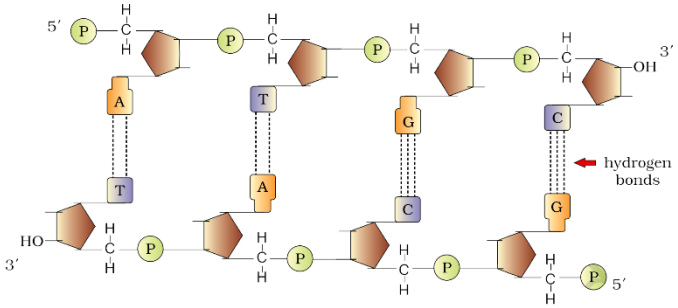
6. MOLECULAR BASIS OF INHERITANCE

THE DNA

STRUCTURE OF THE DNA



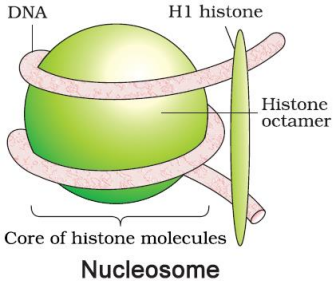
- DNA is made of 2 polynucleotide chains coiled in a right-handed fashion. Its backbone is formed of sugar & phosphates. The bases project inside.
- The 2 chains have **anti-parallel polarity**, i.e. one chain has the polarity **5'→3'** and the other has **3'→5'.**
- The bases in 2 strands are paired through **H-bonds** forming **base pairs (bp).**

A=T (2 hydrogen bonds) **C≡G (3 hydrogen bonds)**

- **Erwin Chargaff's rule:** In DNA, the proportion of A is equal to T and the proportion of G is equal to C.

PACKAGING OF DNA HELIX

- In eukaryotes, there is a set of positively charged, basic proteins called **histones.**
- Histones are rich in positively charged basic amino acid residues ***lysines*** and ***arginines.***
- **8 histones** form **histone octamer.**
- Negatively charged DNA is wrapped around histone octamer to give **nucleosome.**
- A typical nucleosome contains **200 bp.**
- Nucleosomes constitute the repeating unit to form **chromatin.**
- Chromatin has 2 forms:
  - **Euchromatin:** Loosely packed and transcriptionally active region of chromatin. It stains light.
  - **Heterochromatin:** Densely packed and inactive region of chromatin. It stains dark.



THE SEARCH FOR GENETIC MATERIAL

Griffith's Transforming Principle experiment (1928)

Frederick Griffith used **mice** & ***Streptococcus pneumoniae.*** ***Streptococcus pneumoniae*** has 2 strains:

- **Smooth (S) strain (Virulent):** Has polysaccharide mucus coat. Cause pneumonia.
- **Rough (R) strain (Non-virulent):** No mucus coat. Do not cause Pneumonia.

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

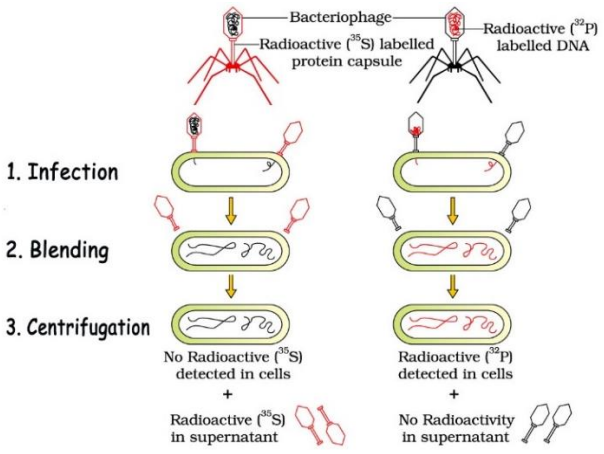
He concluded that some **'transforming principle'** transferred from heat-killed S-strain to R-strain. It enabled R-strain to synthesize smooth polysaccharide coat and become virulent. This must be due to the transfer of genetic material.

Biochemical characterization of transforming principle

- **Oswald Avery, Colin MacLeod** & **Maclyn McCarty** worked to determine the biochemical nature of **'transforming principle'** in Griffith's experiment.
- They purified biochemicals (proteins, DNA, RNA etc.) from heat killed S cells using suitable enzymes.
- They discovered that
  - Digestion of protein and RNA (using *Proteases* and *RNases*) did not affect transformation. It means that 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. It proves that DNA was the transforming principle.

Hershey-Chase Experiment (Blender Experiment)

- **Hershey** & **Chase** grew some bacteriophage viruses on a medium containing radioactive phosphorus (**P^{32}**) and some others on medium containing radioactive sulphur (**S^{35}**).
- Viruses grown in P^{32} got **radioactive DNA** because only DNA contains phosphorus. Viruses grown in S^{35} got **radioactive protein** because protein contains sulphur.
- These preparations were used separately to infect ***E. coli.***
- After infection, the *E. coli* cells were gently agitated in a blender to remove the virus particles from the bacteria.
- Then the culture was centrifuged to separate lighter virus particles from heavier bacterial cells.
- Bacteria infected with viruses having radioactive DNA were radioactive. i.e., DNA had passed from the virus to bacteria. Bacteria infected with viruses having radioactive proteins were not radioactive. i.e., proteins did not enter the bacteria from the viruses. This proves that DNA is the genetic material.



CENTRAL DOGMA OF MOLECULAR BIOLOGY

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

Central dogma

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

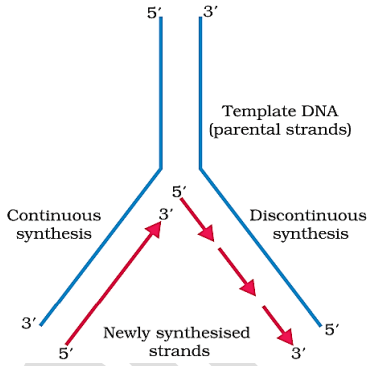
DNA REPLICATION

- Replication is the copying of DNA from parental DNA.

The Machinery and Enzymes for Replication

- During replication, the 2 strands unwind and separate by breaking H-bonds.
- Unwinding of the DNA molecule at a point forms a 'Y'-shaped structure called **replication fork.**
- The separated strands act as **templates** for the synthesis of new strands.

- In presence of an enzyme, DNA dependent ***DNA polymerase,*** many nucleotides join with one another to primer strand and form a polynucleotide chain (new strand).
- During replication, one strand is formed as a continuous stretch in 5'→ 3' direction **(Continuous synthesis).** This strand is called **leading strand.**

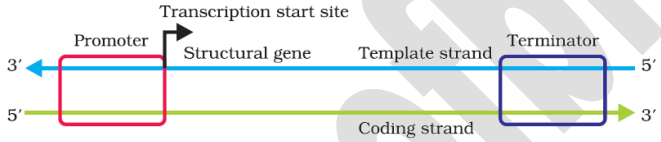


- The other strand is formed in small stretches **(Okazaki fragments)** in 5'→ 3' direction **(Discontinuous synthesis).**
- The Okazaki fragments are then joined together to form a new strand by an enzyme, ***DNA ligase***. This new strand is called **lagging strand.**

TRANSCRIPTION

Transcription Unit

- It is the segment of DNA between the sites of initiation and termination of transcription. It consists of 3 regions:
  - **A promoter:** Binding site for *RNA polymerase.*
  - **Structural gene:** The region between promoter and terminator where transcription takes place.
  - **A terminator:** The site where transcription stops. Located towards 3'-end.



Transcription unit and gene

Structural gene in a transcription unit is 2 types:

- **Monocistronic structural genes (split genes)**: It is seen in eukaryotes. Here, coding sequences ***(exons or expressed sequences)*** are interrupted by ***introns*** (intervening sequences). Exons appear in processed mRNA. Introns do not appear in processed mRNA.
- **Polycistronic structural genes**: It is seen in prokaryotes. Here, there are no split genes.

GENETIC CODE

- It is the sequence of nucleotides in mRNA that contains information for protein synthesis.

Salient features of genetic code

- **Codon is triplet** (three-letter code).
- 61 codons code for amino acids. 3 codons (**UAA, UAG** & **UGA)** do not code for any amino acids. They act as **stop codons (Termination codons or non-sense codons).**
- Genetic code is **universal.** E.g. From bacteria to human UUU codes for Phenylalanine. Some exceptions are found in mitochondrial codons, and in some protozoans.
- **No punctuations** b/w adjacent codons (comma less code). The codon is read in mRNA in a contiguous fashion.
- Genetic code is **non-overlapping.**
- An amino acid is coded by more than one codon (except AUG for methionine & UGG for tryptophan). Such codons are called **degenerate codons.**
- Genetic code is **unambiguous** and **specific.** i.e. one codon specifies only one amino acid.
- AUG has dual functions. It codes for Methionine and acts as **initiator codon.** In eukaryotes, ***methionine*** is the first amino acid and ***formyl methionine*** in prokaryotes.

REGULATION OF GENE EXPRESSION

Lac Operon in *E. coli*

- The operon controlling lactose metabolism.
- It is proposed by **Francois Jacob & Jacque Monod.**

It consists of

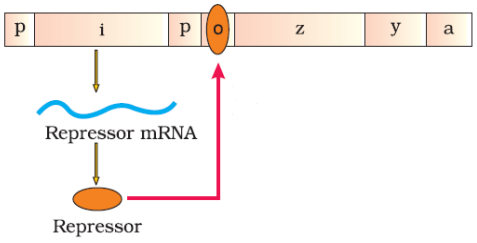
a) A regulatory or inhibitor (i) gene: Codes for repressor protein.

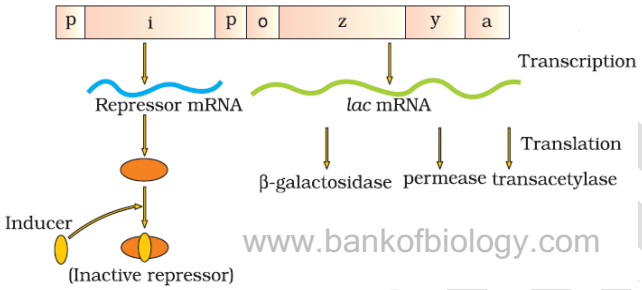
b) 3 structural genes:

i. **z gene:** Codes for ***β galactosidase.*** It hydrolyses lactose to galactose and glucose.
ii. **y gene:** Codes for ***permease.*** It increases permeability of the cell to β-galactosides (lactose).
iii. **a gene:** Codes for a ***transacetylase.***

- Genes in the operon function together in the same or related metabolic pathway.
- If there is no **lactose (inducer),** lac operon remains switched off. The regulator gene synthesizes mRNA to produce **repressor protein.** This protein binds to the **operator** region and blocks *RNA polymerase* movement. So the structural genes are not expressed.
- If lactose or allolactose is provided in the growth medium, it is transported into ***E. coli*** cells by the action of ***permease.*** Lactose (inducer) binds with repressor protein. So repressor protein cannot bind to operator region. The operator region becomes free and induces the *RNA polymerase* to bind with **promoter.** Then transcription starts.
- Regulation of lac operon by repressor is called **negative regulation.**

In the absence of inducer





In the presence of inducer

HUMAN GENOME PROJECT (HGP)

- **Human Genome Project** was the first mega project for the sequencing of nucleotides and mapping of all the genes in human genome.

BAC= Bacterial Artificial Chromosomes
YAC= Yeast Artificial Chromosomes

DNA FINGERPRINTING (DNA PROFILING)

Steps of DNA fingerprinting (Southern Blotting Technique)

a. **Isolation** of DNA (from any cells or blood stains, semen stains, saliva, hair roots, bone, skin etc.).
b. **Digestion** of DNA by **restriction endonucleases.**
c. **Separation** of DNA fragments by **gel electrophoresis.**
d. **Transferring (blotting)** DNA fragments to synthetic membranes such as **nitrocellulose** or **nylon**.
e. **Hybridization** using radioactive labelled **VNTR probe.**
f. **Detection** of hybridized DNA by **autoradiography.**

The autoradiogram gives an image in the form of dark & light bands. It is called **DNA fingerprint.**

Application of DNA fingerprinting

- **Forensic tool** to solve paternity, rape, murder etc.
- For the diagnosis of **genetic diseases.**
- To determine **phylogenetic status** of animals.
- To determine **population and genetic diversities.**