatory protein**.
- *E. coli* prefers **glucose over lactose** as an energy source.
- However, in absence of glucose, **lactose** has to be utilized by E. coli as a **substitute** for energy.
- **Lactose/ β galactoside** is a **dimeric sugar** (disaccharide) consisting of glucose and galactose.

# *Lac* Operon

***lac* gene**

- **Structural gene** which codes for a **polycistronic *lac* mRNA** and **lactose metabolizing enzymes**




**$P_i$** - Promoter of Inhibitory gene

i – Inhibitory gene

- Regulatory gene for lac operon
- Expressed constitutively
- Codes for a **repressor protein**

**P -** Promoter of lac gene

**O -** Operator of lac gene where the repressor protein binds

# *Lac* Operon

## *lacZ* gene

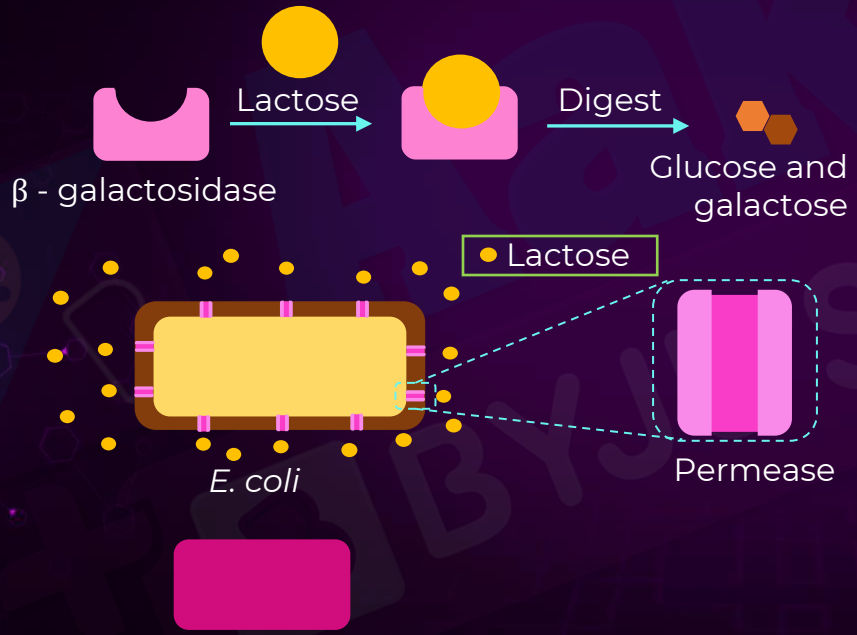
- Lac Z gene codes for **β – galactosidase enzyme**.
- **Lactose** binds to the active site of β – galactosidase.
- Lactose gets digested here into **glucose** and **galactose**.


## *lacY* gene

- Lac Y gene codes for **permease enzyme** which is a cell membrane bound enzyme.
- It make the cell membrane of E. coli **permeable to lactose**.

# *Lac* Operon


***lacA* gene**

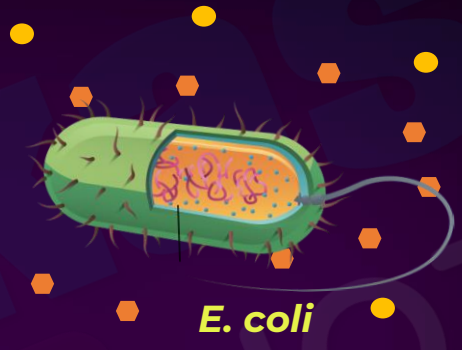



- Lac A gene codes for **transacetylase enzyme**.
- It helps in trans - acetylation reaction.
- Other functions of transacetylase are not known in great detail.

# *Lac* Operon

## Scenario 1 : *E. coli* does not feed on lactose

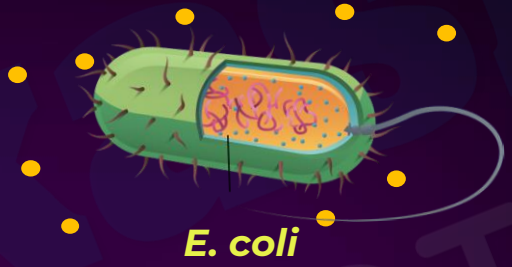
- *E. coli* does not feed on lactose normally because E. coli prefers glucose over lactose.



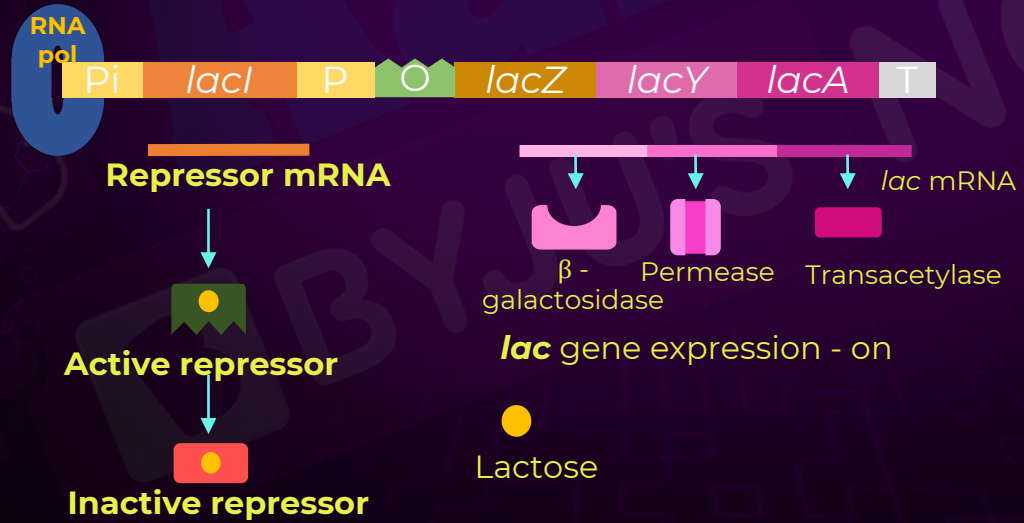

# *Lac* Operon

## Scenario 2 : *E. coli* feeds on lactose

- **RNA polymerase** binds to the **promoter** of **inhibitory gene**, and transcribes repressor mRNA which forms **active repressor**.
- **Lactose** binds to the **active repressor** and makes it **inactive**.




Lactose



# Human Genome Project

A **thirteen years long project** (1990-2003)
**Aim** was to sequence the complete human genome
Also known as **'mega project'**

**Coordinated by:**

- U.S Department of Energy
- National Institute of Health

**Partners:**

- Wellcome Trust (U.K.)
- Japan
- France
- Germany
- China

# Human Genome Project

The **number of base pairs** of the entire human genome is **approx 3 X $10^9$**

**Cost of HGP** was **$ 9 billion US dollar = 900 crores INR**

**Bioinformatics :** Hybrid field that deals with biological data and uses computer science to store, retrieve and analyse them.

**DNA sequencing** – It is a process of identifying the exact sequence of nitrogenous bases in the DNA.

# Human Genome Project : Goals

Identification of approx. **20,000-25,000 genes** in human DNA

Determination of **3 billion chemical base pairs** of human DNA

**Storing the information** in databases

**Improvement of tools** for data analysis

**Transfer related technologies** to other sectors

Address **ethical, legal** and **social issues** that may arise from HGP

# Human Genome Project : Methodology

**Sequence annotation :**

Isolation of DNA

↓

Amplification of DNA or creation of copies

↓

Sequencing of amplified fragmented DNA

↓

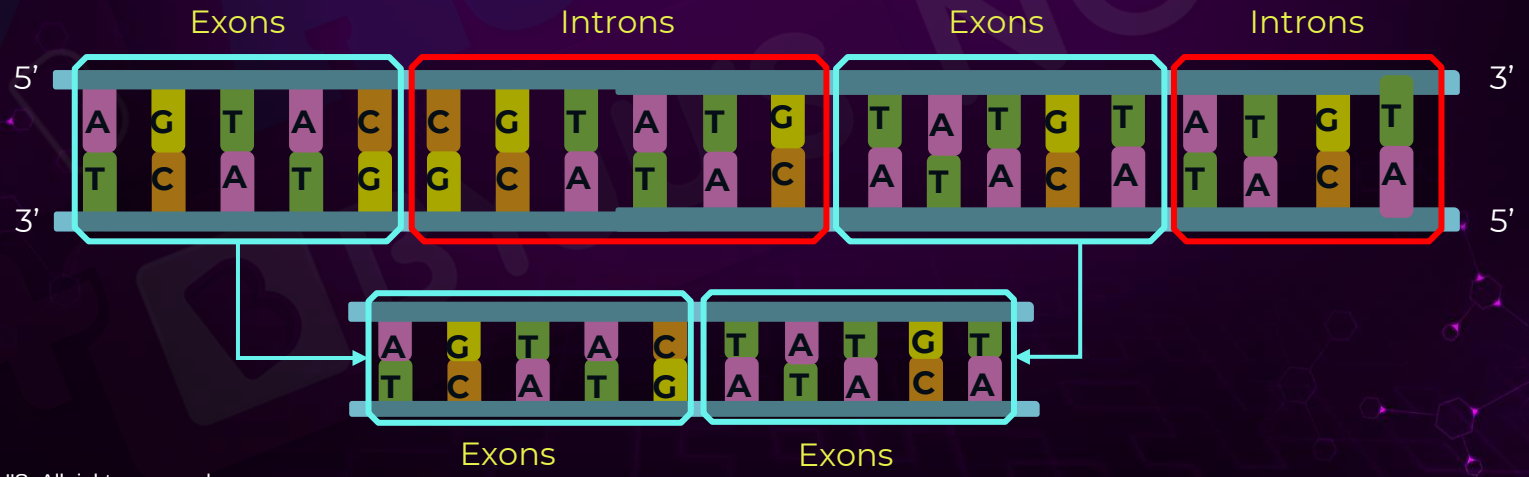
Annotation and assigning of DNA

- Fragmented DNA are then cloned in suitable hosts
- The commonly used host were yeast and bacteria and vectors were :
  - **BAC** (Bacterial Artificial Chromosome)
  - **YAC** (Yeast Artificial Chromosome)

# Human Genome Project : Methodology

**Expressed sequence tags :** Identifying all genes that are expressed as RNA

- **DNA** is **isolated** from the cell.
- **mRNA** is obtained from this DNA.
- Since **introns**, which are present between two exons, are **removed** during mRNA synthesis, they are not sequenced.
- This way, all the **coding genes** are isolated and **sequenced**.



# Human Genome Project : Features

Human Genome contains approx. **3164.7 million bp**

Average size of gene is **3000 bases**

Human genome has **30000 genes**

Function of over **50% genes is unknown**

Only **2%** of genome **codes for proteins**.

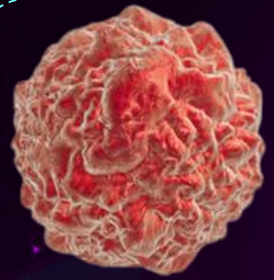
Most of the genome contains **repetitive sequences (VNTRs)**

Repetitive sequences **thought to have no direct coding functions** but shed light on **chromosome structure, dynamics and evolution**

Chromosome 1 has **2968 genes** while Y-chromosome has **231 genes**

**SNPs** were identified which can be used in disease detection and tracing human history

# Human Genome Project : Applications

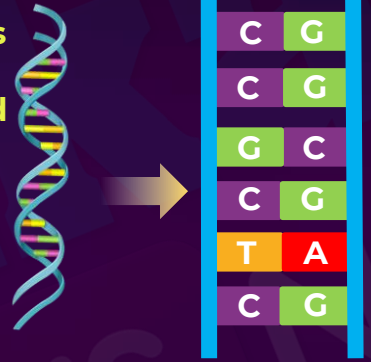


Cancer cell

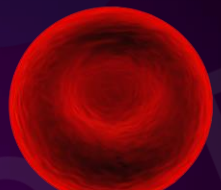
Early diagnosis of cancer cells

Suspect

Suspect's DNA extracted




In forensic medicine, to match DNA samples of suspects to reach the criminals



Normal RBC

Defected RBC

With genomic sequence, disease like sickle cell anemia can be detected

# Repetitive Sequences

- Repetitive elements that **occur multiple times** in the nucleic acid sequences(DNA/RNA)
- In **introns**, the sequences can be both **repetitive** and **non-repetitive**
- Number of these repeats is **different in different individuals**
- **Used in** the technique of **DNA fingerprinting**

5’ CTCATGATGATGATGATGTCATCCCGAAATCGTAGCTA 3’

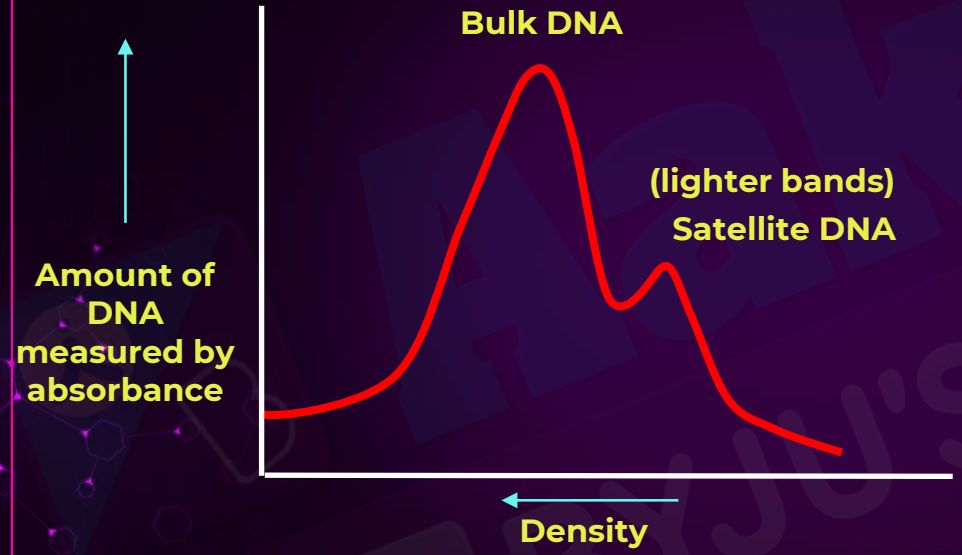
**Repetitive sequence**

5’ CTTAGGATTCAATCCGATTCATCCCGAAATCGT 3’

**Non-repetitive sequence**

# Repetitive Sequences

(Main band is formed by the **denser DNA**)




- These **repetitive DNA are separated** from **bulk genomic DNA** as **different peaks** during **density gradient centrifugation**.

**Satellite DNA:** Highly repetitive DNA sequence that **does not code for proteins** and is used for DNA fingerprinting.

- It is classified on the basis of
  - length of sequence
  - number of repetitive units
  - base composition

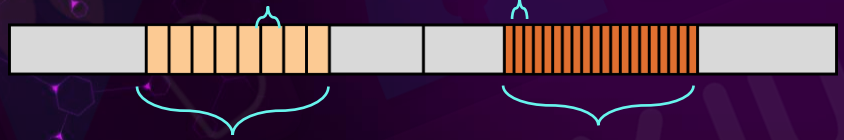
# Satellite DNA - Types

## Micro satellite

**2-6 base pairs** repeating units in tandem repeats

**Short Tandem Repeats**

Repeat unit size = 2 – 6 base pairs



Repeated 8 times

Repeated 20 times

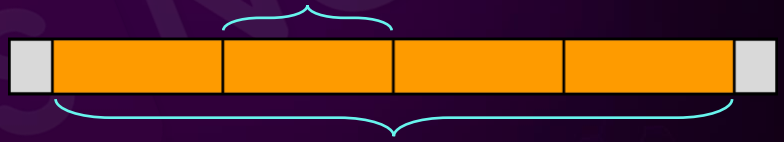
Short Tandem Repeats (STR)

## Mini satellite

**10-100 base pairs** repeating units in tandem repeats

**Variable Number Tandem Repeats**

Repeat unit size = 10-100 base pairs

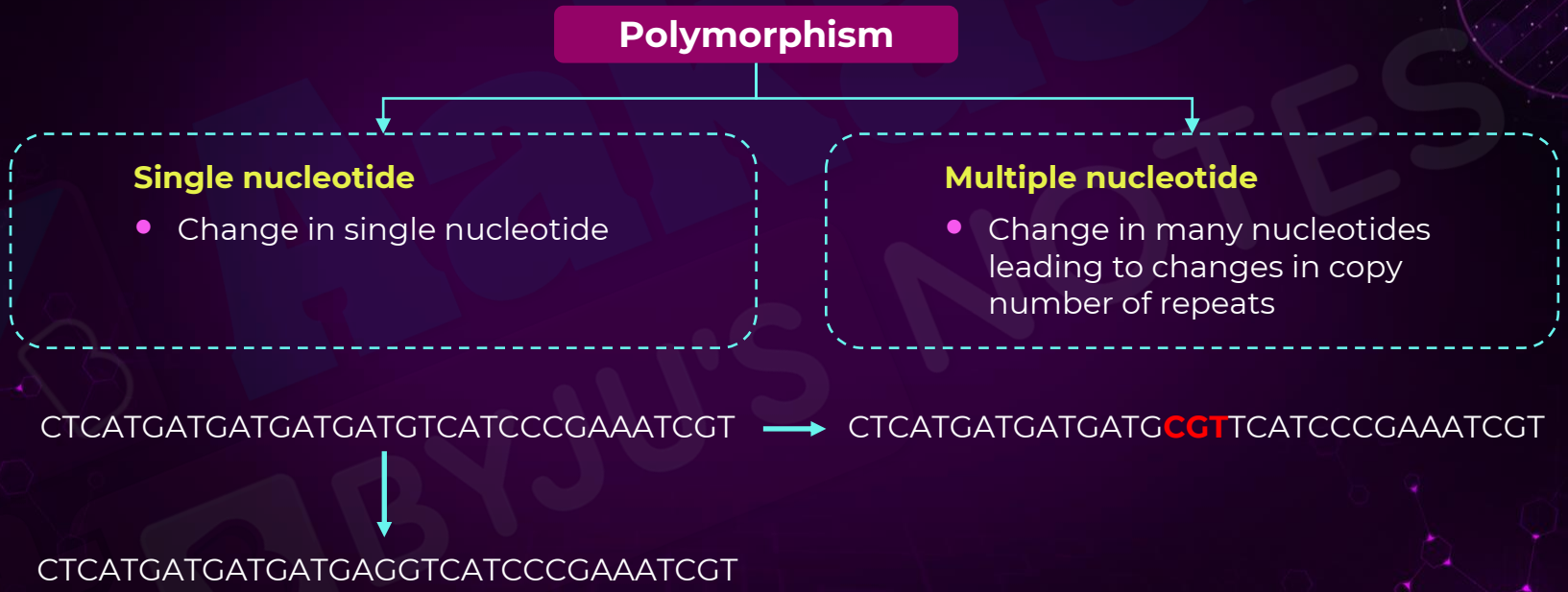


Repeated 4 times

Variable Number Tandem Repeats (VNTR)

# Polymorphism

- It is the **inheritable mutation** observed in a population at a **high frequency (Frequency > 0.01).**
- It plays a **major role in evolution.**



# DNA Fingerprinting

Alec Jeffreys

- A **technique** used to determine the **characteristic of an individual's DNA**
- Used to **compare DNA of two individuals**
- Was discovered by **Sir Alec Jeffreys**
- **Analysing DNA** of two different individuals:
  - 99.9% genome is similar
  - Differ by 0.1% (used for DNA fingerprinting)

# Steps of DNA Fingerprinting

**DNA isolation:** DNA isolation is performed using a biological sample.

DNA isolation

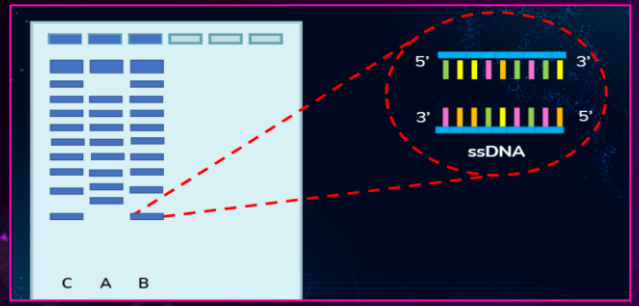
2


Restriction digestion
(Cuts DNA into multiple fragments)

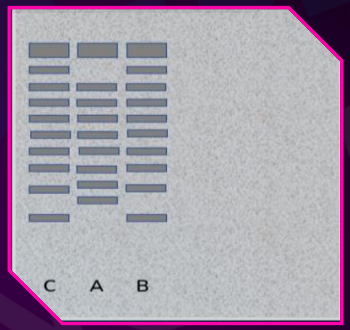
1

# Steps of DNA Fingerprinting

**DNA isolation:** DNA isolation is performed using a biological sample




4



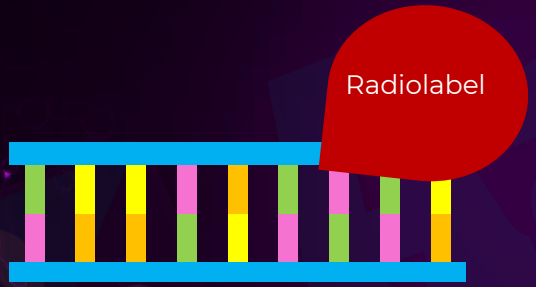

**Southern blot**
**(Transfer of DNA fragments to synthetic medium)**

3

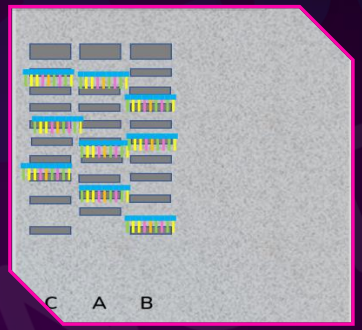
The samples move under the influence of electric charge

**Electrophoresis**
**(Separation of DNA fragments)**

# Steps of DNA Fingerprinting









DNA probes are labelled with radioactive substances

**Autoradiography (Detection of hybridised DNA fragments)**

**Hybridisation (Using labelled VNTR probe)**

As probe binds to the complementary DNA, they send out signal (radiolabels)

↓

The banding pattern obtained after exposure to x-ray is analysed.

# DNA Fingerprinting : Applications

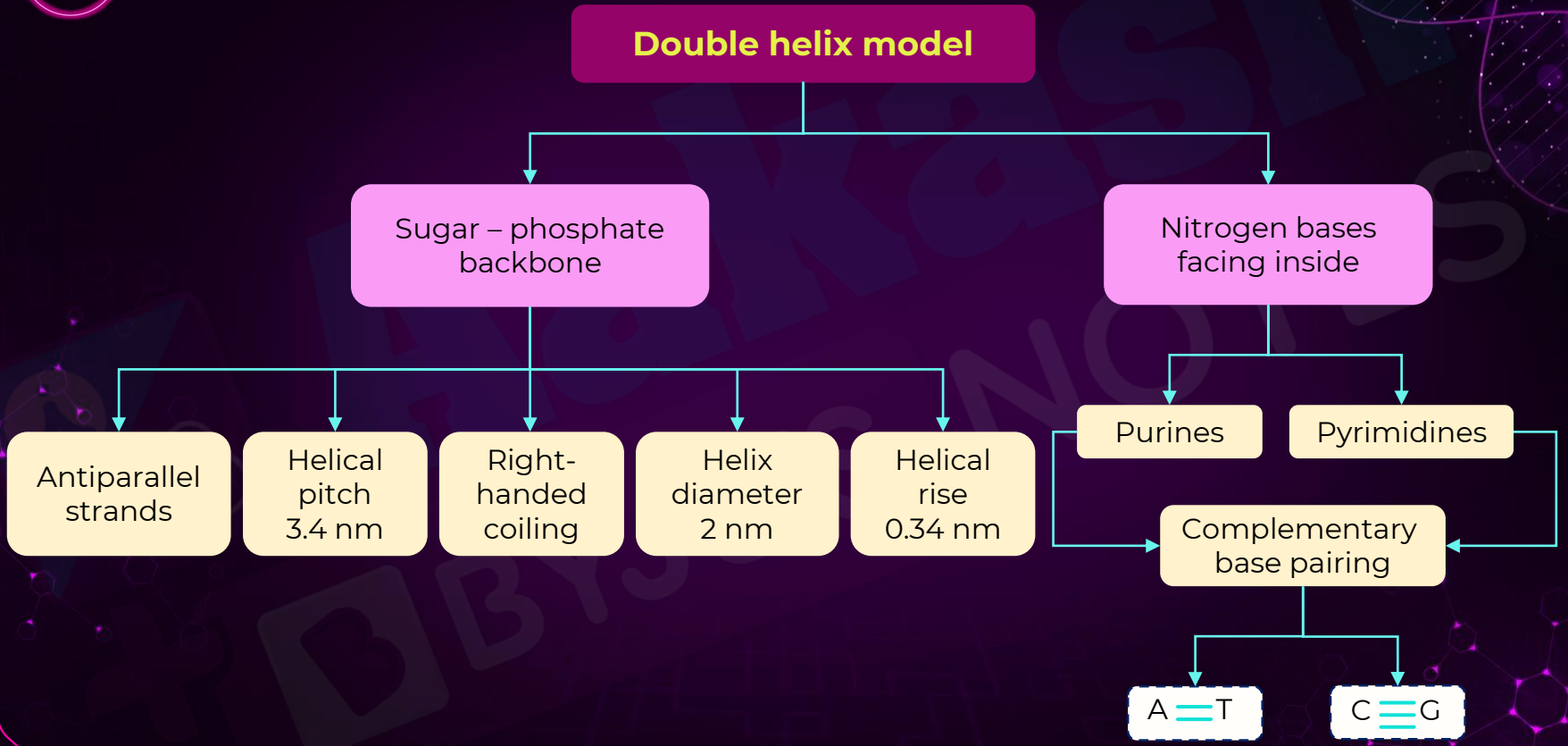
# Summary

**Griffith** concluded that **transfer of genetic material takes place** due to transforming principle

**Avery, Macleod** and **McCarty** experiment proved that **DNA is the genetic material**

**Hershey** and **Chase's** experiment also proved that **DNA is the genetic material**

# Summary



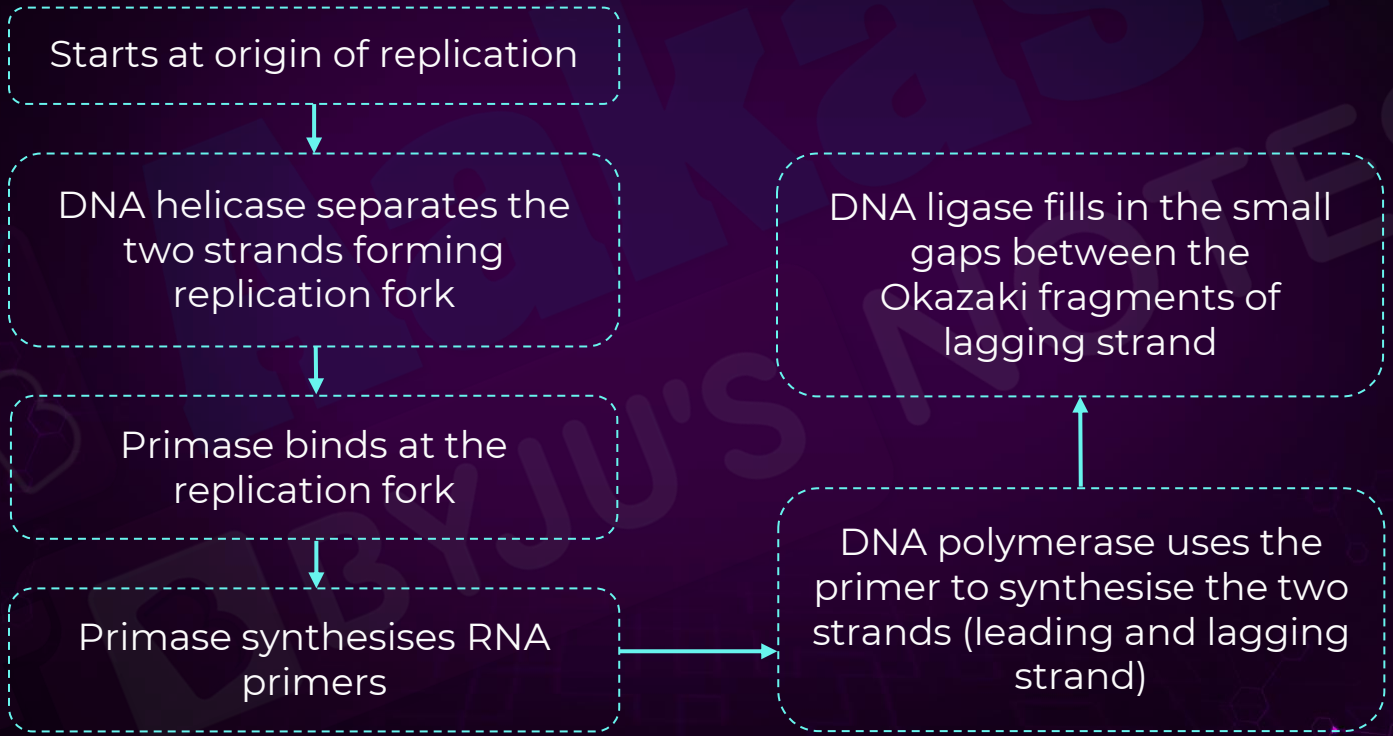
# Summary

## Ideal genetic material

- It should be able to replicate.
- It should be chemically and structurally stable.
- It should provide scope for slow changes (mutation) required for evolution.
- It should be able to express itself in the form of 'Mendelian Characters'.

# Summary

## Process of replication



# Summary

## Transcription steps

### Initiation

- RNA polymerase along with **sigma factor** attaches to the DNA molecule and recognises a **promoter sequence**.
- The DNA double helix unwinds exposing the **bases of DNA template** strand to form new mRNA.

### Elongation

- Nucleotides are added according to the **template strand** that **enables the growth of mRNA**.

### Termination

- RNA polymerase encounters a **terminator sequence**, thus causing the release of RNA from **RNA polymerase** with the help of rho factor.

# Summary

Post-transcriptional modifications

## Splicing

- **Removal of introns** and **joining of exons**

## Clapping

- Addition of an **unusual nucleotide at 5’ end**

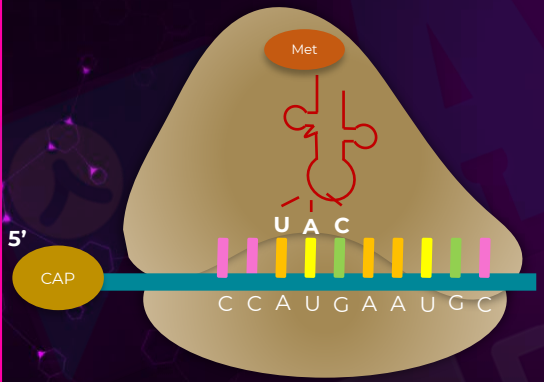
## Tailing

- Adenylate residues **(200-300)** are **added at 3'-end** in a template independent manner

# Summary

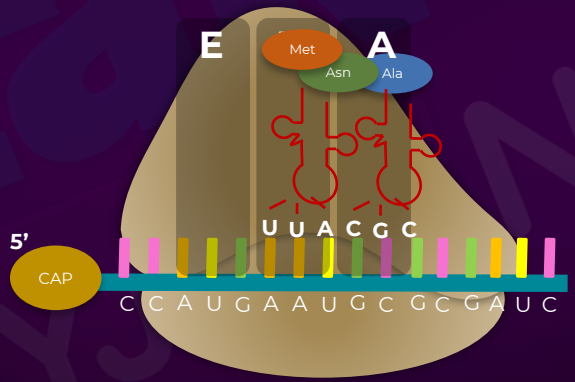
## Steps of translation

**1 Initiation**



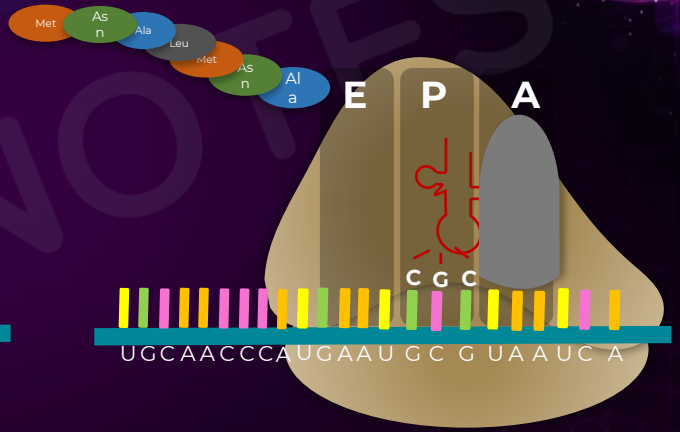

- Assembly of mRNA, ribosome and the initiator tRNA

**2 Elongation**




- Polymerisation of amino acids

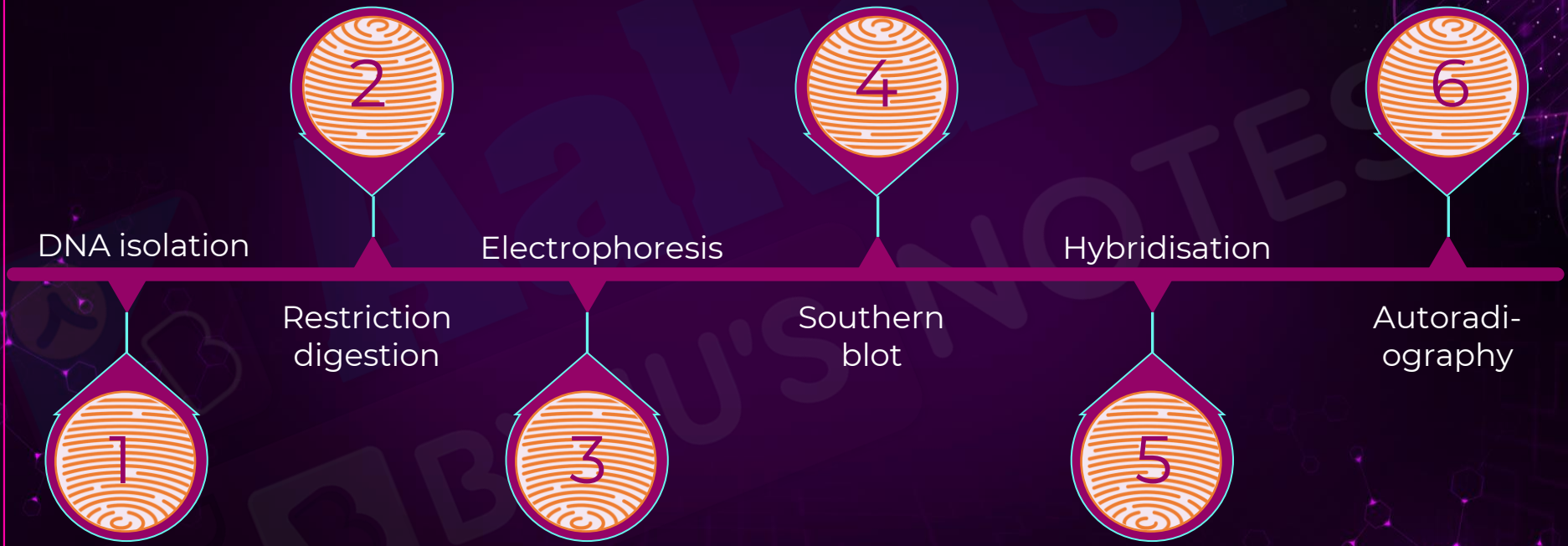
**3 Translation**




- Release of the polypeptide and disassembly of ribosomes and tRNA

# Summary

## Steps of DNA fingerprinting



# Summary