Chapter 6

Molecular Basis of Inheritance

INTRODUCTION

Mendel initially detected and analyzed factors or genes, which were subsequently studied by numerous scientists, tracking their transmission patterns across generations. While these investigations greatly enhanced our understanding of inheritance in living organisms, they did not provide any insights into the structure or molecular composition of these "factors."

In 1926, efforts to unravel the mechanisms behind genetic inheritance delved into the molecular realm, leading to the exploration of the putative genetic material. This journey ultimately revealed that deoxyribonucleic acid (DNA) serves as the genetic material for the majority of organisms, dictating the inheritance of traits and orchestrating their expression across generations.

Living systems universally possess two types of nucleic acids: DNA and ribonucleic acid (RNA). DNA functions as the genetic material in all organisms except certain viruses, while RNA serves as the genetic material in reoviruses and plays various roles in others, such as carrying genetic information, acting as an adapter for amino acids, and serving as a structural or catalytic molecule in certain contexts.

This chapter will delve into the structure of DNA, its replication process, transcription (the synthesis of RNA from DNA), the genetic code governing the sequence of amino acids in proteins, the translation process (protein synthesis), and the fundamental principles underlying their regulation. Additionally, the essentials of human genome sequencing and its implications will be explored in the concluding section.

The DNA (Genetic Material)

The genetic material is the substance responsible for controlling the transfer of traits from one generation to the next and for influencing the development and functionality of these traits.

Properties of Genetic Material

In order to qualify as genetic material, a molecule must meet several criteria:

(i) It must carry hereditary information in a coded form.

(ii) The genetic material should possess the ability to replicate itself accurately and pass on this replicated material to subsequent generations.

- DNA Fingerprinting

(iii) It should have the capacity for variation, including mutations and recombination's, with these variations being stable and inheritable.
(iv) The genetic material should have the capability to produce both copies of itself and new types of molecules.
(v) It must be capable of expressing its effects in the form of Mendelian characters.
These essential characteristics are all present in DNA, leading to its recognition as the primary genetic material.

Structure of Polynucleotide Chain and DNA (Introduction)

In 1953, J.D. Watson and F.H.C. Crick proposed the double helical structure of DNA, drawing upon the findings of M.H.F. Wilkins and colleagues. Their collective research led to the awarding of the Nobel Prize in 1962 for this groundbreaking work.

The double helical structure of DNA exhibits several distinctive features:

1. Each nucleotide comprises a sugar, phosphate, and a nitrogenous base. These nucleotides are connected by phosphodiester bonds to form a polynucleotide chain or strand.
2. Phosphodiester bonds are established between the 5' carbon of the sugar of one nucleotide and the 3' carbon of the sugar of the subsequent nucleotide.
3. The nitrogenous base is affixed to the 1' carbon of the sugar. Purine bases are attached at their 9' position, while pyrimidines are attached at their 3' position.
4. The polynucleotide strand is structured with a backbone composed of sugar and phosphate, constituting its long axis, while the bases are positioned at right angles to this axis.

   In 1950, Chargaff conducted significant observations regarding the composition of DNA, which later became known as Chargaff's rule.

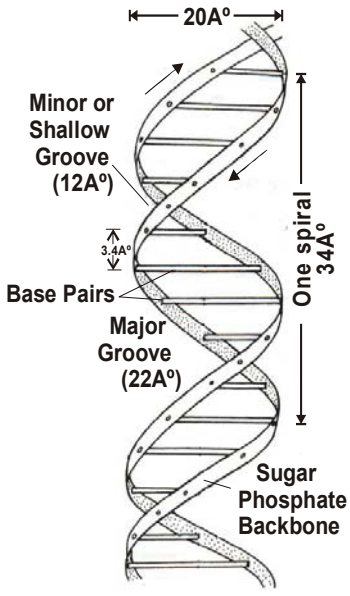


Fig. Structure of DNA- coiling in double helix of DNA

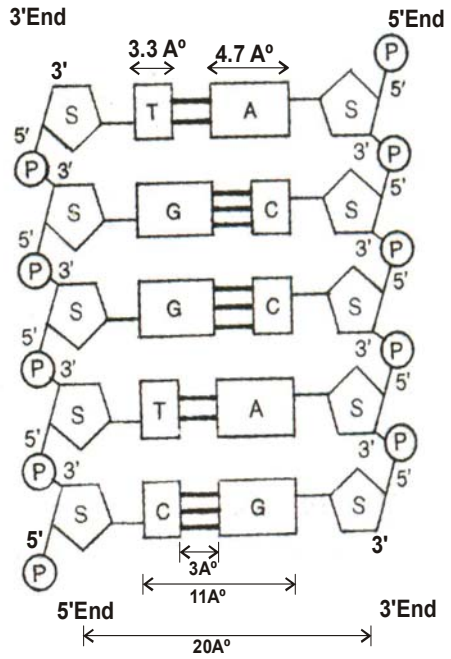


Fig. Arrangement of various Components of DNA

(i.) Purine and pyrimidine base pairs were found to occur in equal amounts, meaning that the sum of adenine and guanine equals the sum of thymine and cytosine.

(ii.) The molar quantity of purine adenine always matches the molar quantity of pyrimidine thymine, similarly, guanine matches cytosine.

(iii.) The sugar deoxyribose and phosphate are present in equimolar proportions.
(iv.) The ratio of A + T to G + C remains constant within a species.

5. According to Chargaff's rule, the base ratio AT is approximately equal to unity, and the GC ratio is also close to unity, indicating that adenine pairs with thymine and guanine pairs with cytosine. This establishes A-T and G-C as complementary base pairs.
6. Thus, if one DNA strand contains A, its complementary strand would contain T, and if it contains G, the complementary strand would contain C. Consequently, if one strand's base sequence is CAT TAG GAC, the other strand's sequence would be GTA ATC CTG. Therefore, these two polynucleotide strands are termed complementary to each other.
7. These complementary strands are joined by hydrogen bonds between their complementary nitrogenous bases, with three hydrogen bonds between cytosine and guanine and two hydrogen bonds between adenine and thymine.
8. The two polynucleotide chains are helically coiled around the same axis, forming a right-handed helix called B-DNA. They are antiparallel, meaning they run in opposite directions in relation to their sugar molecules, with their 5' to 3' and 3' to 5' phosphodiester linkages pointing in opposite directions.
9. A double-stranded DNA molecule has a diameter of approximately 20 angstroms (20Aº), with one complete turn of the helix occurring every 34 angstroms. There are 10 nucleotides per turn of the helix, resulting in a distance of 3.4 angstroms between neighboring base pairs.
10. Since the discovery of the DNA structure, various other forms of DNA have been identified, classified based on the number of base pairs per turn of the helix and the distance of base pairs along the helical axis. These include A-DNA, C-DNA (sometimes referred to as D and E), and Z-DNA. These different forms exhibit both similarities and differences, which are further elaborated upon

S.No.		B	Z	A	C	D
1	Handedness of helix	Right-handed	Left-handed	Right h an de d	Right-handed	Right-handed
2	Pitch of helix per turn	34 Å	46 Å	25 Å	30 Å	24 Å
3	Diameter of helix	20 Å	18 Å (thinnes t)	23 Å (widest)	19 Å	–
4	Stability	Stable and physiologi cally active form	Unstable	Unstable	Unstable	Unstable
5	Base pairs per turn of helix	10	12 (6 dimers)	11	9.33	8
6	Distance between two base pairs	3.4Å	3.8Å	2.5Å	3.3Å	3.03Å
7	Repeating unit	Mononucl eotide	Dinucl eotide	Mononu cl eotide	Mononu cleotide	Mononu cl eoide

Packaging of DNA Helix

The average separation between each adjacent base pair measures approximately 0.34 nanometers (or 3.4 angstroms). For a human diploid cell, with a DNA length of 6.6 x 10^{-9} base pairs, this results in a total length of DNA extending to about 2.2 meters. This length vastly surpasses the spatial confines of a typical nucleus, which is approximately 1 micrometer in size.

In contrast, Escherichia coli, with a genome comprising 4.6 x 10^6 base pairs, yields a total DNA length of 1.36 millimeters. Despite its relatively compact size, the extensive DNA of E. coli, which stretches about 11.1 meters if laid out end to end, is accommodated within the limited confines of its cell primarily through processes of packing or compaction.

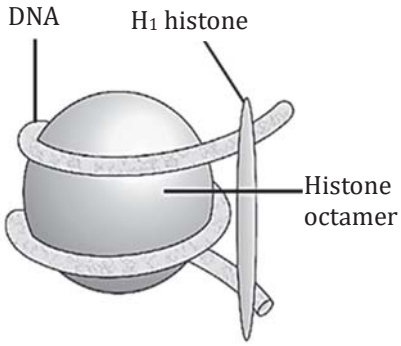
The acidic nature of DNA arises from the presence of a significant number of phosphate groups. Compaction mechanisms involve the folding and attachment of DNA with basic proteins such as polyamines in prokaryotes and histones in eukaryotes. These interactions facilitate the condensation of DNA, allowing for efficient storage within cellular compartments.

DNA packaging in Prokaryotes

Within the cytoplasm, DNA exists in a tightly wound configuration known as a supercoiled state. This coiling is upheld by non-histone basic proteins, such as polyamines. This condensed arrangement of DNA is referred to as the nucleoid or genophore, denoting its compact and organized structure within the cellular environment.

DNA packaging in Eukaryotes

In eukaryotic cells, the organization of genetic material is significantly more intricate compared to prokaryotes. This complexity is orchestrated by a group of proteins known as histones, which possess a high content of positively charged amino acids such as lysine's and arginine's, characterized by their charged side chains. These histones come in five distinct types: H_1, H_2A, H_2B, H_3, and H_4. Typically, four of these histone proteins are present in pairs, resulting in the formation of a histone octamer, also referred to as a nucleosome. This octamer consists of two copies each of H_2A, H_2B, H_3, and H_4. The negatively charged DNA molecule intricately winds around the positively charged histone octamer, creating a compact structure termed a nucleosome.



Core of histone molecules

Fig.: Nucleo Some

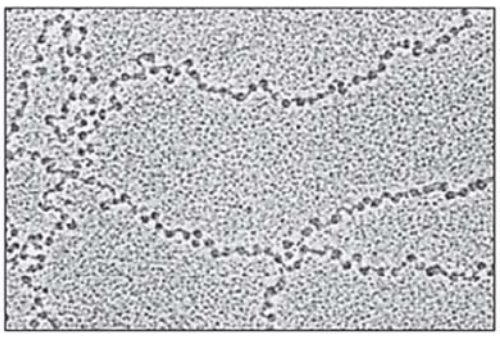


Fig.: EM picture- "Beads–On–String"

Their positively charged terminals extend outward. The negatively charged DNA strand coils around the positively charged histone octamer, forming a structural unit known as a nucleosome. Typically, a nucleosome encompasses 200 base pairs (bp) of the DNA helix. The DNA situated between two adjacent nucleosomes is termed linker DNA, which binds to the H_1 histone protein. The length of linker DNA can vary across different species. Under an electron microscope, a chain of nucleosomes exhibits a "beads on a string" appearance. These nucleosomes further coil to create a structure called a solenoid, which typically has a diameter of 30 nanometers (nm) in chromatin. The "beads on a string" configuration within chromatin is then compacted into chromatin fibers, which are subsequently coiled and condensed during the metaphase stage of cell division to form chromosomes. This higher-level packaging necessitates an additional group of proteins, referred to as non-histone chromosomal (NHC) proteins, which are predominantly acidic in nature.

Non-Histone chromosomal proteins are of three types:

(i) Structural NHC protein

(ii) Functional NHC protein e.g., DNA polymerase, RNA polymerase

(iii) Regulatory NHC protein

Chromatin within a typical nucleus is distinguished into two distinct regions based on its staining behavior:

1. Heterochromatin
   - This area appears darkly stained.
   - Chromatin within heterochromatin is densely packed.
   - It exhibits transcriptional inactivity.
2. Euchromatin
   - Euchromatin is characterized by a lighter staining pattern.
   - Chromatin in this region is loosely packed.
   - It is transcriptionally active.

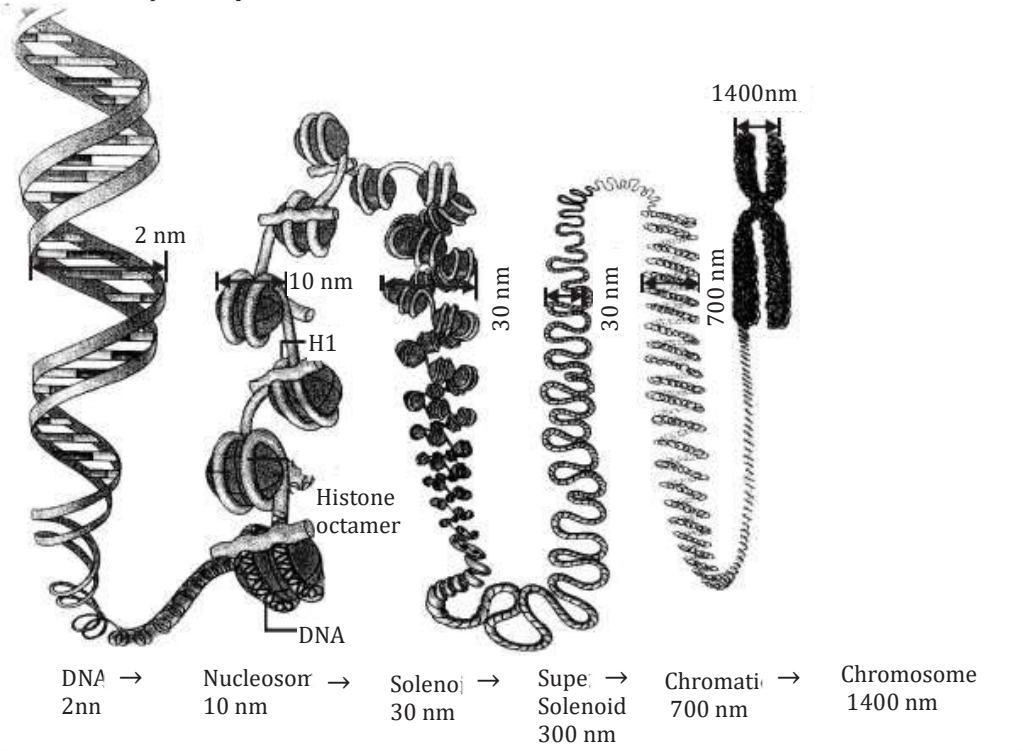


Fig.: Various steps in the folding and super folding of basic chromatin components to generate an eukaryotic chromosome

Within a standard nucleus, certain sections of chromatin exhibit a loose packing arrangement, resulting in a lighter staining appearance, and are labeled as euchromatin. Conversely, chromatin that appears denser and stains darker is termed heterochromatin. Notably, euchromatin is associated with transcriptional activity, whereas heterochromatin is characterized by transcriptional inactivity.

Example: Let's consider a DNA molecule comprising 2000 base pairs. We need to determine:

(a) The quantity of sugar and phosphate molecules.

(b) The count of N-glycosidic linkages.

Solution:

(a) We start by calculating the total number of nucleotides, which is equal to twice the number of base pairs, given that each base pair consists of one nucleotide each for sugar and phosphate. Hence, the total number of nucleotides = 4000. Each nucleotide comprises a nitrogen base, a sugar molecule, and a phosphate molecule.
Therefore, the total count of sugar molecules = 4000, and the total count of phosphate molecules = 4000.

(b) N-glycosidic linkage occurs between the nitrogen base and the sugar molecule. Since each nucleotide contains one N-glycosidic linkage, the total count is 4000.

Example: Calculate the quantity of beaded structures, known as nucleosomes, within the nucleus of a diploid eukaryotic cell containing 2.4×10^6 base pairs.

Solution: To determine the number of nucleosomes, we first ascertain that each nucleosome comprises 200 base pairs.

Given that the total number of base pairs in the nucleus is 2.4×10^6, we divide this value by 200 to find the number of nucleosomes:

$$\frac{(2.4 \times 10^6)}{200} = 1.2 \times 10^4 \text{ or } 12 \times 10^3 \text{ nucleosomes.}$$

Example: Among the given options, identify the bond that is not linked with a deoxyribonucleotide.

(1) Phosphoester bond
(2) Glycosidic bond
(3) Phosphodiester bond
(4) More than one option is correct

Solution: (3) Phosphodiester bond

The presence of a Phosphodiester bond is guaranteed in a deoxyribonucleotide molecule as it connects the sugar and the phosphate group. On the other hand, the formation of a phosphodiester bond occurs between two consecutive deoxyribonucleotides.

Example: Select the accurate sequence depicting the organization of eukaryotic chromosomes:

(1) Nucleosome → Solenoid → Super solenoid
(2) Solenoid → Nucleosome → Chromatid
(3) DNA → Solenoid → Nucleosome
(4) Chromatin → Solenoid → Nucleosome

Solution: (1) Nucleosome → Solenoid → Super solenoid

10nn → 30nn → 300nn