REVISE

MOLECULAR BASIS OF INHERITANCE

Introduction:

- After the years of investigations, it was established that living organisms have two types of nucleic acids, **DNA** (Deoxyribonucleic acid) and **RNA** (Ribonucleic acid).
- DNA is the genetic material in majority of the organisms, whereas RNA in certain viruses along with its additional roles of functioning as a messenger, adapter, catalyst, etc.

Structure of Nucleic Acids:

- DNA and RNA are the long polymers of deoxyribonucleotides and ribonucleotides, respectively.
- A nucleotide has three components, a pentose sugar (ribose in RNA and deoxyribose in DNA), a nitrogenous base and a phosphate group.
- Nitrogenous bases are two types, **Purines** (Adenine and Guanine) and **Pyrimidines** (Cytosine, Uracil and Thymine). Thymine, i.e., 5-methyluracil present only in DNA and Uracil (Demethylated Thymine) only in RNA.
- When nitrogenous base is linked to the pentose sugar through **N-glycosidic linkage**, a nucleoside is formed.

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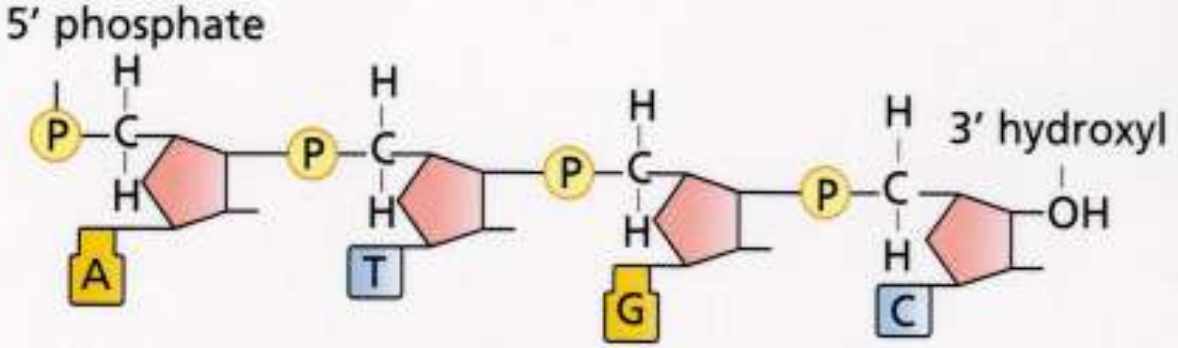
- When a phosphate group is linked to 5'C- OH of a nucleoside through **phosphoester linkage**, a nucleotide is formed.
- When two nucleotides link through 3'-5' phosphodiester linkage, a **dinucleotide** is formed.



A Polynucleotide chain

Detailed Structure of DNA:

- **James Watson and Francis Crick** (1953) gave the double helix model of DNA which is made up of two polynucleotide chains with anti-parallel polarity, i.e., one with 5'→3' and the other with 3'→5'.
- The nitrogenous bases in two chains paired with the help of hydrogen bonds, i.e., Adenine forms two H-bonds with Thymine and Guanine forms three H-bonds with Cytosine.
- The two DNA chains are coiled in a right-handed fashion (B-DNA). The pitch of the helix is 3.4 nm and, in each turn, there are roughly 10 base pairs.

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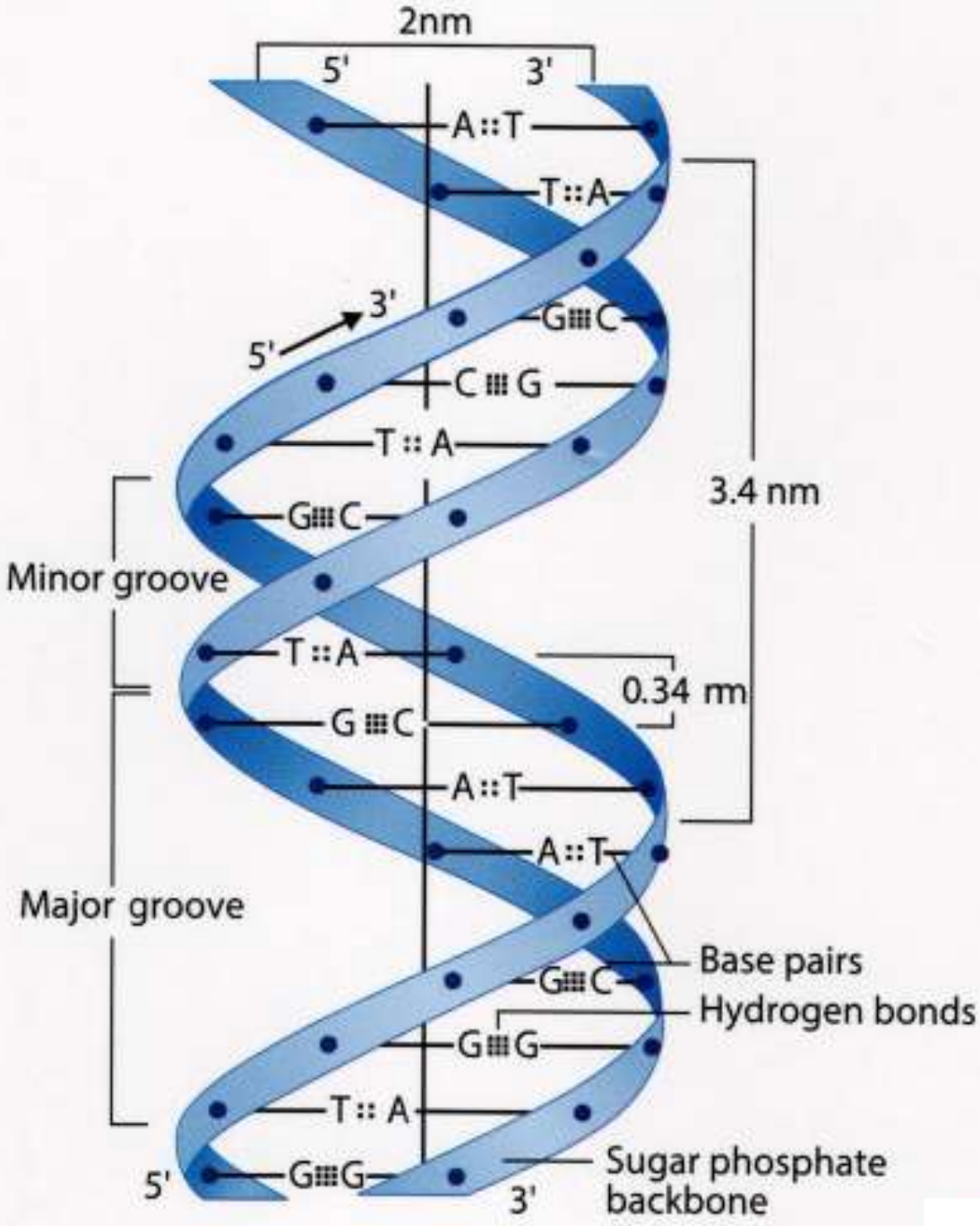
Watson and Crick double helical DNA molecule



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- According to **Chargaff's rule**, the ratio of Adenine and Thymine to Guanine and Cytosine in a double-stranded DNA is one and remains constant.
- The **length of a DNA** double helix is approx. 2.2 meters which is calculated as 6.6×10^9 bp (number of base pairs in a DNA helix) $\times$ 0.34×10^{-9} m/bp (distance between two consecutive base pairs).

Packaging of DNA Helix:

- In **prokaryotes,** the negatively charged DNA is organized in large loops and held with some positively charged proteins in a region called as **'nucleoid'**.
- In **eukaryotes**, the negatively charged DNA wraps around the positively charged histone octamer (rich in lysine and arginine) to form **nucleosome**.
- A nucleosome contains 200bp of DNA helix. These are the repeating unit of **chromatin**.
- In a nucleus, some regions of chromatin are loosely packed and stains light are called **euchromatin** (transcriptionally active chromatin). In some regions, chromatin is densely packed and stains dark are called **heterochromatin** (transcriptionally inactive).
- Search of Genetic Material

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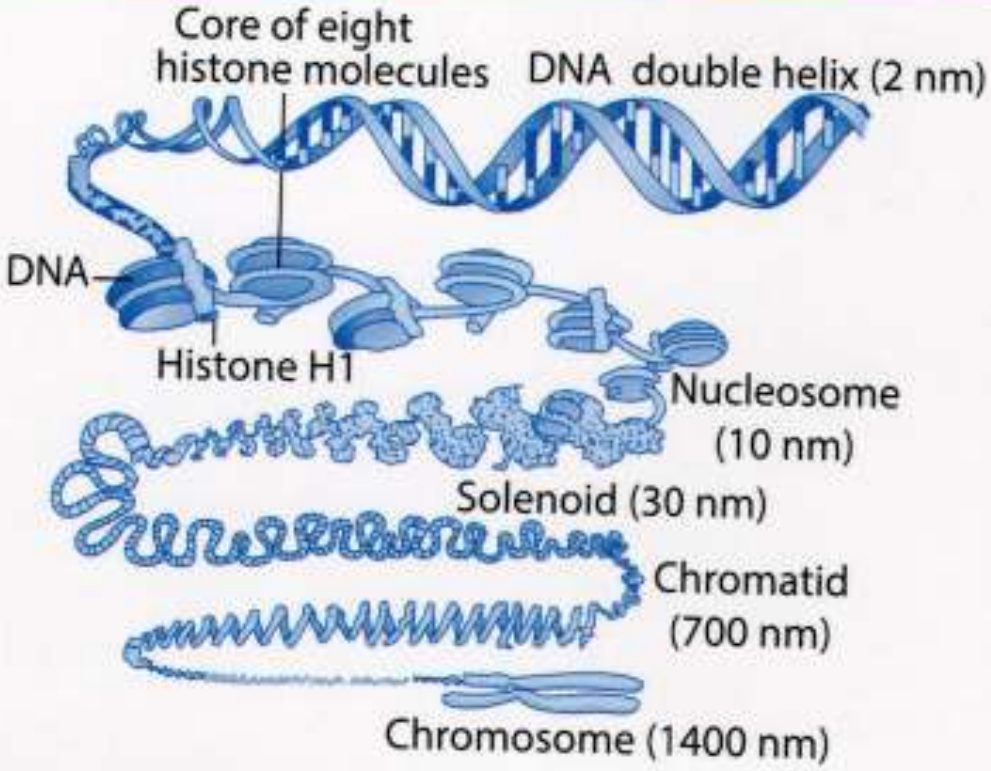
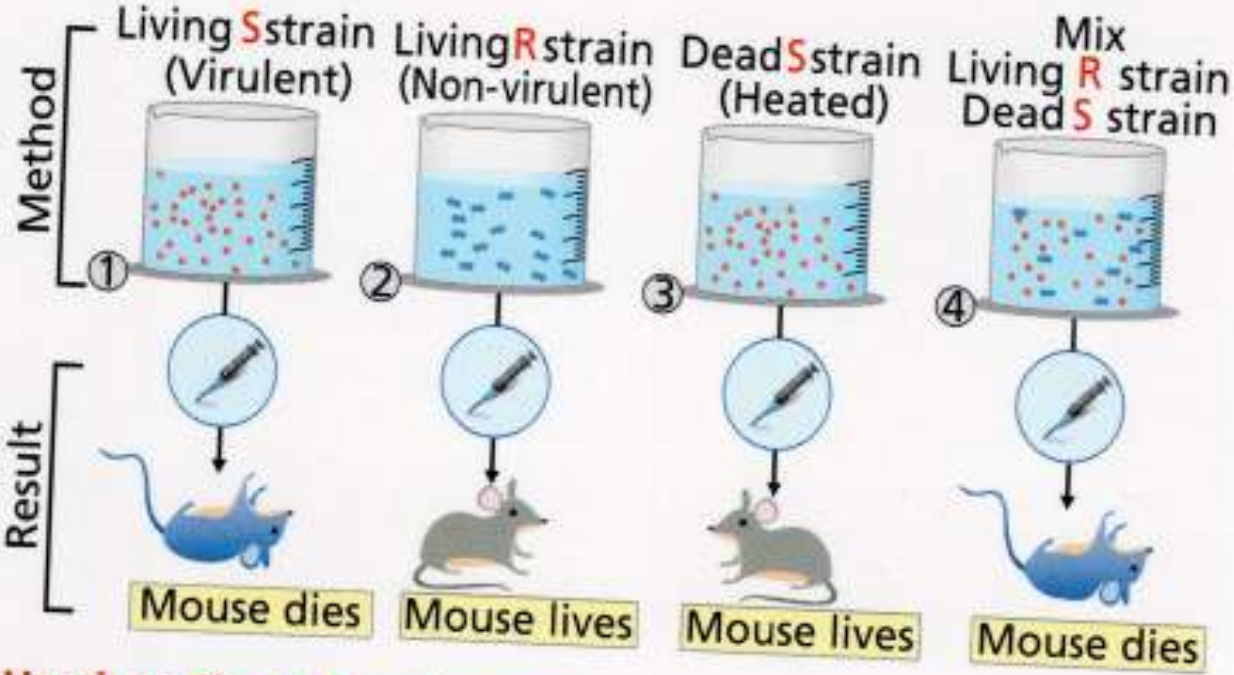


Transforming Principle:

- **Frederick Griffith** conducted a series of experiments with bacterium *Streptococcus pneumoniae*. Two strains of this bacterium were used, **S-strain** (have smooth polysaccharide coat) and **R-strain**.
- Griffith concluded that some 'transforming principle', transferred from heat-killed S strain to R strain and transformed it to virulent. This was due to transfer of the genetic material.
- **Oswald Avery, Colin MacLeod and Maclyn McCarty** purified the DNA, RNA and proteins from the heat-

killed S cells to observe which ones could transform the live R cells to S cells and they observe that among all, DNA alone is responsible for this. Thus, they concluded that the DNA is the hereditary material, by giving Biochemical proof of Griffith Experiment.

➔ The experiment was performed in multiple steps which are as follows:



Hershey-Chase Experiment:

➔ **Alfred Hershey** and **Martha Chase** conducted experiments with bacteriophages and proved that DNA is the genetic material. Their experiment was performed in following steps:

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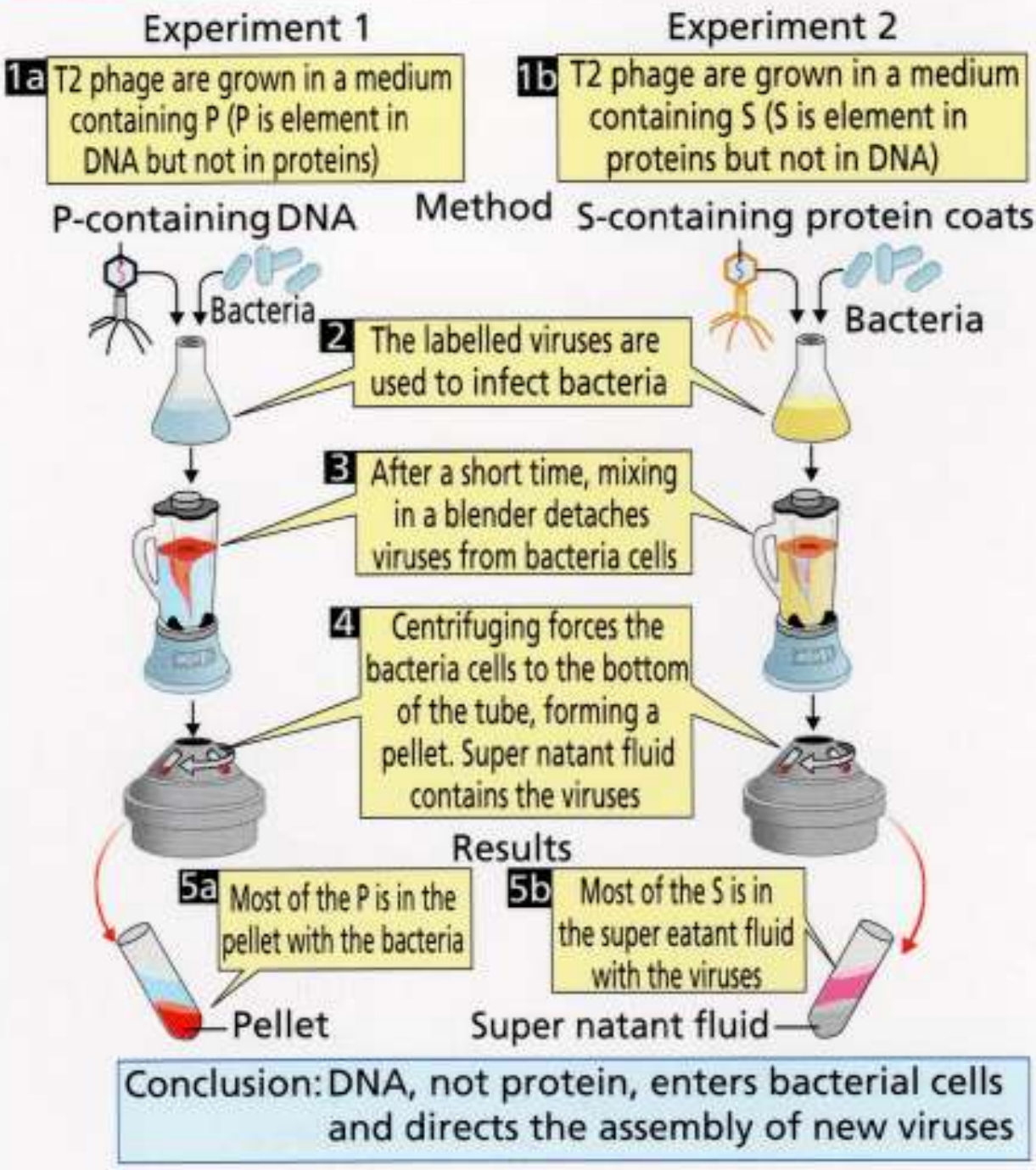

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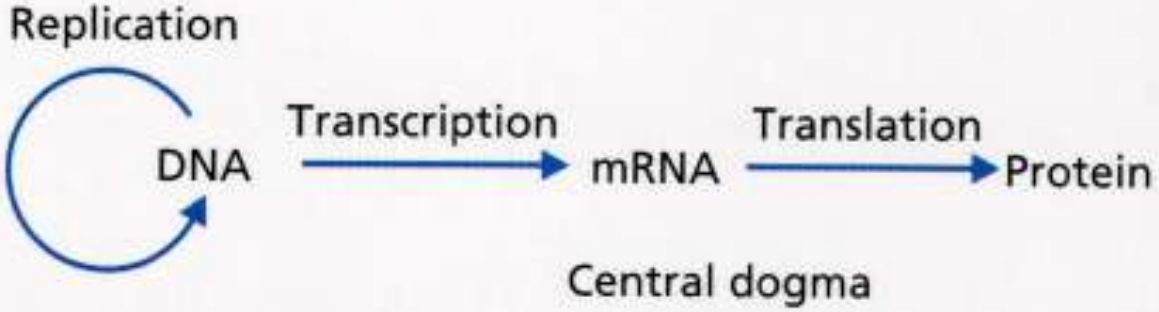
Central Dogma of Molecular Biology:

- This was proposed by **Francis Crick**, according to which genetic information flows from:



Central dogma

- In some viruses such as HIV, flow of information can be in reverse direction which is called as **reverse transcription** (Teminism).

Genetic Code:

- It is the sequence of bases in mRNA, that code for a particular amino acid in the protein synthesis. There are a total of 64 codons, of which 61 code for amino acids.
- Genetic code is **triplet**, nearly universal, degenerate, commaless, non-ambiguous and specific.
- **AUG** is the **initiator codon** as well as codes for the amino acid methionine whereas **UAA**, **UAG** and **UGA** are **terminator codons.**

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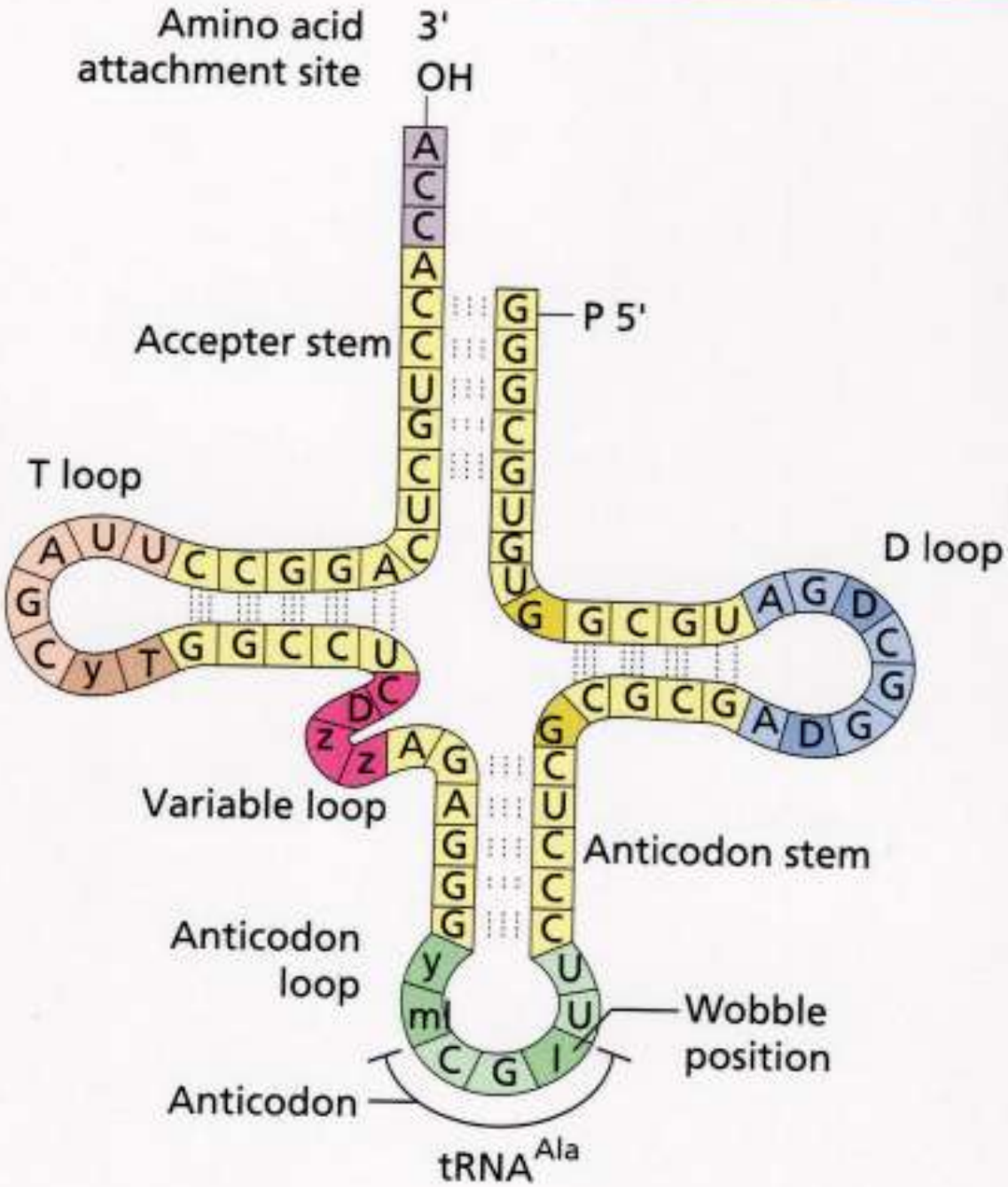
Genetic code table

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

Types of RNA:

- Three types of RNAs are required in the process of translation, **m-RNA** provide the template, **t-RNA** acts as an **adapter,** it brings amino acids and read the genetic code, **r-RNA** play structural and catalytic role.
- **Francis Crick** proposed the presence of tRNA (soluble RNA) whose function is to read the code on one hand and bind to specific amino acids on the other hand.

MOLECULAR BASIS OF INHERITANCE



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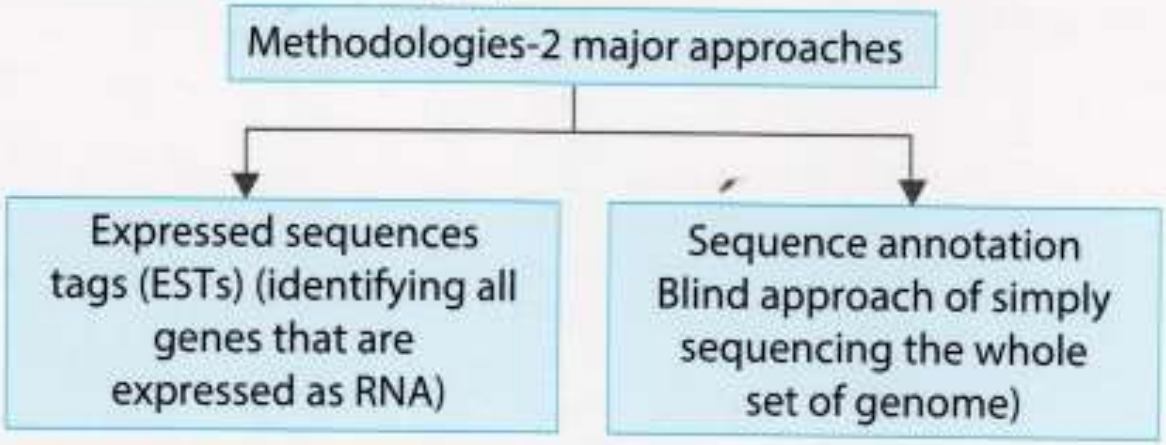
MOLECULAR BASIS OF INHERITANCE

Human Genome Project:

- **Human genome project** (1990-2003) was the first mega project for the sequencing of nucleotides and mapping of all the genes in human genome.
- The important **goals of HGP** are to identify all the estimated genes in human DNA, sequencing of 3 billion chemical base pairs of human DNA, improvement of tools for data analysis, etc.



- **Genome sequencing** procedure involves DNA isolated from cell → Clone in a host using vectors (Bacterial Artificial Chromosomes and Yeast Artificial Chromosomes) for amplification → Sequencing of fragments using automated DNA sequencers → Arrange the sequences based on overlapping regions → Alignment of sequences using computer programs.

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- The sequence of chromosome 1 was completed only in May 2006 (this was the last human chromosomes to be sequenced).
- **Salient features of human genome are:**
  - The human genome has 3164.7 million base pairs.
  - Total 30,000 genes are present with an average of 3000 bases per gene.
  - **Dystrophin** gene is the largest human gene having 2.4 million bases (Present on X-chr).
  - 99.9 % of nucleotides are the same in all people.
  - Less than 2 % of genome codes for proteins.
  - Most genes are found on chromosome 1, i.e., 2968.
  - Least genes are found on the Y chromosome, i.e., 231.
  - There are around 1.4 million locations, where there is a single base difference in DNA; it is called **single nucleotide polymorphism** (commonly called as snips).

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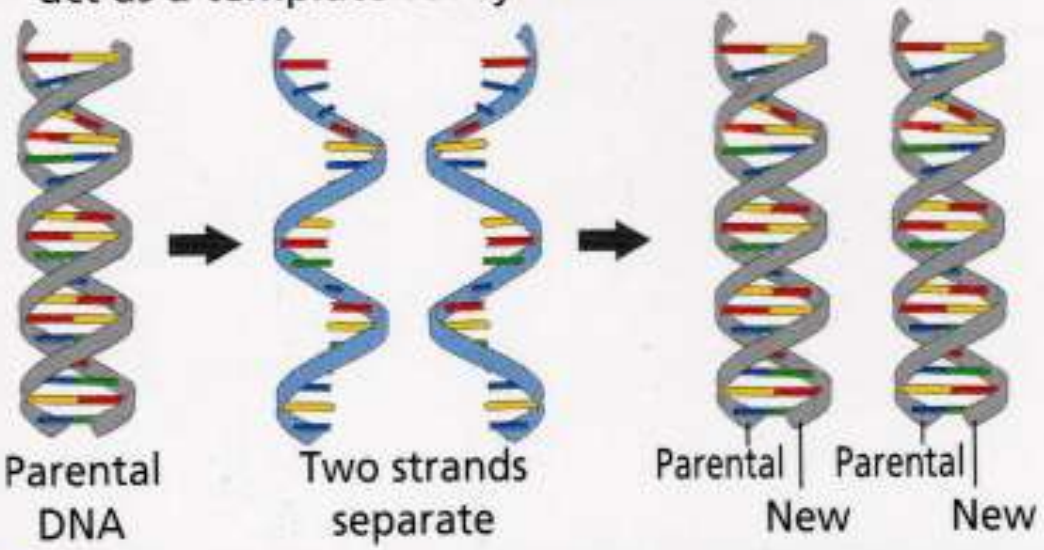
DNA Replication:

- **Watson and Crick** proposed a **semiconservative** scheme for replication of DNA. According to which, parental strands of DNA separate and each act as a template for synthesis of a new strand.



- In DNA replication, **deoxyribonucleoside triphosphates** act as substrate and provide energy for polymerisation reaction. After which, replication initiates at the **origin of replication (ori).**
- A small part of DNA opens up by the enzyme **Helicase** and forms a **replication fork**, where replication occurs and it is catalyzed only in 5′→3′ direction.
- Firstly, **Primase** enzyme helps in the synthesis of **RNA primer** and the enzyme DNA-dependent

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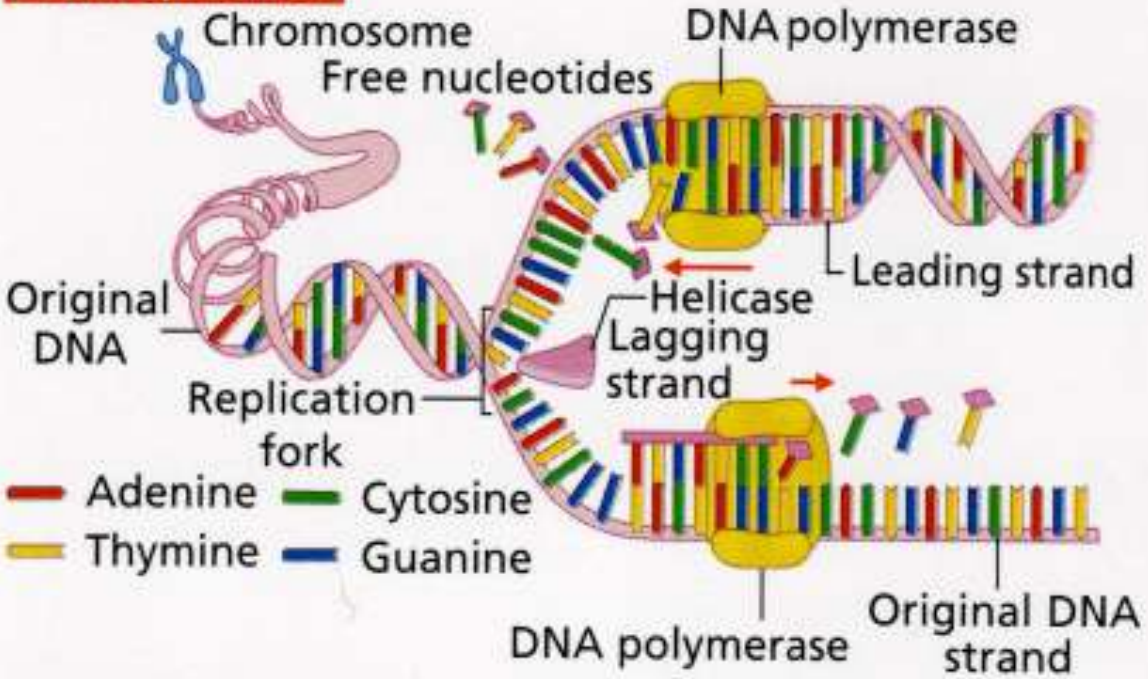
DNA polymerases helps to join the several nucleotides with primer strand and form a polynucleotide chain.

- During replication, a strand is formed in a continuous manner (in 3′→ 5′direction) and called as **leading strand** and other in 5′→ 3′ direction is formed in discontinuous manner in small stretches (**Okazaki fragments**) and called as **lagging strand** which are joined together by enzyme DNA **ligase.**

DNA replication



Experimental Proof for Replication of DNA:

- This proof was first provided by **Matthew Meselson** and **Franklin Stahl.** In 1958, they performed the following experiment:

Hypothesis DNA replication semiconservatively

Method Grow bacteria in ^{15}N (heavy) medium

Transfer some bacteria to ^{14}N (light) medium; bacterial growth continues

Sample at 0 minute | Sample after 20 minutes | Sample after 40 minutes

Samples are taken after 0 minutes, 20 minutes (after) one round of replication, and 40 minimum (Two rounds of replication)

Results

$^{14}N/^{14}N$(light) DNA

$^{14}N/^{15}N$(intermediate) DNA

$^{15}N/^{15}N$(heavy) DNA

Parent (all heavy) | First generation (all intermediate) | Second generation (half intermediate, half light)

Interpretation

Before the bacteria reproduce for the first time in the light medium (at 0 minute), all DNA (parental) is heavy

After two generations, half the DNA was intermediate and half was light only; there was no heavy-only DNA

Conclusion This pattern could only have been observed if each DNA molecule contains a template strand fromthe parental DNA; thus DNA replication is semiconservative

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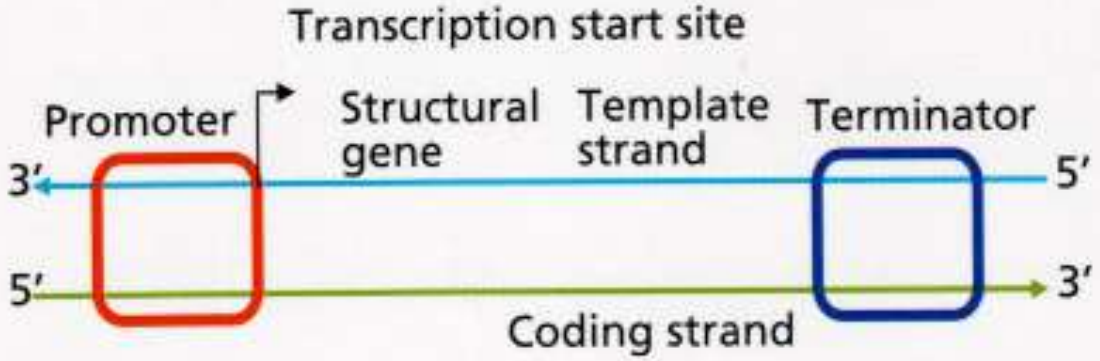
Transcription:

- In transcription, one strand of DNA is copied into RNA. If both strands act as template, they would code for different proteins in both and it complicates the translation or if two RNA molecules are produced, they become double stranded and prevent translation.
- Transcription unit of DNA includes a promoter (at 5′-end upstream), the structural gene (**monocistronic** and **polycistronic** in eukaryotes or prokaryotes, respectively) and a terminator (at 3′- end downstream).



- The strands that has polarity 3′→5′ act as template or antisense strand and other with 5′→3′ polarity is called as coding or sense strand.
- The monocistronic structural gene or split genes have interrupted coding sequences, i.e., **exons** (expressed sequences, appear in mature RNA) and **introns** (intervening sequences, absent in mature RNA).

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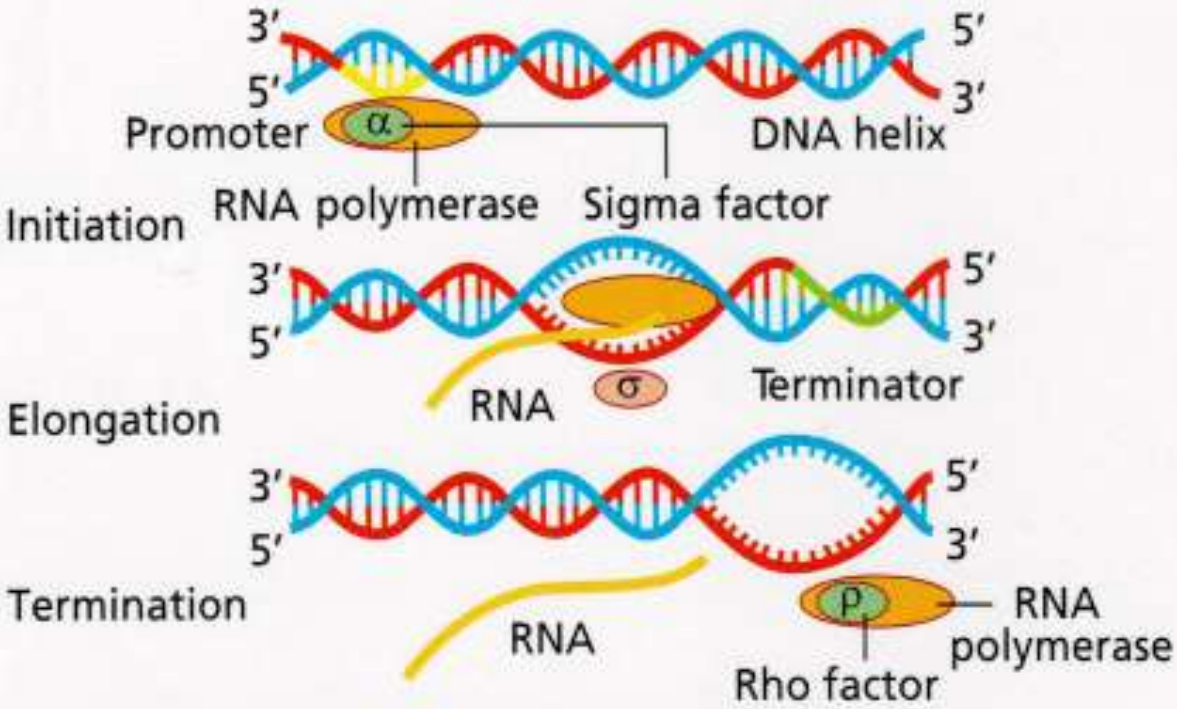
- During transcription in **prokaryotes**, mRNA does not require further processing and translation is coupled with transcription and is initiated before the full transcription of mRNA occurs. There are three steps of transcription:



Transcription in Prokaryotes

- During transcription in **eukaryotes**, complexity increases because of three RNA polymerases, i.e., RNA polymerase I (transcribes rRNAs), RNA polymerase II (transcribes precursor of mRNA, called **hnRNA**), and RNA polymerase III (transcribes tRNA, 5S rRNA and **snRNA**).

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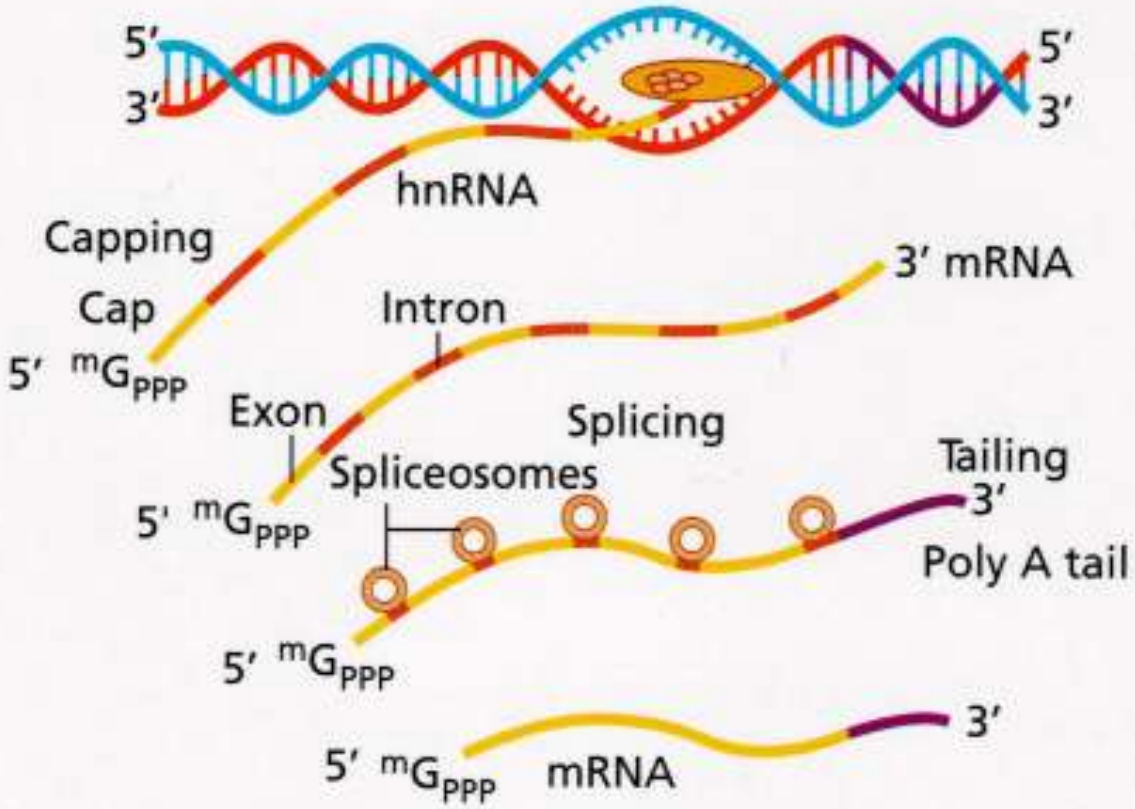
- The second complexity is that both exons and introns are present in primary transcript and are non-functional. Therefore, the primary transcript undergoes processing- **splicing** (removal of introns and joining of exons), **capping** (addition of methyl guanosine triphosphate to the 5′-end) and **tailing** (addition of adenylate residues at 3′-end).



Transcription in Eukaryotes

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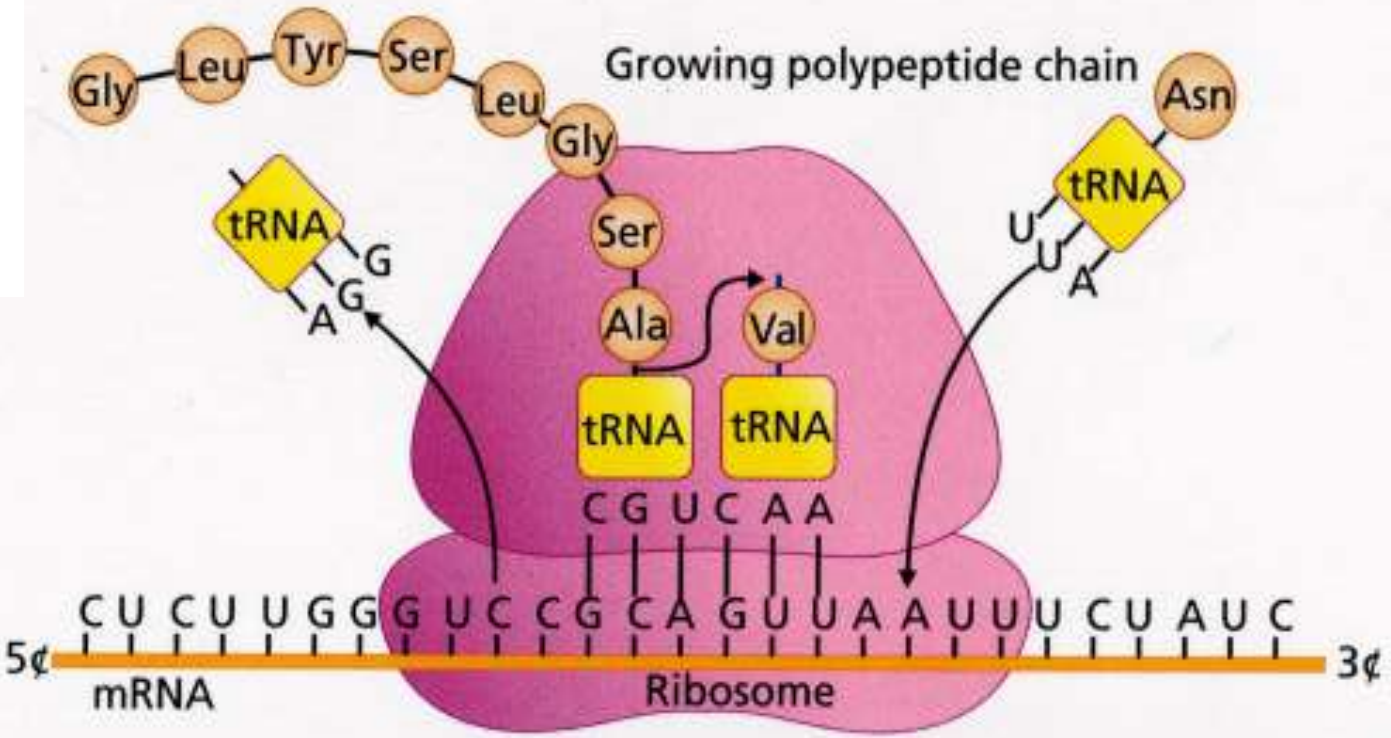
Translation:

- It is the process of polymerisation of amino acids to form a polypeptide based on the sequence of codons in mRNA.
- It requires ribosome, mRNA, tRNA, aminoacyl tRNA synthetase (combines amino acid to particular tRNA) and amino acids.
- An mRNA has some additional sequences that are not translated called **untranslated region** (UTR). Their requirement is crucial for the efficiency of translation process.
- Firstly the **charging (aminoacylation) of tRNA** is must. In this step, amino acids are activated by ATP and then linked to their cognate tRNA in the presence of aminoacyl tRNA synthetase.
- In the **initiation** step, small subunit of ribosome binds to mRNA at the **start codon** that is recognized only by the initiator tRNA. Then large and small subunit binds and forms **translation-initiation** complex. At this stage, two sites are defined, **A-site** (aminoacyl-tRNA site) and **P-site** (peptidyl-tRNA site).

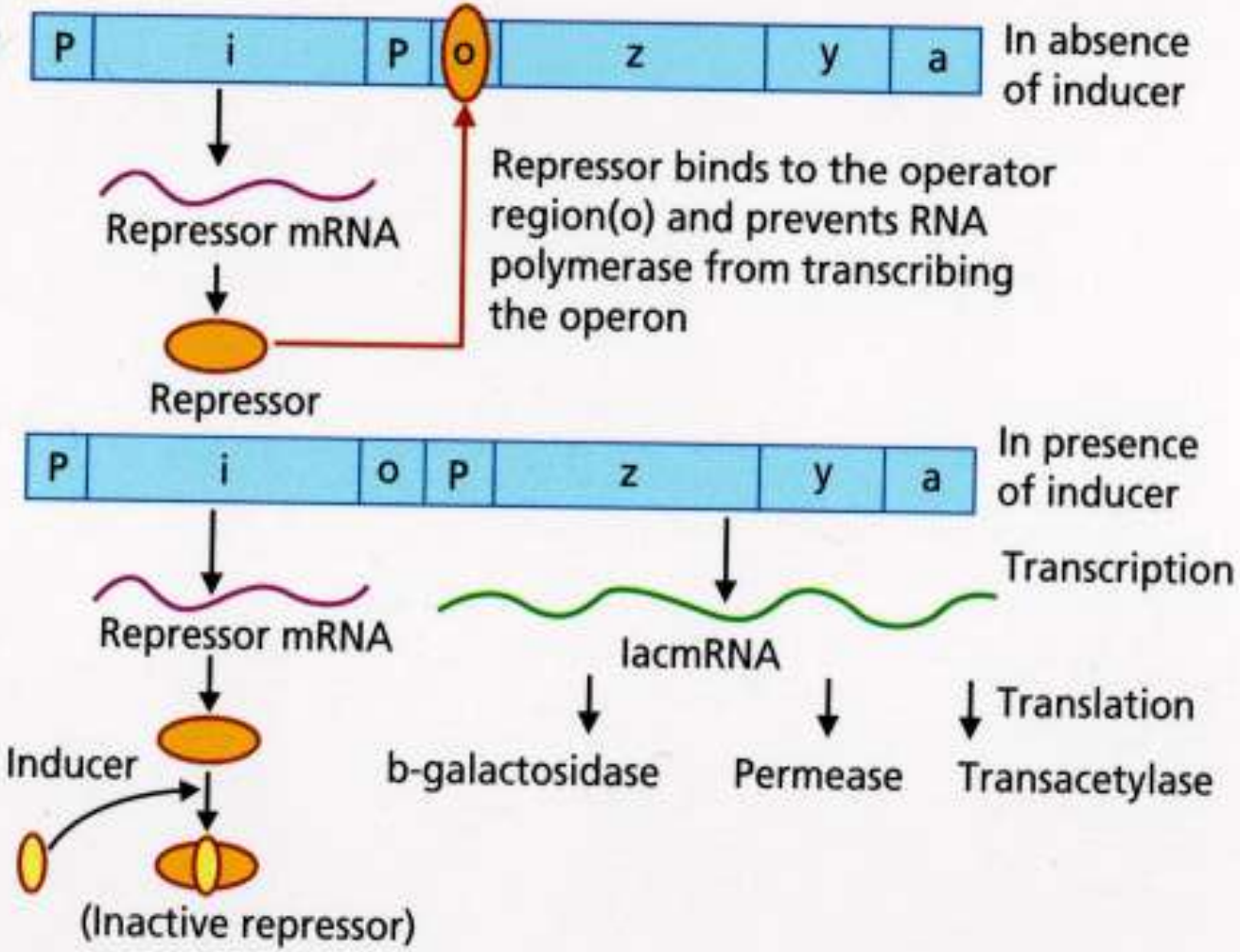


- In the **elongation** step, the amino acid-tRNA complexes bind to the A-site and appropriate codon in mRNA bonds with the tRNA anticodon by complementary base pairing. Then, the ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into Polypeptide sequences.

- In the **termination** step, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

The *Lac* Operon:

- This operon controls lactose metabolism. It consists of one regulatory gene (i gene- codes for repressor), three structural genes (genes z, y and a).
- **Gene z** codes for the enzyme beta-galactosidase which catalyzes the hydrolysis of lactose (β-galoctosides) into glucose and galactose. **Gene y** codes for the enzyme permease which increases the permeability of the cell to β-galactosides. **Gene a** codes for the enzyme transacetylase.
- Lactose is known as the **inducer** of the operon because it regulates the switching on and off of the operon and acts as the substrate for the enzyme beta-galactosidase.



- Regulation of Lac operon by repressor is referred as negative regulation. Operation of Lac operon is also under the control of positive regulation.

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DNA Fingerprinting:

- It is a very quick way to compare the DNA sequence of any two individual. It includes identifying differences in some specific region in DNA sequence called as repetitive DNA.
- Depending upon the base composition, length of segment and number of repetitive units, satellite DNA is classified into micro-satellites, mini-satellites, etc.
- Polymorphism in DNA sequence is the basis for genetic mapping of human genome as well as fingerprinting.
- The technique of DNA fingerprinting was initially developed by Alec Jeffrey. He used a satellite DNA as probe to show high degree of polymorphism was called Variable Number of Tandem Repeats (**VNTR**). The steps involved in the technique are:

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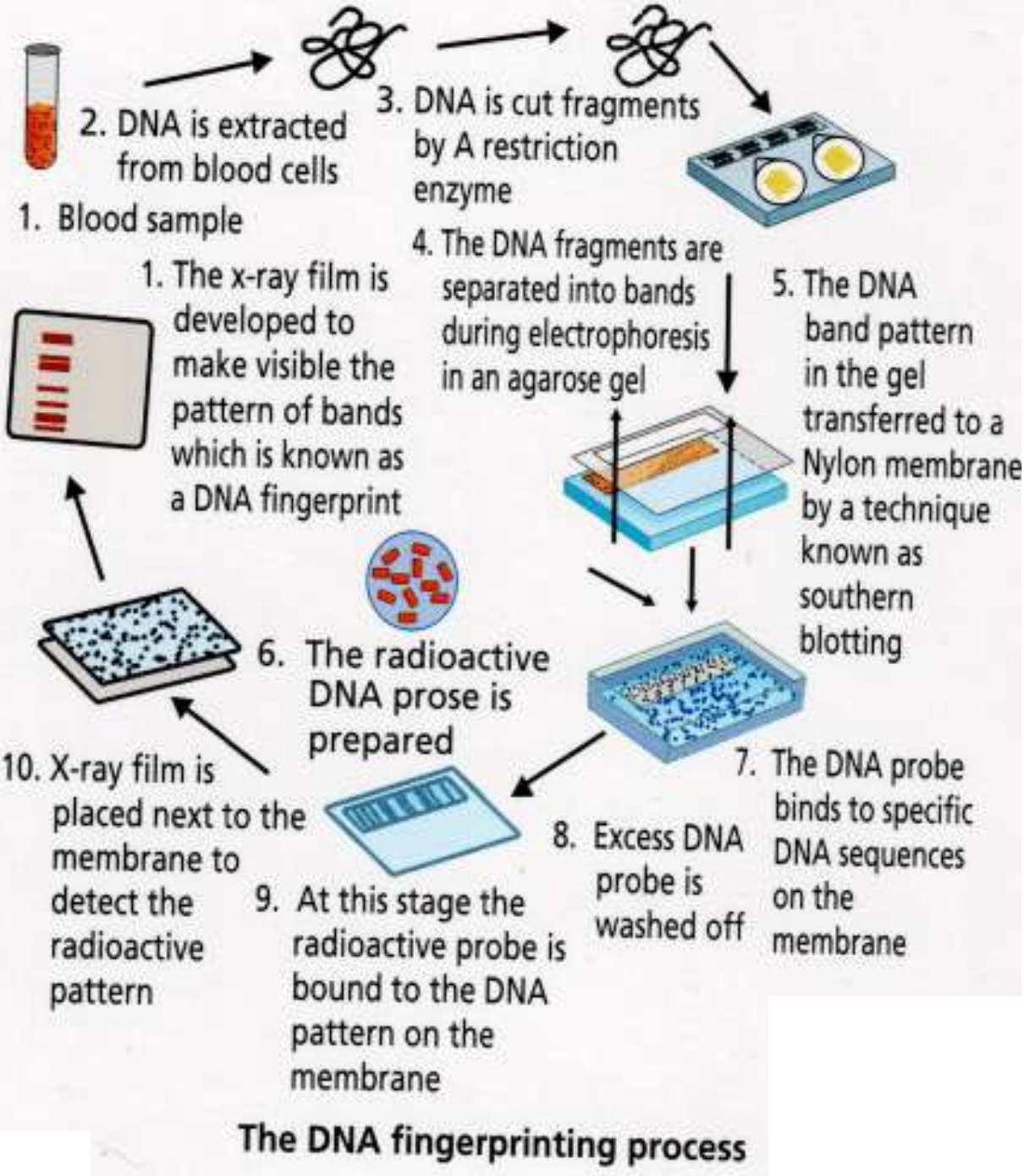

The DNA fingerprinting process

TEST

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1. In prokaryotes, the negatively charged DNA is held with some positively charged proteins in a region called as:
   (a) Nucleus (b) Nucleoid
   (c) Nucleolus (d) Nucleoli
2. Transcriptionally active region in the nucleus is known as _____.
   (a) Euchromatin
   (b) Heterochromatin
   (c) Homochromatin
   (d) None of these
3. A nucleotide or deoxynucleotide is formed when a phosphate group is linked to 5'C–OH of a nucleoside through _____ linkage.
   (a) Glycosidic (b) Phosphoester
   (c) Sulphide (d) Phosphotriester
4. In a bacterium when RNA-polymerase binds to the promoter transcription unit during transcription, it:
   (a) terminates the process
   (b) helps remove introns
   (c) initiates the process
   (d) inactivates the exons

TEST

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Solutions:

1. **Option (b) is correct.**

   In prokaryotes such as E. coli, the negatively charged DNA is organized in large loops and held with some positively charged proteins in a region called as nucleoid.

2. **Option (a) is correct.**

   Transcriptionally active (code for proteins) region in the nucleus is known as euchromatin. These are the regions of chromatin in the nucleus that are loosely packed and are lightly stained.

3. **Option (b) is correct.**

   A nucleotide or deoxynucleotide is formed when a phosphate group is linked to 5'C–OH of a nucleoside through phosphoester linkage. Whereas, a dinucleotide is formed when two nucleotides link through 3'-5' phosphodiester linkage.

4. **Option (c) is correct.**

   In the process of transcription in bacterium, the first step involves the binding of RNA polymerase to the promoter and also associating with initiation factor (σ) for a short period of time, which initiates the process of transcription.

TEST

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5. The proof for semiconservative mode of DNA replication was first provided by:
   (a) Watson and Crick
   (b) George Gamow
   (c) Matthew Meselson and Franklin Stahl
   (d) Hershey and Chase
6. The short fragments of DNA produced in the lagging strand during DNA replication process are known as:
   (a) RNA primer
   (b) Leading strand
   (c) Recombinant DNA
   (d) Okazaki fragments
7. The codon which codes for the amino acid Methionine is:
   (a) CUG
   (b) GUA
   (c) AGU
   (d) AUG
8. _____ chromosome has maximum number of genes in humans.
   (a) Chromosome 20
   (b) Chromosome 1
   (c) Y chromosome

Solutions:

5. **Option (c) is correct.**

The proof for semiconservative mode of DNA replication was first provided by Matthew Meselson and Franklin Stahl. Escherichia coli were the first organism to be used for this experiment and later, it was done in higher organisms such as plants and human cells.

6. **Option (d) is correct.**

The short fragments of DNA produced in the lagging strand during DNA replication process are known as Okazaki fragments. These fragments are later joined together by the enzyme DNA ligase.

7. **Option (d) is correct.**

The codon which codes for the amino acid Methionine is AUG. One of the functions of this codon is that it serves as the start or initiator codon in the process of translation.

8. **Option (b) is correct.**

Chromosome 1 has maximum number of genes in humans i.e., 2968 genes. On the other hand, Chromosome Y has the minimum number of genes i.e., 231.