6. MOLECULAR BASIS OF INHERITANCE

NUCLEIC ACID

- DNA and RNA (genetic material).
- DNA – in most of the organisms.
- RNA – in some viruses only.

Structure of nucleic acid

Nucleoside → Nucleotide → Polynucleotide

a. Nucleoside:

Nucleoside = Nitrogen base + Pentose sugar

b. Nucleotide:

Nucleotide = Nitrogen base + Pentose sugar + Phosphate group

c. Polynucleotide:

Nucleotide + Nucleotide = Dinucleotide

Several nucleotides = Polynucleotide.

Bond present between nucleotides = Phosphodiester bond.

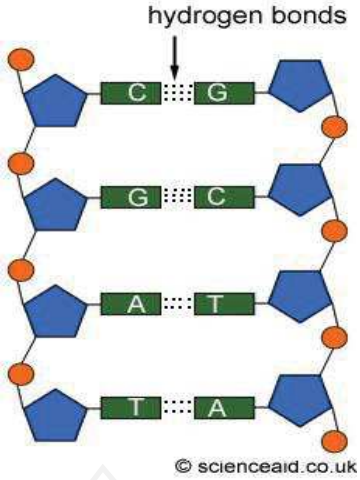
THE DNA DOUBLE HELIX MODEL

- Proposed by **Watson** and **Crick**.
- Contains **2 polynucleotide chains**.
- Nitrogen bases – **ATGC**.
- Sugar – **Deoxyribose**.
- **Hydrogen bond** – Connect Nitrogen bases.

  Between A and T – 2 hydrogen bond.

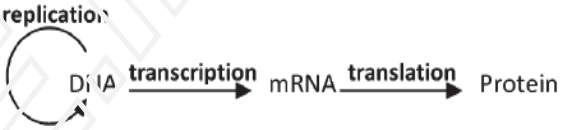
  Between G and C – 3 hydrogen bond.
- **Phosphodiester bond** – Connect Sugar and phosphate.
- One chain has **5'-3'** polarity.

  Other chain has **3'-5'** polarity.
- The two chains coiled in a right handed fashion.
  - Pitch of helix 3.4nm
  - 10 bp in each turn.
  - Distance between base pairs is 0.34nm.



CENTRAL DOGMA

- **Francis Crick** proposed central dogma.



- But in certain viruses RNA is the genetic material.
- They form DNA from RNA by reverse transcription. eg: In retro viruses, reverse transcription occurs with the help of enzyme reverse transcriptase.

PACKAGING OF DNA DOUBLE HELIX

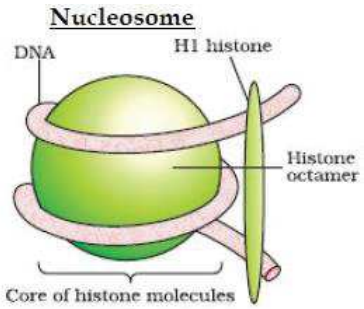
Prokaryotes

- No well defined nucleus.
- DNA is held with some proteins in nucleoid region.

Eukaryotes

- **Histones:**
  - Positively charged proteins.
  - Rich in arginine and lysine.
  - 5 types-H1, H2A, H2B, H3 H4.
- **Histone octamer:**
  - A pair of 4 histones.
- **Nucleosome:**

  - DNA (-ve charge) makes two complete turns around the histone octamer (+ve charge) to form a nucleosome.



Nucleosomes

⬇

Chromatin is packaged

⬇

Chromatin fibres

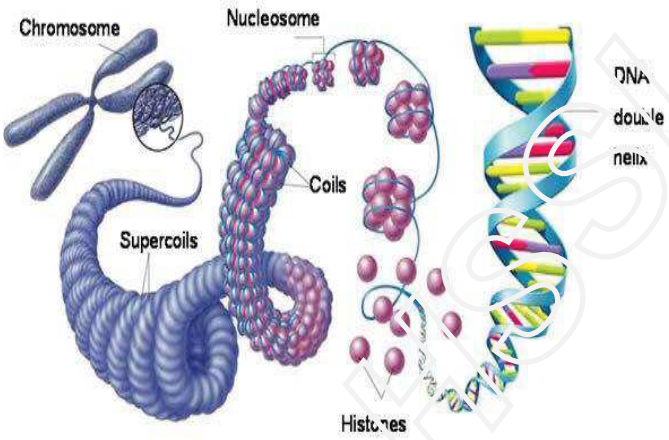
⬇

Coiled and condensed at metaphase stage

⬇

Chromosomes

- The nucleosome in the chromatin is seen as '**beads-on-string**'.



NHC Proteins:

- The packaging of chromatin fibres at higher level requires additional set of proteins called **Non-histone chromosomal proteins (NHC Proteins).**

LENGTH OF DNA

- Length of DNA = Total no: of base pairs x Distance

  = 6.6 x 10 bp x 0.34 x 10 m/bp

  = 2.2 m (human DNA)

- Dimension of nucleus is 10 approximately. The 2.2m DNA is packaged in a nucleus of about 10 m.

Organism	No: of base pair
174 bacteriophage	5386 bp
E.coli	4.6 x 10 bp
Lambda bacteriophage	48502 bp
Human DNA (haploid content)	3.3 x 10 bp

ERWIN CHARGAFF'S RULE

- This rule was proposed by **Erwin Chargaff**.
- It states that, in a double stranded DNA, the ratio between adenine and thymine, and guanine and cytosine are equal.

  i.e, A=T and G=C

  If G = 30% then G = 30%, A = 20% and T = 20%

HETEROCHROMATIN AND EUCHROMATIN

Heterochromatin	Euchromatin
Densely packed regions of chromatin	Loosely packed regions of chromatin
It stains dark	It stains light
It is transcriptionally inactive	It is transcriptionally active

EXPERIMENTAL EVIDENCES TO PROVE THAT DNA IS THE GENETIC MATERIAL

A. Bacterial transformation experiment (Griffith's experiment)

Materials used:

- Mice.
- *Sterptococcus pneumoniae* bacteria.
  - 2 strains- Rough (R) and smooth (S) strain.
  - R strain does not cause pneumonia.
  - S strain causes pneumonia.

Experiment:

S-strain → Inject into mice → Mice die
R-strain → Inject into mice → Mice live
S-strain (Heat killed) → Inject into mice → Mice live
S-strain (Hk) + R-strain (live) → Inject into mice → Mice die

Conclusion:

Some "factors" transferred from heat killed S strain to R strain. This made **R strain** to get transferred into pathogenic **S strain**.

"The phenomenon by which a trait is transferred from one bacterium to another one directly is called bacterial transformation or Griffith effect."

Avery, Mac Leody, Mc Carty experiment

They discovered that:

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

B. Viral infection experiment (Hershey and Chase experiment)

Materials used:

- Bacteriophage (Virus that infect bacteria).
- E. coli bacteria.
- Medium containing radioactive phosphorus(P-32).
- Medium containing radioactive sulphur (S-35).
- Blender.
- Centrifuge.

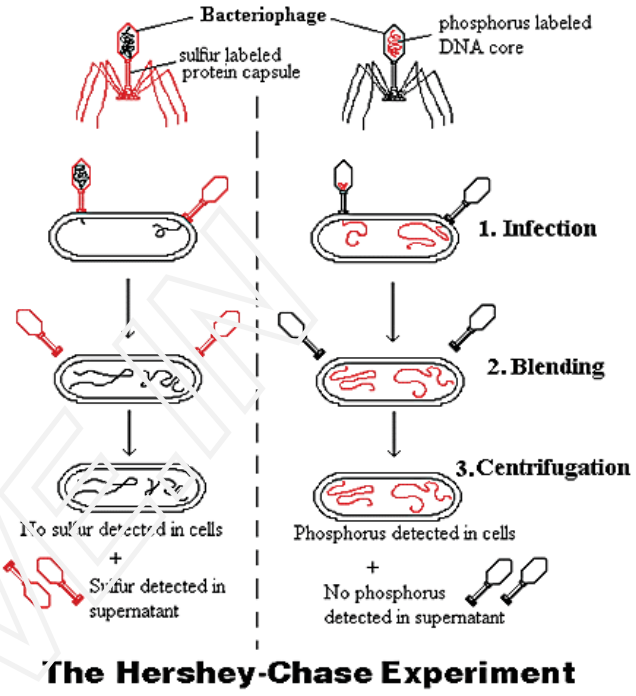
Experiment:

i. They grew some viruses on a medium that contained **radioactive phosphorus** (P-32) and some others on a medium that contained **radioactive sulphur** (S-35).
ii. These preparations were used separately to **infect** E.coli.
iii. After infection, the E.coli cells were agitated in a **blender**.
iv. Then it is **centrifuged**.
v. After centrifugation, heavier bacterial cells are formed as a pellet at the bottom. Lighter viral components remained as supernatant at the top.

Conclusion:

They found that:

- ✓ Supernatant contains viral protein labeled with S-35. Ie, the **viral protein** had **not entered** the bacterial cells.
- ✓ Pellet contains bacterial cells labeled with P-32. Ie, the **viral DNA** labeled with P-32 had **entered** the bacterial cells. This proves that DNA is the genetic material.



The Hershey-Chase Experiment

Criteria required for a molecule to be genetic material

1. It should be able to generate its replica.
2. It should be chemically and structurally stable.
3. It should provide scope for slow changes (mutation) that are required for evolution.
4. It should be able to express itself in the form of 'Mendelian characters'.

DIFFERENCES BETWEEN DNA AND RNA

DNA	RNA
Double stranded	Single stranded
Deoxyribose sugar	Ribose sugar
Nitrogen bases are ATGC	Nitrogen bases AUGC
Purines and pyrimidines are present in equal amount	Purines and pyrimidines are not present in equal amount
Seen in nucleus, mitochondria and chloroplast	Seen in cytoplasm, ribosome and nucleolus

DNA versus RNA

DNA	RNA
1. DNA can replicate. 2. DNA is **stable**. ie, it does not change with different stages of life cycle, age or with change in physiology of an organism. The presence of thymine gives additional stability to DNA. 3. DNA is chemically **less reactive**. 4. DNA can **mutate**. **5.** DNA is **dependent on RNA** for the **synthesis of proteins**.	1. RNA can also replicate. 2. RNA is **not stable**. Because, 2 OH group present at every nucleotide in RNA is a reactive group and makes RNA liable and easily degradable. 3. RNA is **reactive**. 4. RNA **mutates** at **faster** rate. Because, RNA is unstable. Therefore, viruses having RNA genome and having shorter life span mutate and evolve faster. 5. RNA can **directly** code for the **synthesis of proteins**, hence can easily express the characters. So for the transmission of genetic information RNA is better.

The DNA is structurally more stable and chemically less reactive when compared to RNA. Therefore, DNA is a better genetic material.

DNA REPLICATION

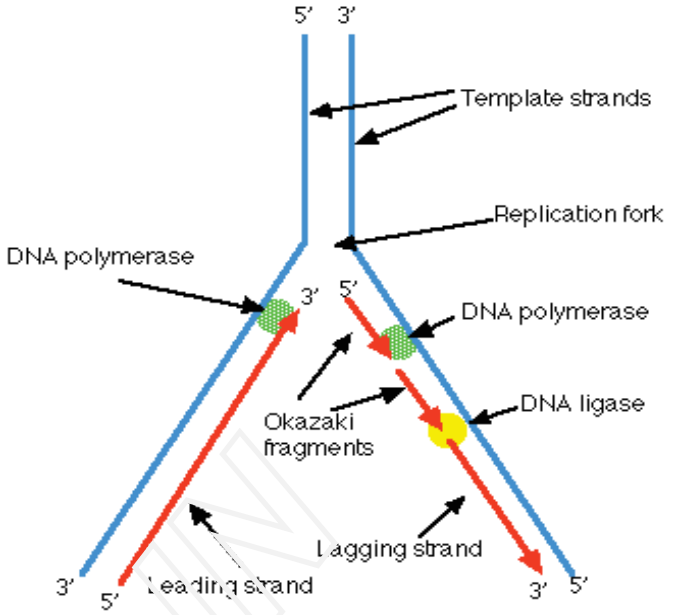
- Proposed by Watson and Crick.
- It is the synthesis of new DNA molecules from the pre-existing DNA.
- It is semi conservative type.

What is semi conservative replication?

The newly formed DNA will have one parental strand and one newly synthesized strand. As one strand is conserved, it is called semi conservative replication.

Replication process

- In **eukaryotes**, DNA replication takes place at **S phase** of the cell cycle.



- **Origin of replication** – starting point of replication.
- **Helicase** enzyme will unwind DNA.
- Hydrogen bond breaks down.
- Separated strands become template strand.
- **Primer** (Short RNA) is formed first.
- **DNA polymerase** enzyme adds nucleotides to the primer. As a result leading strand and lagging strand is formed.

✓ **Leading strand:**
- It is formed in 5'-3' direction.
- Its formation is continuous.

✓ **Lagging strand:**
- It is formed in 5'-3' direction, but away from the replication fork.
- Its formation is discontinuous.
- Small fragments (**Okazaki fragments**) are formed first.
- Okazaki fragments are joined by DNA **ligase** enzyme.
- RNA primer is replaced with DNA.

Replication fork:

- When the DNA helix is unwind up to a point, it appears to be a 'Y'shaped structure called **replication fork**.

EXPERIMENTAL PROOF FOR DNA REPLICATION (Messelson and Stahl experiment)

- E. coli was grown in a medium containing **heavier isotope** of nitrogen (**N 15 medium**).
  - **Result**: N_{15} was incorporated into both strands of DNA of E. coli. Thus DNA becomes heavier. This DNA is called **heavier DNA**.

- E. coli with heavier DNA is transferred to a medium containing **lighter isotope** of nitrogen (**N 14 medium**).
  - **Result**: **After first generation**: The DNA of daughter cells contained **one heavier** strand and **one lighter** strand.
    - ✓ Heavier strand – strand containing N_{15}.
    - ✓ Lighter strand – strand containing N **14.**
    - This indicated the semi – conservative method of DNA replication.
  - **After second generation**: **Half** of the DNA molecules were **hybrid** (each DNA having one heavier and one lighter strand) and the **other half** were completely **new** (each DNA with lighter strands only).

TRANSCRIPTION

- The process of copying genetic information from one strand of DNA into RNA is termed as **transcription**.

Requirements for transcription

a. Enzyme.
b. DNA.
c. Transcription unit.

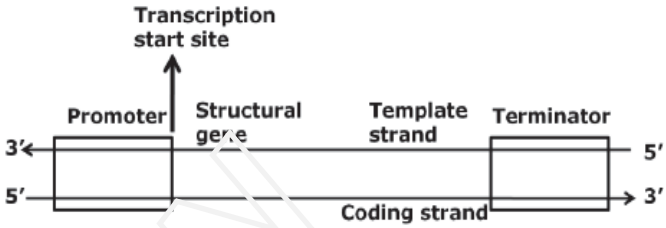
a. Enzyme:

- The enzyme that catalyzes transcription is **RNA polymerase**.
- In **bacteria** there is only **one** RNA polymerase.
- In **eukaryotes** there are **three** RNA polymerases.
  - RNA polymerase I (involved in the synthesis of RNA).
  - RNA polymerase II (involved in the synthesis of precursor of mRNA, ie, hnRNA).
  - RNA polymerase III (involved in the synthesis of tRNA, 5sRNA, SnRNA).

b. DNA:

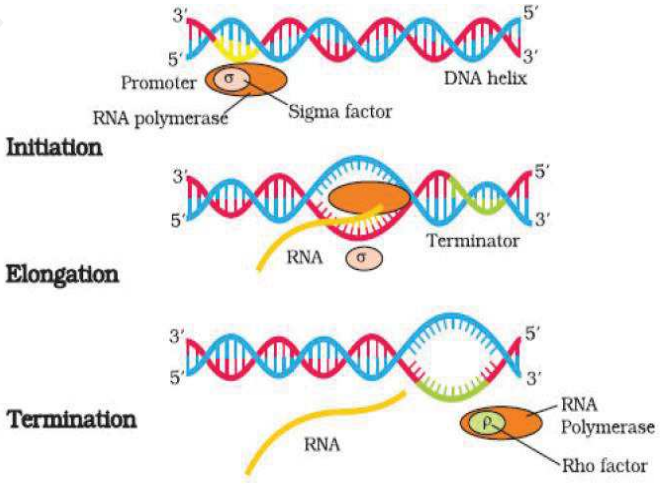
- DNA is needed for the synthesis of RNA.

c. Transcription unit:



- Transcription unit contains – promoter, structural gene and terminator.
  - **Promoter** - It provides binding site for RNA polymerase.
  - **Structural gene** – It provides template strand for the formation of RNA.
  - **Terminator** – It defines the end of the process of transcription.

Steps in the process of transcription



a. Initiation

- Transcription is initiated from the promoter region.
- RNA polymerase binds to the promoter region of DNA. As a result DNA unwinds. RNA formation starts.
- In bacteria, only one **initiation factor sigma (σ)** is required.
- In eukaryotes, several initiation factors are required.

b. Elongation

- The RNA chain is synthesized in 5'- 3' direction.
- The nucleotides are added to the growing chain.

c. Termination

- Termination occurs at terminator region.
- A **termination factor rho** (**ρ**) binds to the RNA polymerase and terminates transcription.

RNA PROCESSING IN EUKARYOTES

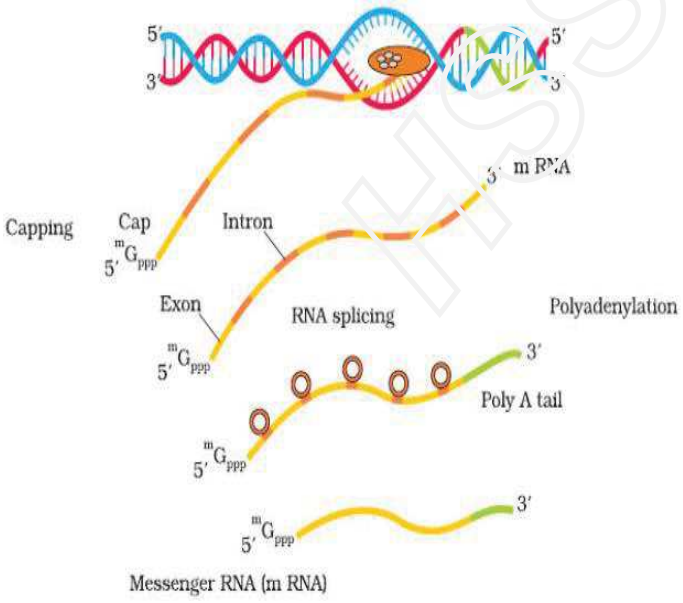
❖ The processing events include the following –
  - **Capping** of the 5' end of the RNA.
  - **Splicing**.
  - **Tailing** (polyadenylation of the 3' end of the RNA).

Capping - an unusual nucleotide (methyl guanosine triphosphate) is added to the 5' end of the hnRNA.

Splicing - the introns (non – functional) are removed and the exons are joined together.

Tailing - adenylate residues are added at the 3'end of the RNA. It is mediated by an enzyme called **Poly A Polymerase**.

The hnRNA is now called mRNA, which is transported out of the nucleus for translation.



PROPERTIES OF RNA

- Complementary to the template strand of the DNA duplex.
- Identical to the non – template strand (coding strand).

eg: Template strand – 3' TACGTACGTACGTACG 5'

Coding strand – 5' ATGCATGCATGCATGC 3'

mRNA strand – 5' AUGCAUGCAUGCAUGC 3'

Template strand	Coding strand
It acts as a template for the synthesis of mRNA during transcription.	It is a sequence of DNA that has the same base sequence as that of mRNA (except thymine is replaced by uracil).
It runs from 3' – 5' direction	It runs from 5' – 3' direction.

EXONS AND INTRONS

Exons: Coding regions.

Introns: Non coding regions.

CISTRON

- **Cistron:** It is a **segment of DNA**, coding for a polypeptide.
- **MONOCISTRONIC** (In eukaryotes): Codes for only one polysaccharide.
- **POLYCISTRONIC** (In prokaryotes): Codes for more than one polysaccharide.

GENETIC CODE

- The sequence of nitrogen bases in mRNA which contains information for protein synthesis is called genetic code.
- The code is made up of 3 nitrogen bases. This is called **triplet code**. Eg: Code for Phenyl alanine is UUU and UUC.
- **Codon** – The sequence of 3 bases determining a single amino acid is called codon.

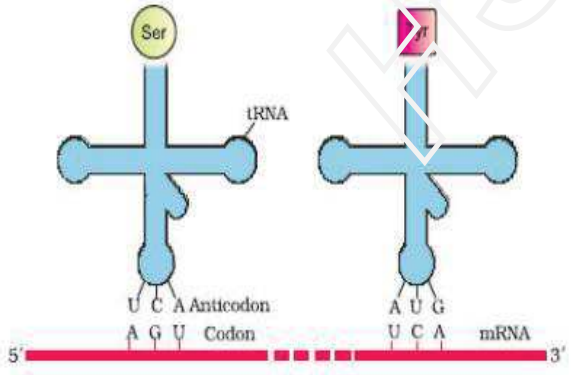
- There are **64 codons** for 20 naturally occurring amino acids.
- **George Gamow**, **Marshall Nirenberg**, **Severo Ochoa**, **Hargobind Khorana** etc have made significant contributions to decipher the genetic code.

- **George Gamow:** Suggested that for coding 20 amino acids, the code should be made up of 3 nucleotides.
- **Har Gobind Khorana:** Developed the chemical method in synthesizing RNA molecules with defined combinations of bases (homopolymers & copolymers).
- **Marshall Nirenberg:** Developed cell-free system for protein synthesis.
- **Severo Ochoa (polynucleotide phosphorylase) enzyme** is used to polymerize RNA with defined sequences in a template independent manner.

Salient features of the genetic code.

- Genetic code is **triplet** code.
- Genetic code is **universal** in nature.
- Genetic code is **unambiguous**, ie, is one codon codes for only one amino acid.
- Genetic code is **degenerate**, i.e., a single amino acid is represented by many codons.
- Genetic code is **comma less**.
- Genetic code has initiation codon and termination codon.
  - **Initiation codon** - AUG
  - **Termination codon** -UAA, UAG, UGA

tRNA



tRNA has

- **An Anticodon (NODOC) loop** that has bases complementary to the code.
- **An amino acid acceptor end** to which amino acid binds.
- For initiation, there is another tRNA called **initiator tRNA.**
- There are no tRNAs for stop codons.
- **Secondary (2-D)** structure of tRNA looks like a **clover-leaf. 3-D structure** looks like **inverted 'L'.**

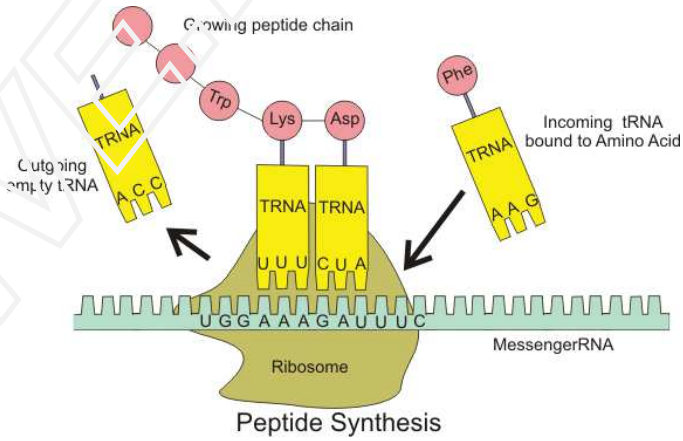
TRANSLATION

- The process by which proteins are derived from RNA is called translation.

Requirements for translation

- Messenger RNA (mRNA)
- Ribosomes (70s ribosome in prokaryotes and 80s ribosome in eukaryotes)
- Transfer RNA (tRNA)
- Amino acids
- Enzymes
- Energy source

Steps in the process of translation



1. Charging of tRNA

Amino acids are linked to their tRNA in the presence of amino acyl tRNA synthetase enzyme. So the tRNA becomes charged.

2. Initiation

- It begins at the **5' end** of the mRNA.
- The small subunit of ribosome binds to mRNA.
- The initiation codon in mRNA is **AUG**.
- The initiator tRNA (methionyl tRNA) having **UAC** at the anticodon site blinds to the initiation codon on the mRNA.
- The large subunit of ribosome then blinds to mRNA.
- The large subunit has two blinding sites for tRNA – **A site** and **P site**.
- The initiator tRNA is founds at the P site.
- All other **tRNA first binds to A site** and **then shift to the P site.**

3. Elongation

- Then another tRNA complex with an appropriate amino acid enters the ribosome and attaches at the **A site**.
- **Methionine** from the first tRNA is now attached to the amino acid of the second tRNA through peptide bond.
- The first tRNA is removed from the **P site**.
- The second tRNA is moved to the P site from A site (translocation).
- Then again third tRNA with amino acid bind at the A site.
- This process of peptide bond formation and translocation are repeated.

4. Termination

- When the tRNA reaches the **termination codon** (UAA, UAG, UGA) termination of protein synthesis occurs.
- A release factor binds to the termination codon (stop codon).
- This leads to the release of the polypeptide chain of amino acids and tRNA from the ribosome.

Untranslated regions (UTRs)

- They are the sections of the RNA before the start codon and after the stop codon that are not translated.
- The UTRs are present at both 5' end and 3' end.

REGULATION OF GENE EXPRESSION

In eukaryotes:

In eukaryotes the regulation could be exerted at,

I. Transcriptional level
II. Processing level
III. Transport of mRNA from nucleus to the cytoplasm
IV. Translational level

In prokaryotes:

Operon Concept

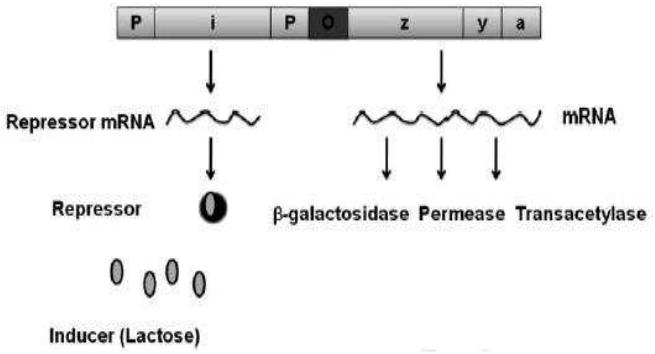
- It was put forward by **Jacob** and **Monod**.

The Lac Operon

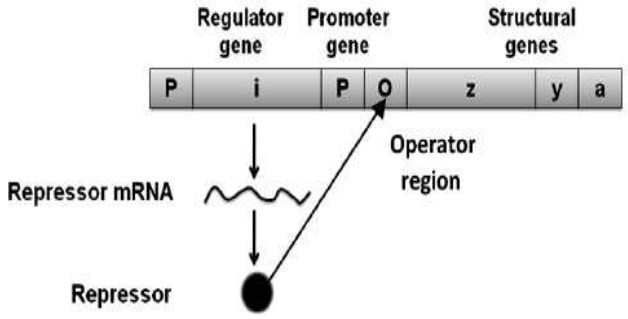
- It controls the **degradation of lactose** in E.coli.
- The operon consists of:
  - **3 structural genes – z, y and a.**
  - **Operator gene**
  - **Regulator gene ie, 'i' gene.**
  - **Promoter region**
- The structural genes transcribe together and form mRNA.
- This mRNA directs the synthesis of 3 enzymes:
  - **β galactosidase**
  - **Galactosidase permease**
  - **Galactosidase transacetylase**
- The regulator gene synthesizes a regulator protein called ''**lac repressor**".
- The regulation can be positive or negative.

Positive regulation	Negative regulation
• It occurs when **lactose is present** in the medium and **enzymes are required** to metabolize it. • Lactose serves as the inducer and it **inactivates** the **lac repressor**. • Lac repressor **cannot bind** to the operator region. • The structural gene **undergoes transcription** and translation to produce the enzymes.	• It occurs when **lactose is absent** in the medium and **enzymes are not required**. • **Lac repressor** is **active**. • Lac repressor **binds** to the operator region. • The structural gene **cannot undergo transcription** and translation and the enzymes are not produced.

In the presence of inducer:



In the absence of inducer:



HUMAN GENOME PROJECT (HGP)

- It was started in the year 1990 **to map the entire genome** and completed in 2003.

Why HGP is called a mega project?

- Because: The total estimated cost of the project was around **9 billion US Dollars**. Around **3300 books** were required for storing the information of DNA sequence of a single human cell. A large amount of data was stored in **computers**. For this bioinformatics is used.

Goals of HGP

- To **identify all the genes** in human DNA.
- To **determine the sequence** of 3 billion base pairs in human DNA.
- To **store this information** in databases.
- To address **ethical**, **legal** and **social issues** (**ELSI**) that may arise from the project.

Methodologies

Two major approaches were used. They are:

- **Expressed sequence tags (ESTs)**: Sequencing the genes that are expressed as RNA.
- **Sequence annotation**: Sequencing the whole genome that contain coding and non-coding sequences.
-

Procedure:

Isolate total DNA from a cell → Convert into random fragments → Clone in suitable host (e.g. BAC & YAC) for amplification → Fragments are sequenced using Automated DNA sequencers (using Frederick Sanger method) → Sequences are arranged based on overlapping regions → Alignment of sequences using computer programs

Features of human genome

- The human genome contains **3164.7 million nucleotides**.
- **Chromosome I** has **most genes** (2968) and the **Y** has **fewest** (231).
- The **largest human gene is dystophin** having 2.4 million base pairs.

Repeated sequences – They are stretches of DNA sequences that are repeated many times. They have no direct coding function.

Application of HGP

- Biological systems can be well studied by the knowledge of DNA sequences.
- HG sequence used to develop a new approach to biological research.

DNA FINGER PRINTING

- The technique was developed by **Alec Jeffry**.

Repetitive DNA	Satellite DNA
• A small stretch of DNA is repeated several times in the total DNA of a cell.	• Highly repetitive non-transcribed region of DNA.

DNA Polymorphism: It is an inheritable mutation.

VNTR (Variable Number of Tandem Repeats): The repetitive sequences are called VNTR.

PCR (Polymerase Chain Reaction): It is used to amplify DNA.

Steps in DNA finger printing

1. Isolation of DNA
2. Digestion of DNA into fragments by restriction endonuclease
3. Separation of DNA fragments by electrophoresis
4. Transferring of separated DNA fragments into nitrocellulose paper (blotting)
5. Hybridization using labeled VNTR probe
6. Detection of hybridized DNA fragments be auto radiography

Application of DNA finger printing

- ✓ Used in forensic science to solve the problems of paternity, rape, murder etc.
- ✓ Used to diagnose genetic disorders.

PREPARED BY ARIFA T M A (HSST ZOOLOGY)
DEMHSS THALANGARA, KASARAGOD.