NUCLEIC ACIDS

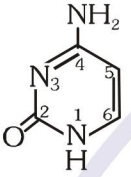
- F. Meischer discovered nucleic acid in nucleus of pus cell and called it "nuclein".
- Nucleic acids are polymer of **nucleotides**.

Nucleotide = Nitrogen base + pentose sugar + phosphate

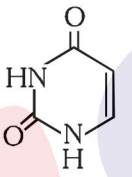
Nucleoside = Nitrogen base + pentose sugar

Nitrogen base :- On the basis of structure nitrogen bases are broadly of two types :–

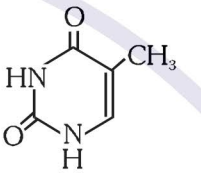
(1) **Pyrimidines** – Consist of one pyrimidine ring. (2N + 4C) e.g. Cytosine, Thymine and Uracil.



CYTOSINE

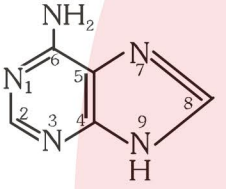


URACIL

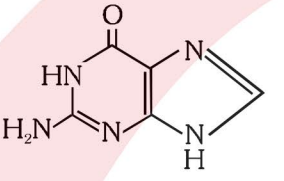


THYMINE

2. **Purines -** Consist of two rings i.e. one pyrimidine ring (2N + 4C) and one imidazole ring (2N + 3C) e.g. Adenine and Guanine.

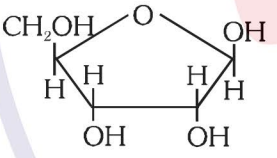


ADENINE

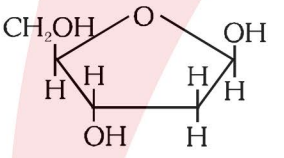


GUANINE

Pentose Sugar :–

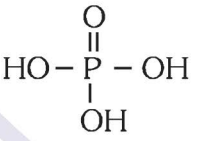


Ribose



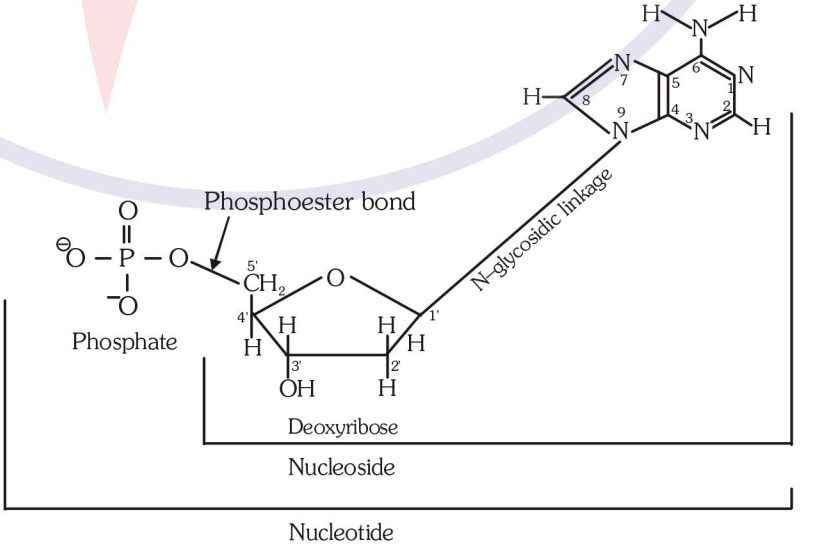
Deoxy ribose

Phosphate (Acidic) :-

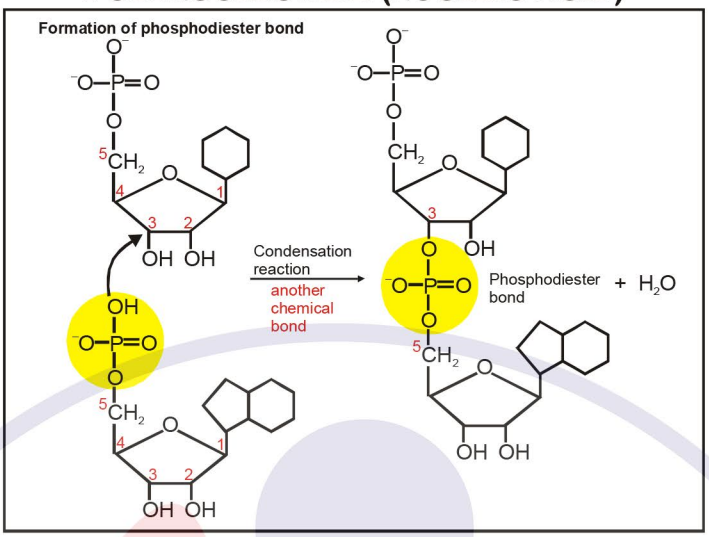


Nitrogen base forms bond with first carbon of pentose sugar to form a nucleoside. Nitrogen of **first place** (N_1) forms bond with sugar in case of pyrimidines while in purines nitrogen of **ninth place** (N_9) forms bond with sugar.

Phosphate forms ester bond (covalent bond) with fifth Carbon of sugar to form a complete nucleotide.

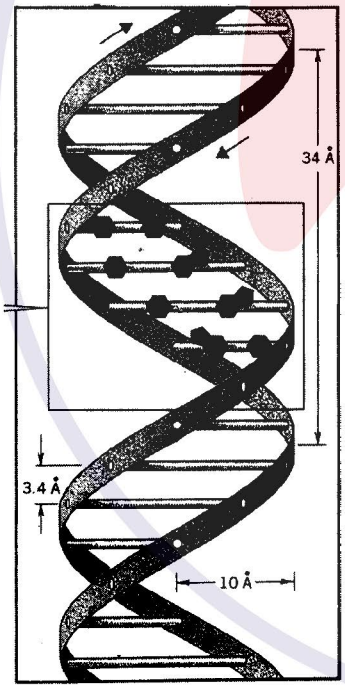


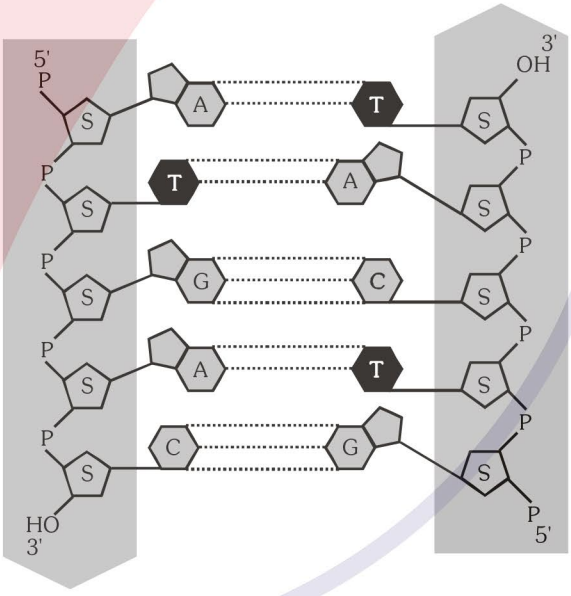
POLYNUCLEOTIDE (NUCLEIC ACID)



DNA

- In DNA pentose sugar is deoxyribose sugar and four types of nitrogen bases A,T,G,C
- **Wilkins** and **Franklin** studied DNA molecule with the help of **X-Ray crystallography.**
- Discovered by F. Meischer.





- With the help of this study, **Watson** and **Crick** (1953) proposed a double helix molel for DNA.
- One main hallmark (main point) of double helix model is complementary base pairing between purine and purimidine.
- According to this model, DNA is composed of two polynucleotide chains.
- Both polynucleotide chains are complementary and antiparallel to each other.
- In both strand of DNA direction of phosphodiester bond is opposite. i.e. If direction of phosphodiester bond in one strand is 3'-5' then it is 5'-3' in another strand.

- Both strand of DNA held together by hydrogen bonds. These hydrogen bonds are present between nitrogen bases of both strand.
- Adenine binds to thymine by two hydrogen bonds and cytosine binds to guanine by three hydrogen bonds.
- In a DNA molecule one purine always pairs with a pyrimidine. This generates approximately uniform distance between the two strands of DNA.
- In DNA plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure of DNA.
- **Chargaff's equivalency rule -** In a double stranded DNA amount of purine nucleotides is equals to amount of pyrimidine nucleotides.

  Purine = Pyrimidine

  [A] + [G] = [T] + [C]

$$\frac{[A]+[G]}{[T]+[C]} = 1$$

Configuration of DNA Molecule :–

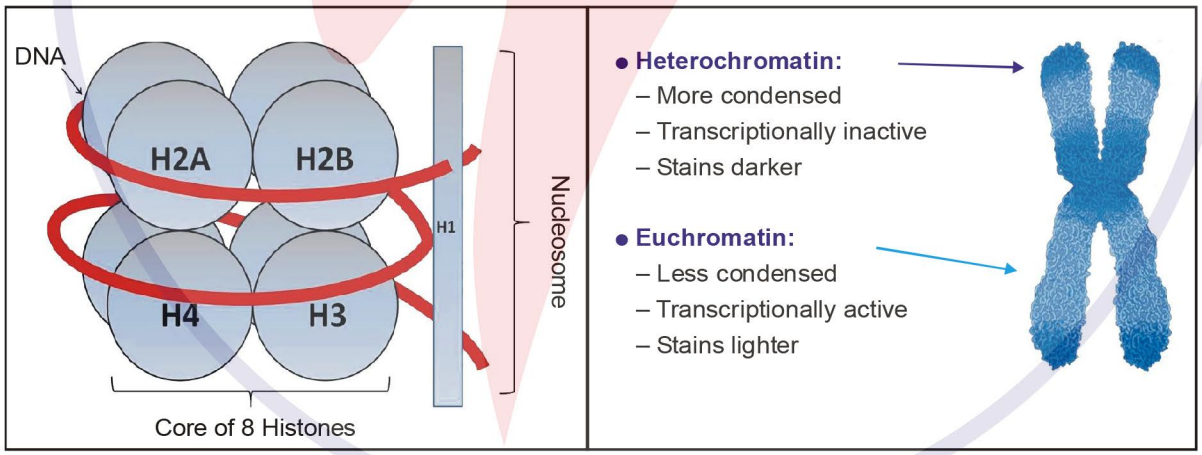
- Distance between two successive steps is 3.4 A^0 . In one complete turn of DNA molecule there are such 10 steps (10 pairs of nitrogen bases). So the length of one complete turn is 34 A^0. This is called helix length.
- Diameter of DNA molecule i.e. distance between phosphates of two strands is 20A^0.

Packaging of DNA Eukaryotes :

Histones (Positively charged, Lysine and arginine rich) are organised to form a histone octamer [$(H_2A + H_2B + H_3 + H_4) \times 2$]. The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome.

Nucleosomes constitute the repeating unit of a structure in nucleus called chromatin.

The nucleosomes in chromatin are seen as "beads on string" structure when viewed under electron microscope with the help of non-histone chromosomal protein chromatin fibre coiled and condeged to form chromosome.



THE SEARCH FOR GENETIC MATERIAL

I. Frederick Griffth's Transformation Experiment :-

Experimental organism : Streptococcus pneumoniae (He used S-III and R-II strains)

The experiment can be described in following steps :

(A) Live S strain → injected into mice → Mice died

(B) Live R strain → injected into mice → mice lived

(C) S strain (heat killed) → Injected into mice → Mice lived

(D) S strain (heat killed) + R strain (living) → injected into mice → Mice died

Conclusion : (from IVth step) – Some rough bacteria (R-II) were transformed into smooth type (Virulent S-III) of bacteria.

Biochmical characterisation of transforming principle : by Avery, Macleod and McCarty.

They purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material, but not all biologists were convinced.

II. TRANSDUCTION EXPERIMENT OF HERSHEY & CHASE

Used a virus (T2 bacteriophage) to infect bacteria (E.coli).

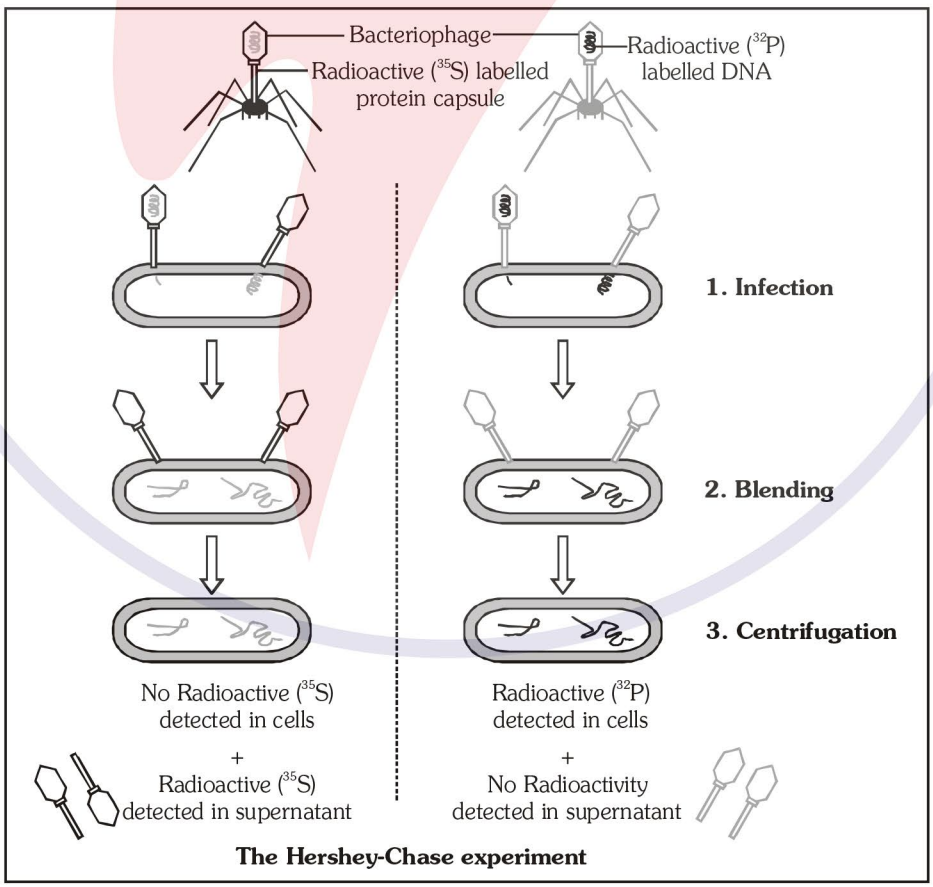
Viruses were labeled with radioactive sulphur (^{35}S) or radioactive phosphorus (^{32}P)

Viral protein coats contain Sulphur but no Phosphorus.

DNA contains Phosphorus but no Sulphur.

Labelled strains of virus were allowed to infect bacteria, mixture agitated and then centrifuged to create solid pellet in liquid supernatant.

The unequivocal proof that DNA is the genetic material.



The Hershey-Chase experiment

Special Points :

- A molecule that can act as a genetic material must fulfil the following criteria–

  (i) It should be able to generate it's replica (replication)

  (ii) It should chemically and structurally be stable

  (iii) It should has property of mutation.

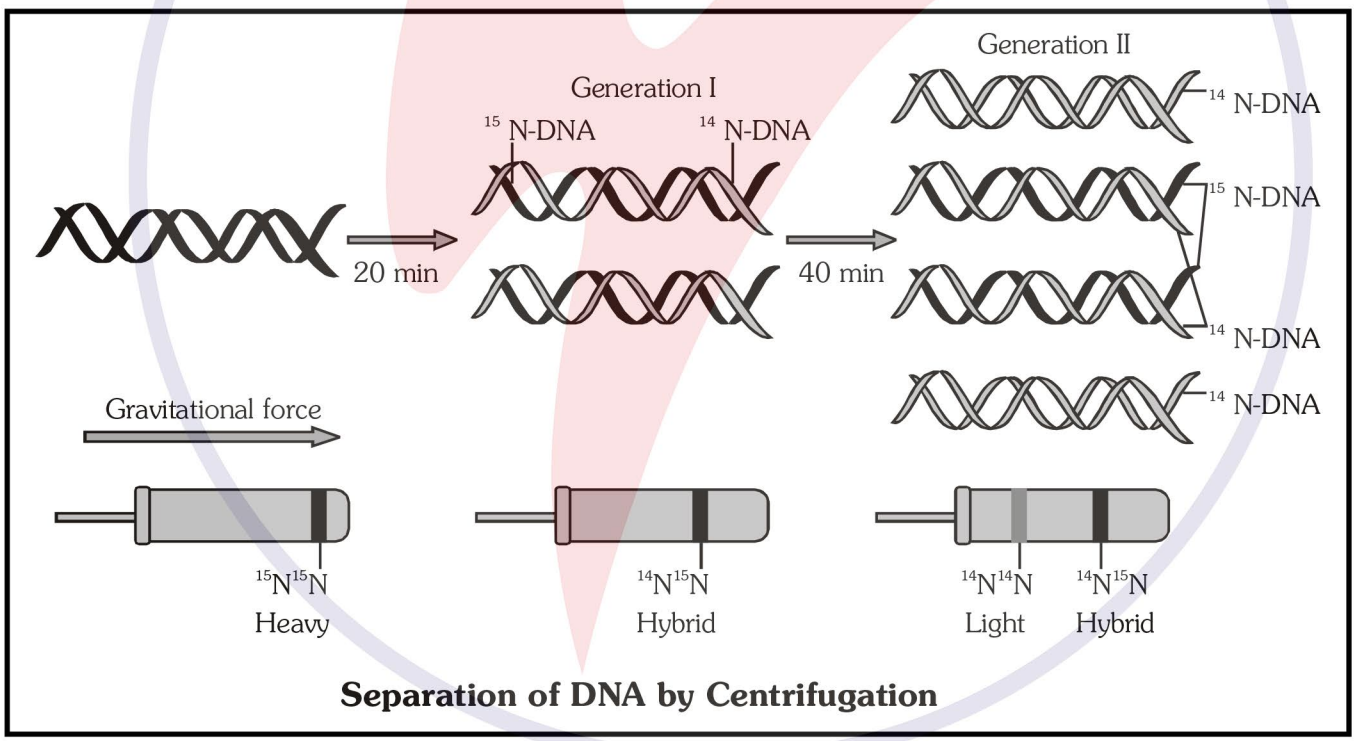
  (iv) It should be able to express itself in the form of "Mendelian Characters".

DNA REPLICATION (DNA ⟶ DNA)

- DNA replication takes place in "S - Phase" of the cell cycle.

SEMI CONSERVATIVE MODE OF DNA REPLICATION

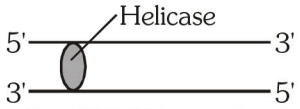
First proposed by **Watson & Crick**. Later on it was experimentally proved by **Meselson & Stahl** (1958) on ***E - Coli*** and ***Taylor*** on ***Vicia faba* (1958)**.To prove this method, Taylor used Radiotracer Technique in which Radioisotopes (tritiated thymidine = $_1H^3$) were used. Meselson and Stahl used heavy isotope (N^{15}).



Separation of DNA by Centrifugation

MECHANISM OF DNA REPLICATION

Helicase unwinds origin of Relication.



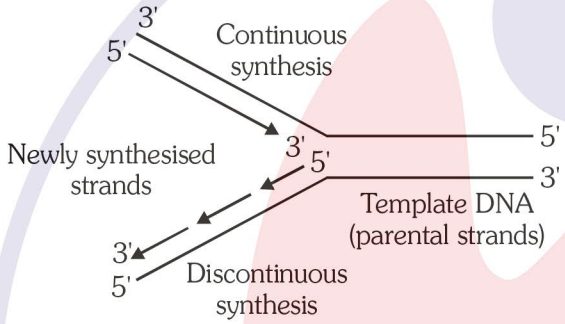
Topoisomerase prevents Supercoiling

Single strand Binding (SSB) Protein binds to single stranded region of DNA to prevent reformation of H-bonds in separated strands.

RNA primers are synthesised by Primase.

RNA primer provide free 3'OH group for the addition of new nucleotides or for the initiation of replication.

DNA poly III starts the elongation is 5' ⟶ 3' direction.



On 3' ⟶ 5' template strand synthesis of New DNA is continuous (leading strand) and on 5' ⟶ 3' template synthesis is discontinuous (lagging strand/Okazaki fragments).

After synthesis of DNA by DNA poly III, RNA primers are removed and gaps are filling by DNA poly–I.

Finally all okazaki fragments are Joined by ligase.

Special Point :

Deoxy ribonucleoside triphosphate

Provide energy — act as substrate

- DNA poly II is least reactive enzyme. It is helpful in DNA repairing in absence of DNA poly I and DNA poly III.
- Any failure in cell division after DNA replication result into polyploidy.
- Difference between DNAs and DNase is that DNAs menas many DNA and DNase means DNA digestive enzymes.

RIBO NUCLEIC ACID (RNA)

Structure of RNA is fundamentally same as DNA, but there are some differences. The differences are as follows:-

(1) In place of De-oxyribose sugar in DNA, there is present Ribose sugar in RNA.

(2) In place of nitrogen base Thymine in DNA, there is present uracil in RNA.

- The presence of thymine at the place of uracil also provide additional stability to DNA.

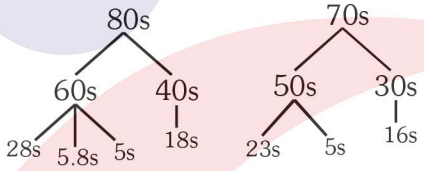
(3) RNA is made up of only one polynucleotide chain i.e. R.N.A. is **single stranded**.

- 2-OH groups present at every nucleotide in RNA as reactive group and makes RNA labile and easily degradable and RNA also has catalytic function so it is more reactive so DNA is chemically less reactive and structurally more stable as compared to RNA. Therefore DNA has evolved from RNA with chemical modification that make it more stable. DNA being double stranded and having complementary strand further resists changes by evolving a process of repair.

Types of RNA :

(A) Ribosomal RNA (r - RNA) :-

- 80% of the cell's total RNA
- It is found in ribosomes.
- It is the most stable form of RNA .



Function :–

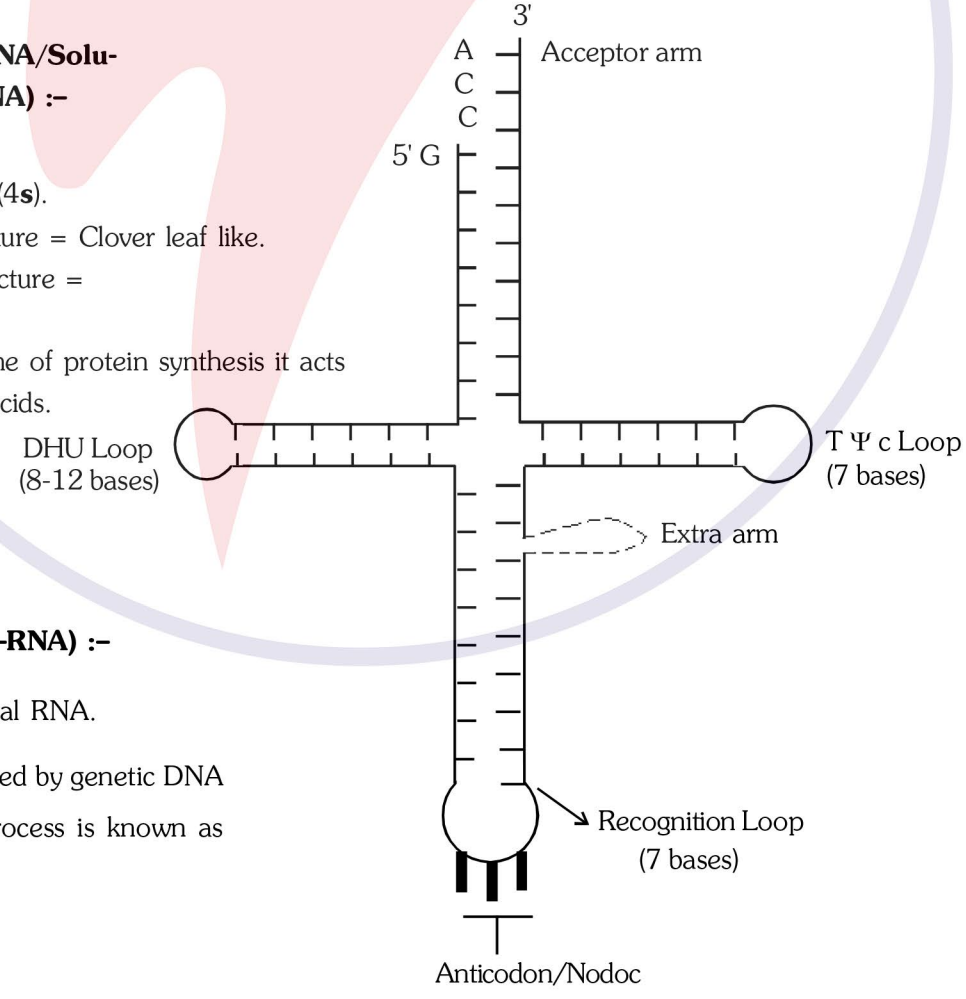
- At the time of protein synthesis, r–RNA provides attachement site to t–RNA and m–RNA and attaches them on the ribosome.

(B) Transfer – RNA (t–RNA/Soluble RNA/Adapter RNA) :–

- 10-15% of total RNA.
- It is the smallest RNA (4**s**).

Two dimensional structure = Clover leaf like.
Three dimensional structure = inverted L-shaped.

Function :– At the time of protein synthesis it acts as a carrier of amino-acids.



(C) Messenger RNA (m –RNA) :–

- 1 - 5% of the cell's total RNA.
- The m - RNA is produced by genetic DNA in the nucleus. This process is known as Transcription.
- It is least stable RNA.

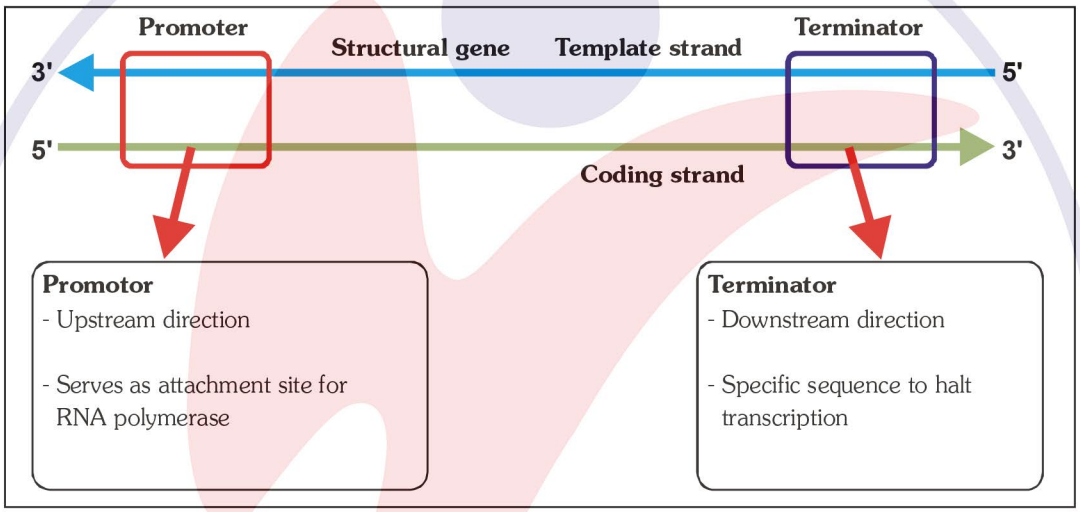
TRANSCRIPTION (DNA ⟶ RNA)

Cistron – Functional unit of DNA involved in transcription.

Transcription Unit
- Promotes
- Structural gene
- Terminator

Only one strand of DNA participates in transcription Called 'Antisense strand' (3' ⟶ 5' strand)

GENE : TRANSCRIPTION UNIT OF DNA



MECHANISM OF TRANSCRIPTION

With the help of σ factor RNA polymerase attached with promoter site of DNA.

↓ RNA polymerase breaks H – bonds

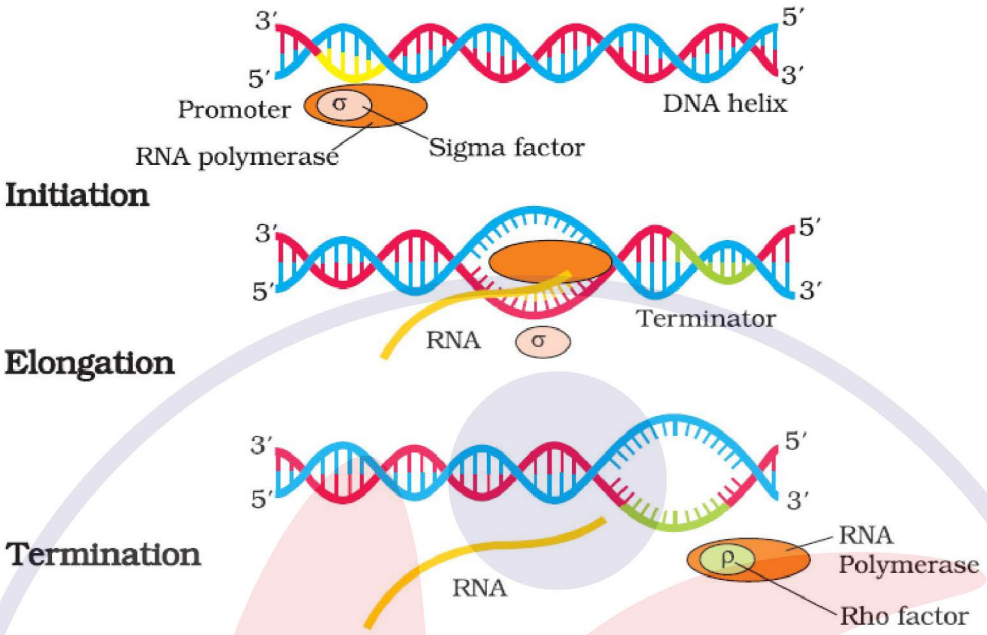
RNA utilizes poly ribonucleosides triphospates for formation of new RNA in 5' ⟶ 3' direction.

↓ Release σ factor

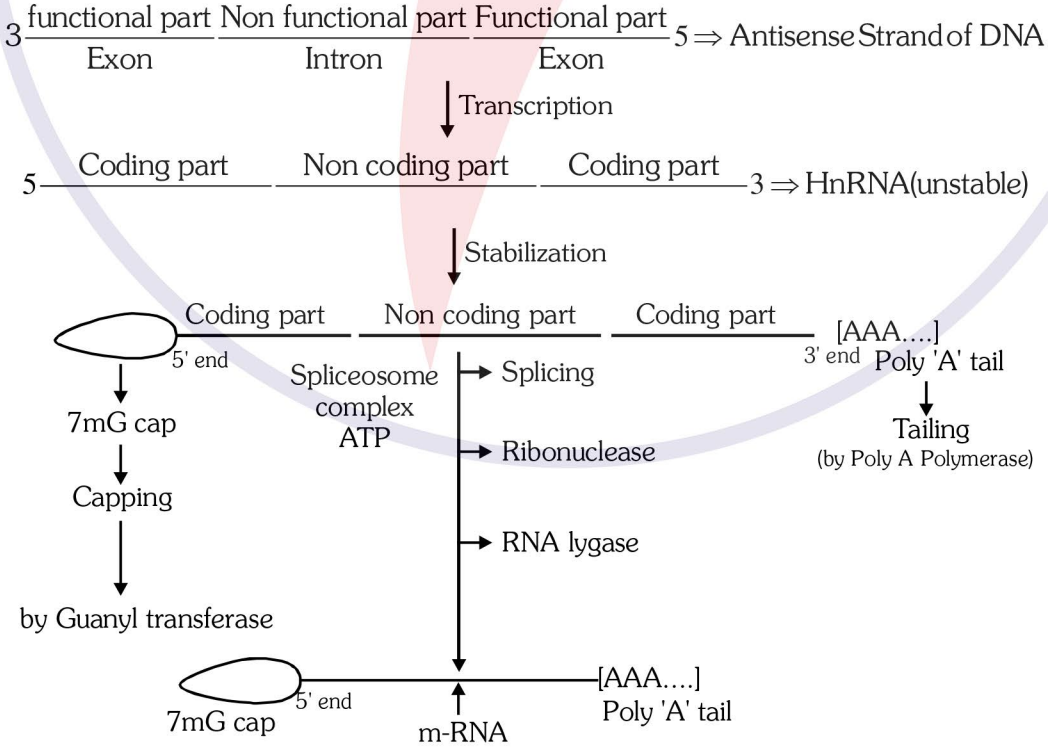
The sequence of Nucleotide bases in newly synthesised m–RNA is complementory to the template strand.

↓

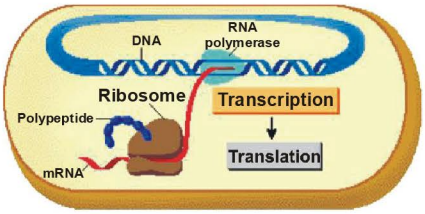
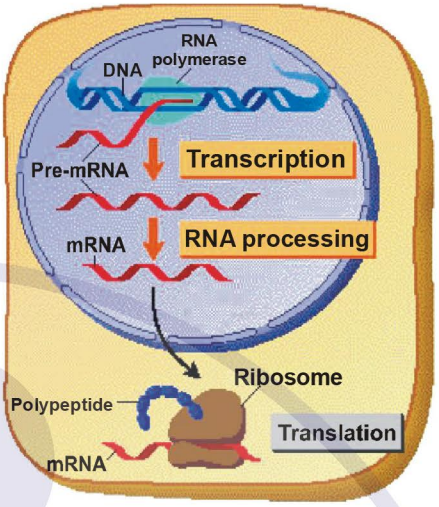
In most cases RNA polymerase recognise terminator site and when RNA polymerase reaches at terminator site it separates from DNA. But in prokaryotes terminator site is recognised by Rho factor.



RNA PROCESSING



PROKARYOTIC VS EUKARYOTIC TRANSCRIPTION

	 Prokayotic Transcription	 Eukayotic Transcription
1.	Coupled transcription-translation is the rule.	Coupled transcription translation is not possible.
2.	Occurs in the cytoplasm.	Occurs in the nucleus.
3.	A single RNA polymerase synthesises all the three types of RNA (mRNA, tRNA, rRNA)	The RNA polymerases I, II and III synthesizes rRNA, mRNAand tRNA respectively.

GENETIC CODE

- Term Given by ***George Gamow***.
- Discovered by Nirenberg, Mathai and Khorana.
- The relationship between the sequence of amino acids in a polypeptide chain and nucleotide sequence of DNA or m–RNA is called ***genetic code***.
- Genetic code is triplet i.e. one codon consists of three nitrogen bases

  Triplet code = $4^3 = 4 \times 4 \times 4 = 64$ codons
- H.G. Khorana artificially synthesized an mRNA.
- **Severo ochoa enzyme** (RNA polymerase enzyme) is also helpful in polymerising RNA with defined sequences in a template independent manner.

Characteristics of Genetic Code :–

(i) **Triplet in Nature :–**

- One code is made up of three N_2-bases.

(ii) **Universality :–**

The genetic code is applicable universally.

(iii) **Non – Ambiguous :–**

- One codon specifies only one amino acid and not any other.
- Exception GUG codon which codes both valine and methionine amino acids.

(iv) **Non – Overlapping :–**

A nitrogen base is a constituent of only one codon.

(v) **Comma less :–**

- There is no punctuation (comma) between the adjacent codon i.e. each codon is immediately followed by the next codon.

(vi) **Degeneracy of Genetic code :–**

- There are 64 codons for 20 types of amino acids, so most of the amino acids (except two) can be coded by more than one codon. Single amino acid coded by more than one codon is called ***"Degeneracy of genetic code"***.
- Only two amino acids ***Tryptophan*** and ***Methionine*** are specified by single codon.

[UGG for Tryptophan, AUG for Methionine.]

- Degeneracy of genetic code is related to third position (3′– end of triplet codon) of codon. The third base is described as ***"Wobbly base"***.

Chain Initiation and Chain Termination Codon :–

- AUG codes methionine amino acid in eukaryotes and in prokaryotes AUG codes N–formyl methionine.
- Out of 64 codons 3–codons are stopping or nonsense or termination codon.

Nonsense codons do not specify any amino acid.

UAA (Ochre)
UAG (Amber) } **Non–Sense Codons or Stop codons**
UGA (Opal)

- So only 61 codons are sense codons which specify 20 amino acid.

First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gin	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gin	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Fig : **Triplet codons of mRNA for amino acids represented in tabular form.**

TRANSLATION (m-RNA ⟶ Protein)

Process of polymerisation of Amino-acid to form a polypeptide.

(I) Activation of Amino acid :–

$$\text{Amino acid} + \text{ATP} \xrightarrow[\text{t-RNA Synthetase}]{\text{Amino acyl}} \text{Amino acyl AMP–enzyme complex} + \text{PP}$$

(II) Charging of t–RNA (Loading of t-RNA) :–

$$\text{Amino acyl AMP–enzyme complex} + \text{t–RNA} \rightarrow \text{Amino acyl t–RNA complex} + \text{AMP} + \text{enzyme}$$

(III) Process of Translation

$$30\text{ s ribosome} + \text{m} - \text{RNA} \xrightarrow[mg^{+2}]{} 30\text{s m} - \text{RNA complex}$$

↓

$$30\text{s m} - \text{RNA complex} + \text{formyl methionyl t} - \text{RNA complex} \xrightarrow{\text{GTP}} 30\text{s} - \text{m} - \text{RNA formyl methinonyl t} - \text{RNA complex}$$

↓ Now larger subunit of ribosome joins this complex

First t–RNA attaches to P site and next t–RNA attaches to 'A' site of ribosome.

↓

Peptide bond taken place between COOHgp of 'P' site amino acid and NH_2 gp of 'A'-site amino acid by peptidyl transferase (23s-r-RNA).

↓

Now t–RNA of 'A' site is transferred to 'P' site and 'A' site become empty.

↓

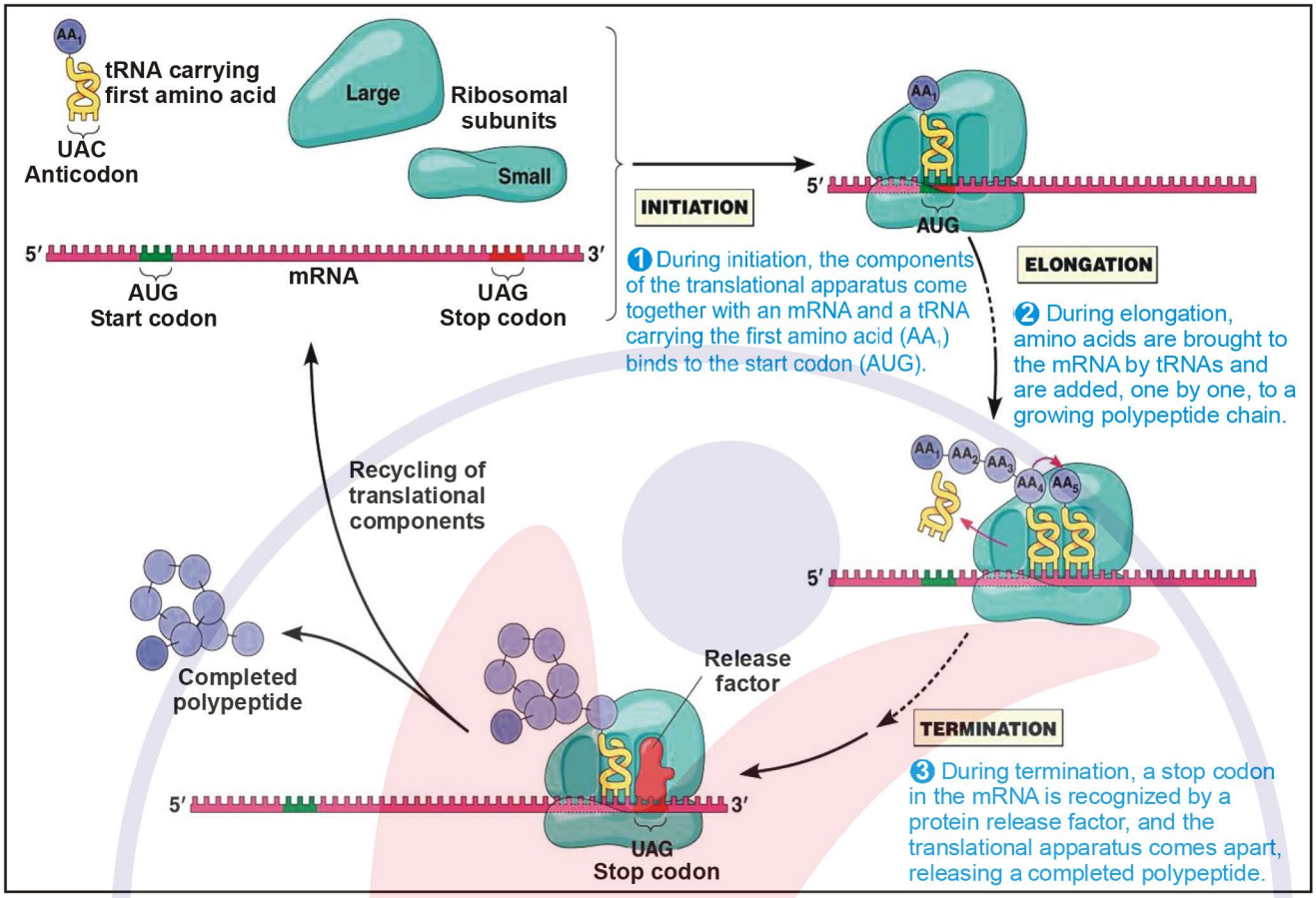
Ribosome slides over m–RNA in 5' ⟶ 3' (translocation by translocase + GTP)

↓

Termination happens when a stop codon in m–RNA (UAA, UAG, UGA) enters the 'A' site.

↓

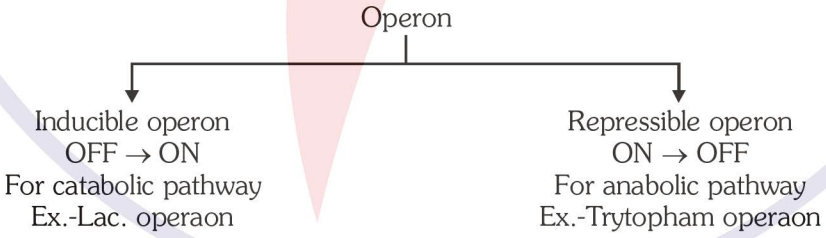
Release factors with the help of GTP separates the polypeptide chain from t–RNA and newly made polypeptide chain is released.



REGULATION OF GENE EXPRERSION

OPERON CONCEPT

– **Francis Jocob** and **Jacques Monad** proposed **operon model** for the regulation of gene action in *E. coli.*
An operon is a part of genetic material or DNA, which acts as a single regulated unit having one or more structural genes-an operator gene, a promoter gene, a regulator gene.



Lac operon of E-coli :

An inducible operon system consists of four types of genes

(i) **Structural genes -** These genes synthesise mRNAs. The lac operon (lactose operon) of *Escherichia coli* contains three structural genes (Z, Y and A). They transcribe a polycistronic mRNA molecule that helps in the synthesis of three enzymes-β galactosidase, lactose permease and transacetylase.

(ii) **Operator gene -** It lies adjacent to the structural genes and directly controls the synthesis of *mRNA* over the structural genes. It is switched off by the presence of a repressor.

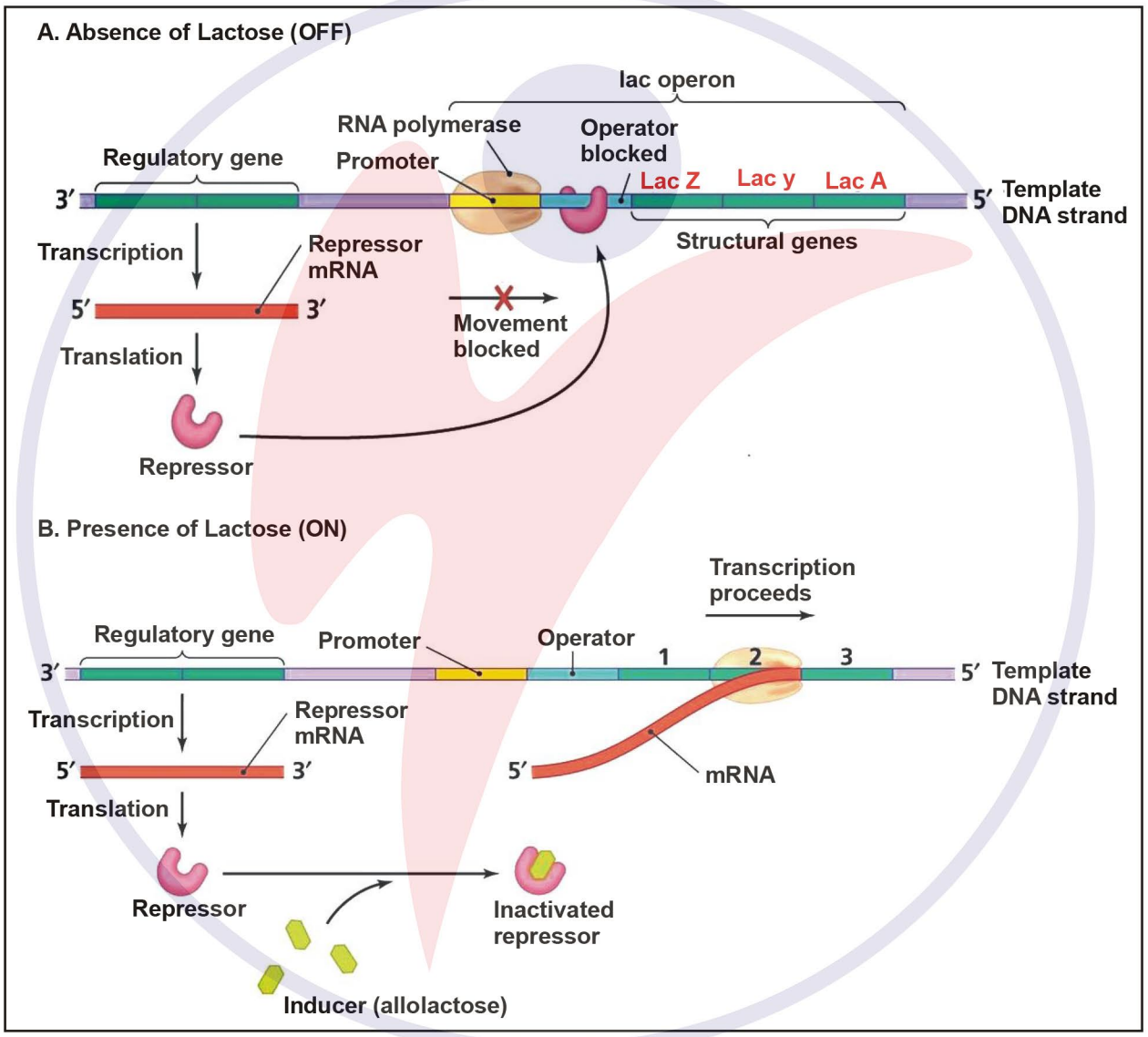
(iii) **Promoter gene -** This gene is the site for initial binding of RNA polymerase.

(iv) Regulator gene - It produces a repressor that binds to operator gene and stops the working of the operator gene.

Repressor - It is a protein, produced by the regulator gene. Repressor has two allosteric site (1) operator gene (2) effective molecule (inducer/corepressor)

Inducer - It is a chemical (substrate, hormone or some other metabolite) which after coming in contact with the repressor, forms an **inducer repressor complex**. This complex cannot bind with the operator gene, which is thus switched on.

Active repressor + inducer = inactive repressor



DNA FINGER PRINTING / DNA TYPING / DNA PROFILING/ DNA TEST

The technique of DNA Fingerprinting was initially developed by Alec Jeffreys. He used a satellite DNA that shows very high degree of polymorphism. It was called as Variable Number of Tandem Repeats (VNTR).

- DNA of human is almost the same for all individuals but very small amount that differs from person to person that forensic scientists analyze to identify people.
- The VNTR belongs to a class of satellite DNA referred to as mini-satellite. A small DNA sequence is arranged tandemly in many copy numbers. The copy number varies from chromosome to chromosome in an individual. The numbers of repeat show very high degree of polymorphism. As a result the size of VNTR varies in size from 0.1 to 20 kb.

Technique of DNA Finger printing involves the following major stpes.

(i) isolation of DNA,

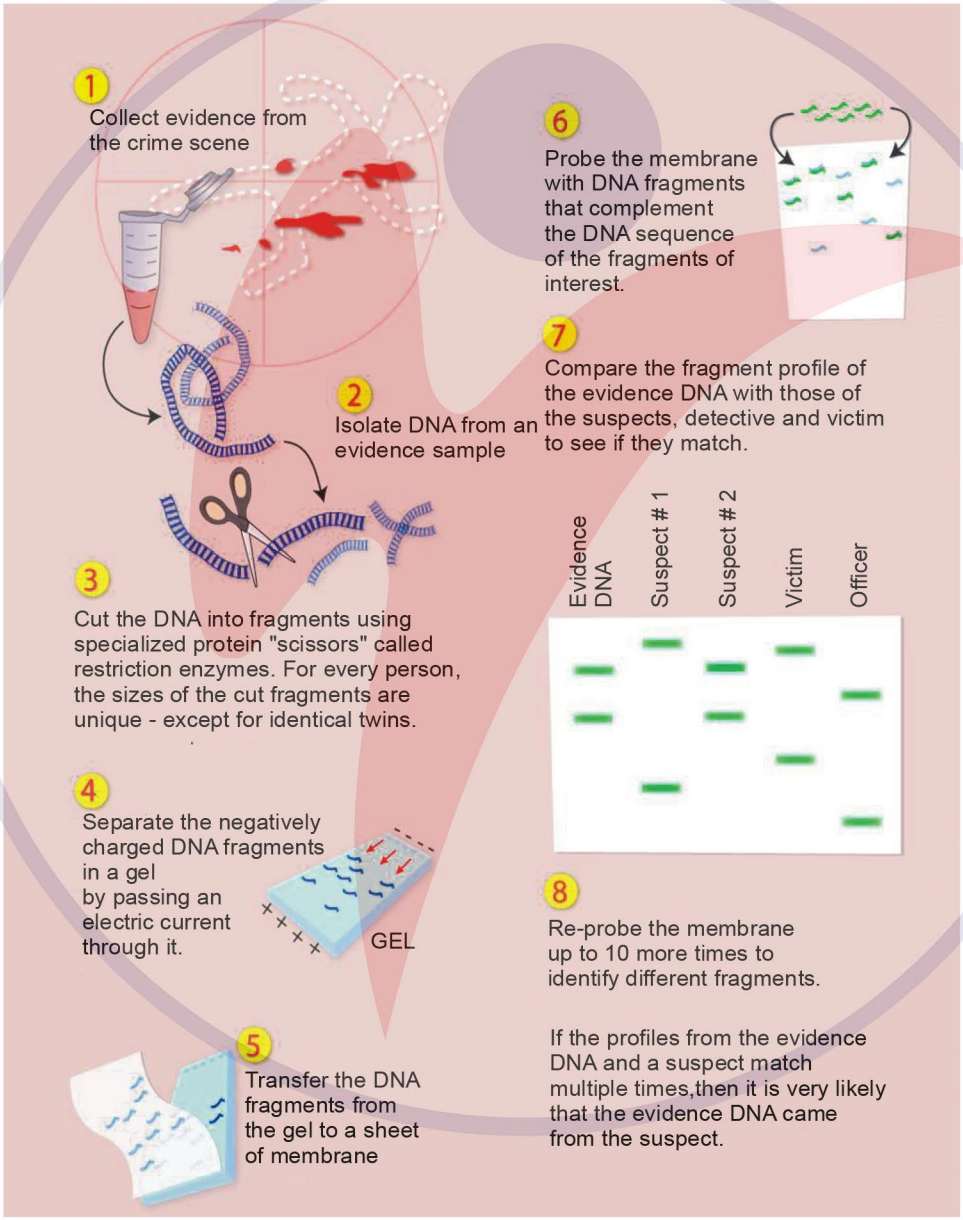
(ii) digestion of DNA by restriction endonucleases,

(iii) separation of DNA fragments by electrophoresis,

(iv) transferring (blotting) of separated DNA fragments to synthetic membranes, such as nitrocellulose or nylon,

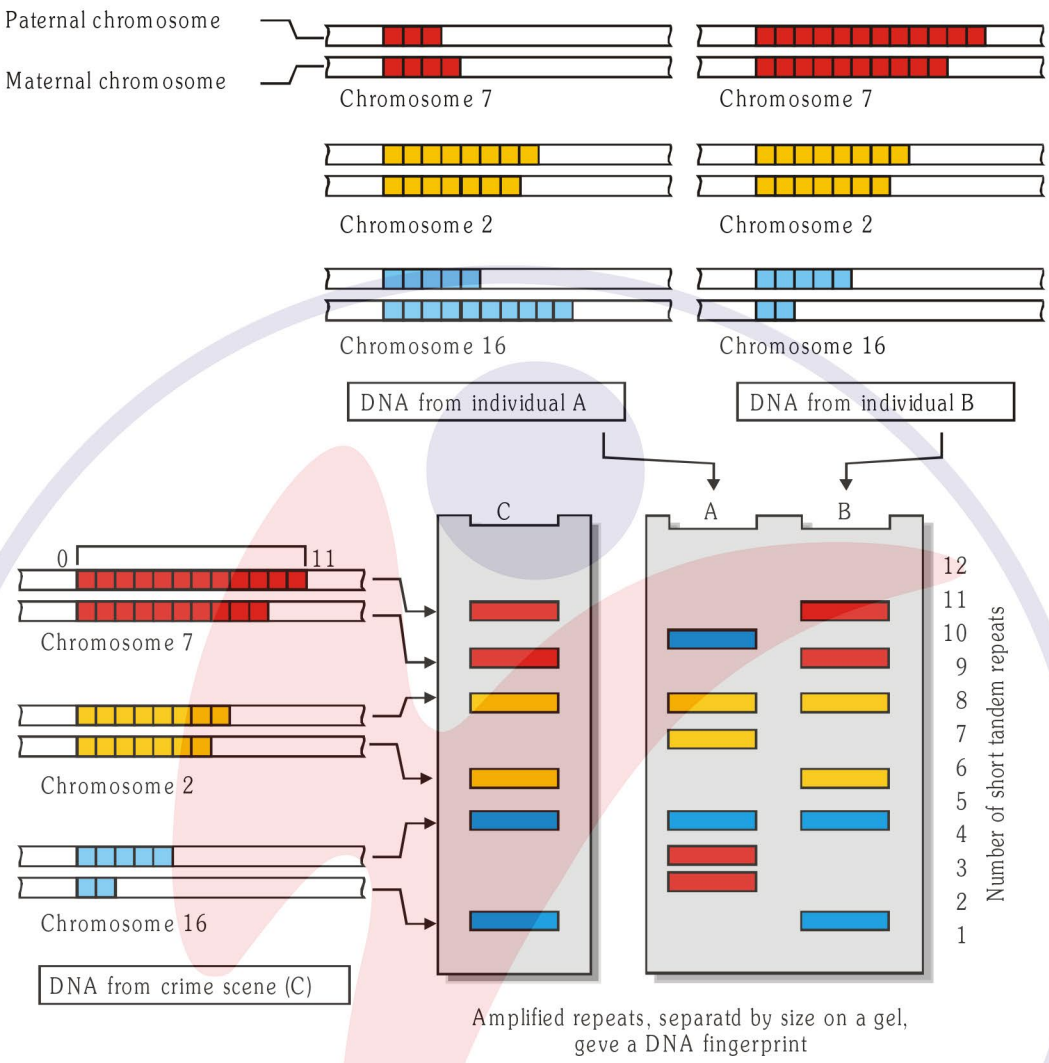
(v) hybridisation using labelled probe, and

(vi) detection of hybridised DNA fragments by autoradiography



Application of DNA Finger printing

1. Paternity tests.
2. Identification of the criminal.



Schematic representation of DNA fingerprinting : Few representative chromosomes have been shown to contain different copy number of VNTR.

HUMAN GENOME PROJECT

Human Genome Project (HGP) was called a mega project launched in year 1990. The project was completed in 2003. HGP was closely associated with the rapid development of a new area in biology called as **Bioinformatics.**

Goals of HGP

Some of the important goals of HGP are as follows :

(i) Identify all the genes in human DNA.

(ii) Determine the sequences of the 3 billion chemical base pairs that make up human DNA.

(iii) Store this information in databases.

(iv) Improve tools for data analysis.

(v) Transfer related technologies to other sectors, such as industries.

(vi) Address the ethical, legal, and social issues (ELSI) that may arise from the project.

Methodologies : The methods involved **two** major approaches.

(1) **Expressed Sequence Tags** (ESTs) - Identifying all the genes that expressed as RNA.

(2) **Sequence Annotation -** The blind approach of simply sequencing the whole set of genome that contained all the coding and non-coding sequence, and later assigning different regions in the sequence with functions. The commonly used hosts were bacteria and yeast, and the vectors were called as **BAC** (bacterial artificial chromosomes), and **YAC** (yeast artificial chromosomes).

The fragments were sequenced using automated DNA sequencers that worked on the principle of a method developed by **Frederick Sanger**. These sequences were then arranged based on some **overlapping regions** present in them.

The sequence of chromosome I was completed only in May 2006 (this was the last of the 24 human chromosomes -22 autosomes and X and Y- to be sequenced).

Salient Features of Human Genome –

Some of the salient observations drawn from human genome project are as follows :

(i) The human genome contains 3164.7 million nucleotide bases.

(ii) The average gene consists of 3000 bases.

(iii) The total number of genes is estimated at 30,000.

(iv) Less than 2 per cent of the genome codes for proteins.

(v) Repeated sequences make up very large portion of the human genome.

(vi) Chromosome 1 has most genes (2968). and the Y has the fewest (231).

(vii) Scientists have identified about 1.4 million locations where single-base DNA differences **(SNPs- single nucleotide polymorphism,** pronounced as 'snips') occur in humans,

Mutation (Sudden heritable change in genetic material**)**

Chromosomal mutation
[Change in number of chromosome & change in structure of chromosome]

Heteroploidy mutation
(Change in number of chromosome)

Chromosomal aberration
(Change in structure of chromosome)

Deletion

Terminal

Intercalary or interstitial

Duplication

Inversion

Paracentric

Pericentric

Translocation

Simple Translocation

1 2 3 4 5 6 7 8
A B C D E F G H
1 2 3 4 5 6
A B C D E F G H 7 8

Reciprocal Translocation

1 2 3 4 5 6 7 8
A B C D E F G H
1 2 3 4 5 6 G H
A B C D E F 7 8

Gene mutation
(Change in Nucleotide)

Substitution mutation
(Replacement of one nitrogenous base by another nitrogenous base
Ex. : Sickle cell anaemia)

Frame shift mutation
(Loss or addition of one or rarely more than one nitrogenous bases in structure of DNA
Ex : Thalassemia)

Euploidy
(change in number of sets of chromosomes)

Monoploidy
[Loss of set of chromose]
(2n–n)

n
n → n

Polyploidy
(Addition of sets of chromosomes)

eg. 2n + n = 3n triploid
2n + 2n = 4n tetraploid
2n + 3n = 5n pentaploid
2n + 4n = 6n hexaploid
2n + 5n = 7n heptaploid
2n + 6n = 8n octaploid

Aneuploidy
(change in number of chromosomes in sets)

Hypoaneuploidy
(Loss of chromosomes in sets)

Monosomy [2n – 1] | Double monosomy [2n – 1 –1] | Nullisomy [2n – 2]

Hyperaneuploidy
(Add.of chromosomes in sets)

Trisomy [2n + 1] | Double trisomy [2n + 1 +1] | Tetrasomy [2n + 2]