

CHAPTER 5: MOLECULAR BASIS OF INHERITANCE

1. Introduction to DNA & Nucleic Acids

DNA (Deoxyribonucleic Acid) is the fundamental molecule of inheritance present in the cell nucleus of most organisms. It carries genetic instructions across generations.

- **Discovery:** Friedrich Miescher (1869) discovered a specific substance in the nucleus of pus cells and named it "**Nuclein**".
- **Naming:** Altmann later termed it **Nucleic Acid**.
- **Types (P.A. Levene):** Nucleic acids are divided into two primary types:
 - **DNA** – Deoxyribonucleic acid (genetic material in most organisms)
 - **RNA** – Ribonucleic acid (genetic material in specific viruses like Tobacco Mosaic Virus, HIV, etc.)

2. Molecular Structure of DNA

DNA is composed of three chemical components:

1. **Pentose Sugar:** Deoxyribose sugar in DNA (lacks an oxygen atom at Carbon 2' position; –H instead of –OH) and Ribose sugar in RNA.
2. **Nitrogenous Bases:** Divided into two chemical classes:
 - **Purines (2-ring structure):** Adenine (A) and Guanine (G).
 - **Pyrimidines (1-ring structure):** Cytosine (C), Thymine (T) [in DNA], and Uracil (U) [in RNA].
3. **Phosphoric Acid (H_3PO_4):** Provides acidic nature and negative charge to the nucleic acid structure.

Base Pairing Rules:

- In DNA: Purines (A, G) pair with Pyrimidines (C, T).
- In RNA: Uracil (U) replaces Thymine (T) to pair with Adenine (A).

3. Nucleosides and Nucleotides

- **Nucleoside:** Pentose Sugar + Nitrogenous Base (Linked via *N-glycosidic bond* at Carbon 1').
- **Nucleotide:** Nucleoside + Phosphoric Acid (Linked via *Phosphoester bond* at Carbon 5').

Nomenclature of Nucleosides and Nucleotides

Base	Nucleoside (DNA)	Nucleotide (DNA)	Nucleoside (RNA)	Nucleotide (RNA)
Adenine (A)	Deoxyadenosine	Deoxyadenylic acid	Adenosine	Adenylic acid
Guanine (G)	Deoxyguanosine	Deoxyguanylic acid	Guanosine	Guanylic acid
Cytosine (C)	Deoxycytidine	Deoxycytidylic acid	Cytidine	Cytidylic acid
Thymine (T)	Deoxythymidine	Deoxythymidylic acid	-	-
Uracil (U)	-	-	Uridine	Uridylic acid

4. Polynucleotide Chain Architecture

Multiple nucleotides polymerize through **3'-5' phosphodiester linkages** to form a continuous polynucleotide chain. One end has a free phosphate group at 5'-carbon (5' end), while the other has a free -OH group at 3'-carbon (3' end).

5. Double Helix Model of DNA

Proposed by **James Watson & Francis Crick (1953)** based on X-ray diffraction data from Rosalind Franklin & Maurice Wilkins.

- **Double Stranded:** Composed of two polynucleotide chains with *antiparallel polarity* ($5' \rightarrow 3'$ and $3' \rightarrow 5'$).
- **Helical Coiling:** Right-handed helix. Pitch of helix is **3.4 nm** (34 Å), containing ~10 base pairs per turn.
- **Base Distance:** Distance between consecutive base pairs is **0.34 nm** (3.4 Å).
- **Diameter:** Constant helix diameter of **2.0 nm** (20 Å).
- **Complementary Hydrogen Bonding:** A pairs with T via 2 Hydrogen bonds ($A = T$); G pairs with C via 3 Hydrogen bonds ($G \equiv C$).

Chargaff's Rule (Erwin Chargaff, 1950): In double-stranded DNA, the ratio of Adenine to Thymine and Guanine to Cytosine is equal and constant: $[A] = [T]$, $[G] = [C]$, and $(A + T) / (G + C) = 1$.

6. Central Dogma & Packaging of DNA

Central Dogma: Proposed by Francis Crick (1958). Outlines the flow of genetic information:

DNA → RNA → Protein (Replication → Transcription → Translation).

Reverse Transcription (Teminism): Temin & Baltimore (1970) discovered that retroviruses transcribe RNA back into DNA using **Reverse Transcriptase**.

Packaging of DNA in Eukaryotes & Prokaryotes

Human diploid cell contains 6.6×10^9 bp, spanning approximately **2.2 meters** in total length, packed into a ~10 µm nucleus.

- **Prokaryotes:** Lacks defined nucleus. Negatively charged DNA is held in large loops with positively charged non-histone proteins in a region called **Nucleoid**.
- **Eukaryotes:** Wrapped around positively charged basic proteins called **Histones** (rich in Lysine and Arginine residues).
- **Nucleosome Structure:** 8 histone subunits form a *Histone Octamer* (2 each of H2A, H2B, H3, H4). Negatively charged DNA (~200 bp) wraps around the octamer 1.75 times.
- **Chromatin:** "Beads-on-string" structure connected by Linker DNA and H1 histone.
 - **Euchromatin:** Loosely packed, light-staining, transcriptionally active.
 - **Heterochromatin:** Densely packed, dark-staining, transcriptionally inactive.

7. Search for Genetic Material

1. **Griffith's Transformation Experiment (1928):** Used *Streptococcus pneumoniae* S-strain (virulent, smooth) and R-strain (non-virulent, rough) in mice. Demonstrated that heat-killed S-strain transformed live R-strain into virulent S-strain.
2. **Avery, MacLeod & McCarty (1934-44):** Proved biochemically that **DNA** is the transforming agent (DNase enzyme destroyed transforming activity, while Proteases/RNases did not).

Hershey-Chase Blending Experiment (1952)

Alfred Hershey and Martha Chase conclusively proved DNA is the genetic material using Bacteriophages infected into *E. coli*.

- Phages grown on **Radioactive Phosphorus (^{32}P)** labeled DNA.
- Phages grown on **Radioactive Sulfur (^{35}S)** labeled Protein coat.
- **Results:** After infection, blending, and centrifugation, radioactive ^{32}P was recovered inside bacterial cells (pellet), while radioactive ^{35}S remained in the supernatant (ghost coats).

Criteria for Genetic Material (DNA vs RNA)

Property	DNA	RNA
Replication	Capable of self-replication	Capable of self-replication
Chemical Stability	High (lacks 2'-OH, presence of Thymine)	Low (2'-OH makes it reactive & labile)
Mutation Rate	Slow, controlled mutation	Fast mutation rate (rapid viral evolution)
Role	Storage of genetic information	Transmission & protein synthesis

8. DNA Replication

DNA replicates via a **Semi-Conservative Mechanism** (Watson & Crick). Proven experimentally by **Meselson & Stahl (1958)** using heavy isotope ^{15}N and normal ^{14}N in *E. coli* with CsCl density gradient centrifugation.

- **Helicase:** Unwinds double helix by breaking Hydrogen bonds.
- **SSB Proteins:** Prevent single strands from re-annealing.
- **Topoisomerase:** Relieves torsional tension ahead of replication fork.
- **DNA Polymerase:** Synthesizes new strand strictly in 5'→3' direction.
 - *Leading Strand:* Continuous synthesis (3'→5' template).
 - *Lagging Strand:* Discontinuous synthesis producing **Okazaki Fragments**, joined by **DNA Ligase**.

9. Transcription & Types of RNA

Transcription is the process of copying genetic code from one template strand of DNA into RNA catalyzed by RNA Polymerase.

Transcription Unit Components

1. **Promoter:** Binding site for RNA polymerase at 5' end of coding strand.
2. **Structural Gene:** Region coding for RNA/protein (Monocistronic in Eukaryotes, Polycistronic in Prokaryotes).
3. **Terminator:** Defines end of transcription at 3' end of coding strand.

Major Types of RNA

RNA Type	Abundance	Primary Function
mRNA (Messenger)	3–5%	Carries genetic template code from DNA to ribosome.
tRNA (Transfer / Soluble)	10–15%	Acts as adapter molecule reading code and picking amino acids.
rRNA (Ribosomal)	80%	Structural & catalytic role inside ribosomes (e.g., 23S rRNA peptidyl transferase).

10. Genetic Code

Deciphered by George Gamow, Marshall Nirenberg, Har Gobind Khorana, and Severo Ochoa.

- **Triplet Nature:** 3 nitrogen bases form 1 codon ($4^3 = 64$ total codons).
- **Code Assignments:** 61 codons code for 20 amino acids; 3 are **Stop Codons** (UAA, UAG, UGA).
- **Start Codon:** AUG codes for Methionine and serves as initiator codon.
- **Degeneracy:** Single amino acid can be coded by multiple codons.
- **Unambiguous & Universal:** A specific codon always codes for same amino acid across all organisms.

11. Mechanism of Translation

Translation is the process of synthesizing a polypeptide chain from mRNA sequence.

1. **Activation & Charging of tRNA:** Amino acid + ATP → Aminoacyl-AMP. Binds to 3'-CCA end of tRNA (charging/aminoacylation).
2. **Initiation:** Small ribosomal subunit (30S/40S) binds mRNA at AUG. Initiator tRNA carrying Methionine occupies P-site.
3. **Elongation:** Successive aminoacyl-tRNAs enter A-site. Peptidyl transferase forms peptide bonds. Ribosome translocates in 5'→3' direction.
4. **Termination:** Release factor binds stop codon at A-site, releasing completed polypeptide chain.

12. Regulation of Gene Expression (Lac Operon)

Proposed by **François Jacob and Jacques Monod (1961)** in *E. coli* for lactose metabolism.

Components of Lac Operon:

- **i-gene (Inhibitor):** Codes for Repressor protein.
- **Promoter (P):** Binding site for RNA polymerase.
- **Operator (O):** Switch site where repressor binds.
- **Structural Genes:**
 - **z gene:** Codes for β-galactosidase (breaks lactose into glucose + galactose).
 - **y gene:** Codes for Permease (increases membrane permeability to lactose).
 - **a gene:** Codes for Transacetylase.

Operon Mechanism: In the absence of Inducer (Lactose), Repressor binds operator and turns operon *OFF*. When Lactose is present, it binds repressor, preventing operator binding and turning operon *ON*.