BIO- ZOOLOGY

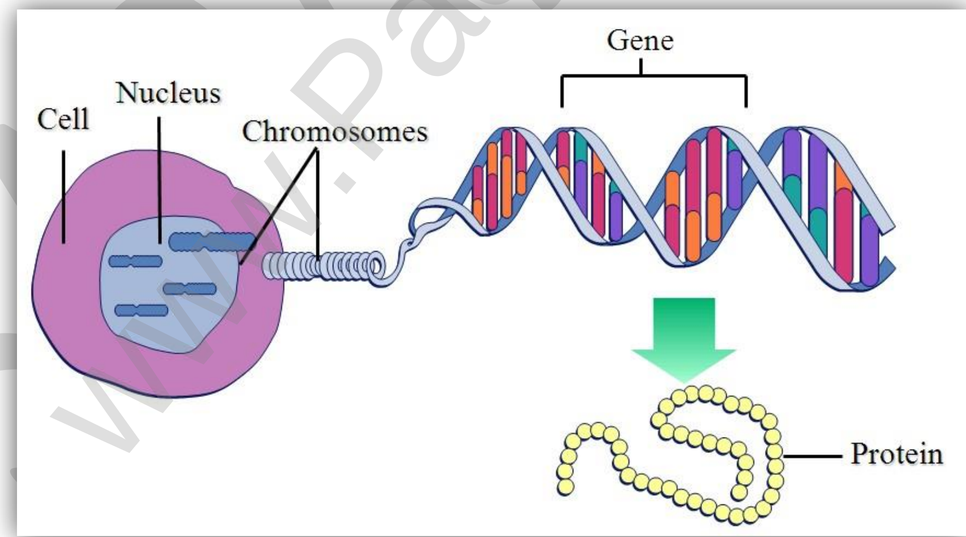
CHAPTER-5 MOLECULAR GENETICS

Gene as the functional unit of inheritance

- A gene is a basic physical and functional unit of heredity.
- The concept of the gene was first explained by **Gregor Mendel in 1860's. He never used the term 'gene'. He called it 'factor'.**
- In 1909, the Danish biologist **Wilhelm Johannsen**, coined the term '**gene**'. Classical concept of gene introduced by **Sutton in 1902**.

Properties of Genes

- Number of genes in each organism is more than the number of chromosomes; hence several genes are located on the same chromosome.
- The genes are arranged in a single linear order like beads on a string.
- Each gene occupies a specific position called locus.
- Genes may exist in several alternate forms called alleles.
- Genes may undergo sudden change in positions and composition called mutations.
- Genes are capable of self-duplication producing their own copies



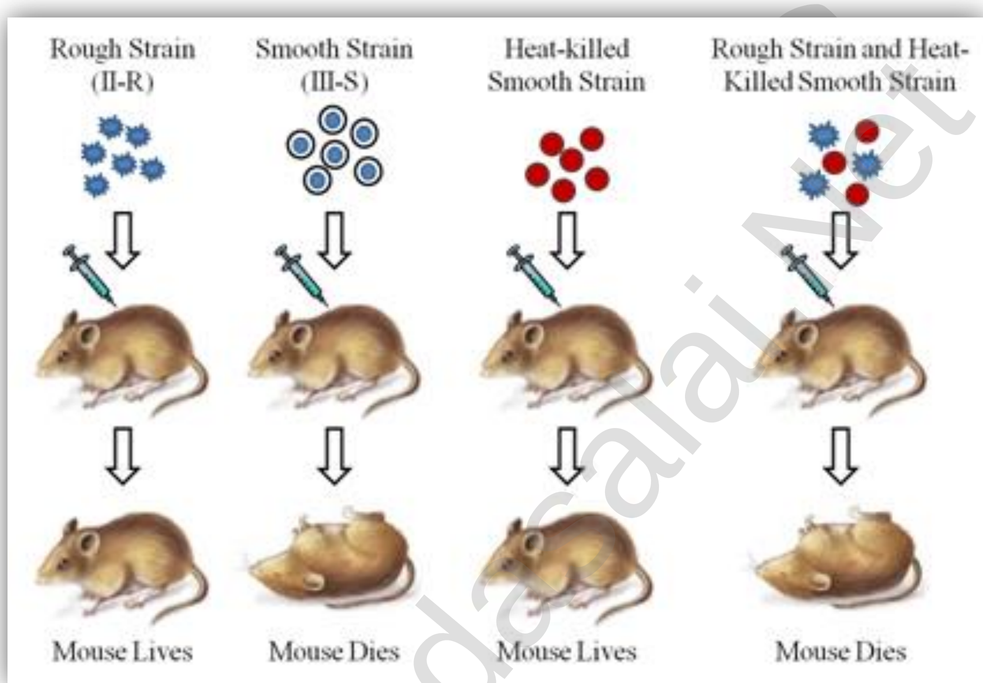
In search of the genetic material

- **Wilhelm Hofmeister**, a German botanist, had observed that cell nuclei organize themselves into small, rod like bodies during mitosis called chromosomes.
- **Friedrich Miescher**, a Swiss physician, isolated a substance from the cell

nuclei and called it as nuclein.

- It was renamed as nucleic acid by **Altman** (1889), and is now known as DNA.
- chromosomes are made up of proteins and DNA.
- 1928 **Griffith's experiments** (bacterial transformation) proved that DNA is the Genetic material - cause of transformation and bio chemical nature of genetic material was not defined.

Griffith's experiment



- ✓ He used 2 strains of ***Streptococcus pneumonia***:
  - **Type S** (smooth) strain(virulent - capsulated)
  - **Type R** (rough) strain (avirulent –non capsulated)

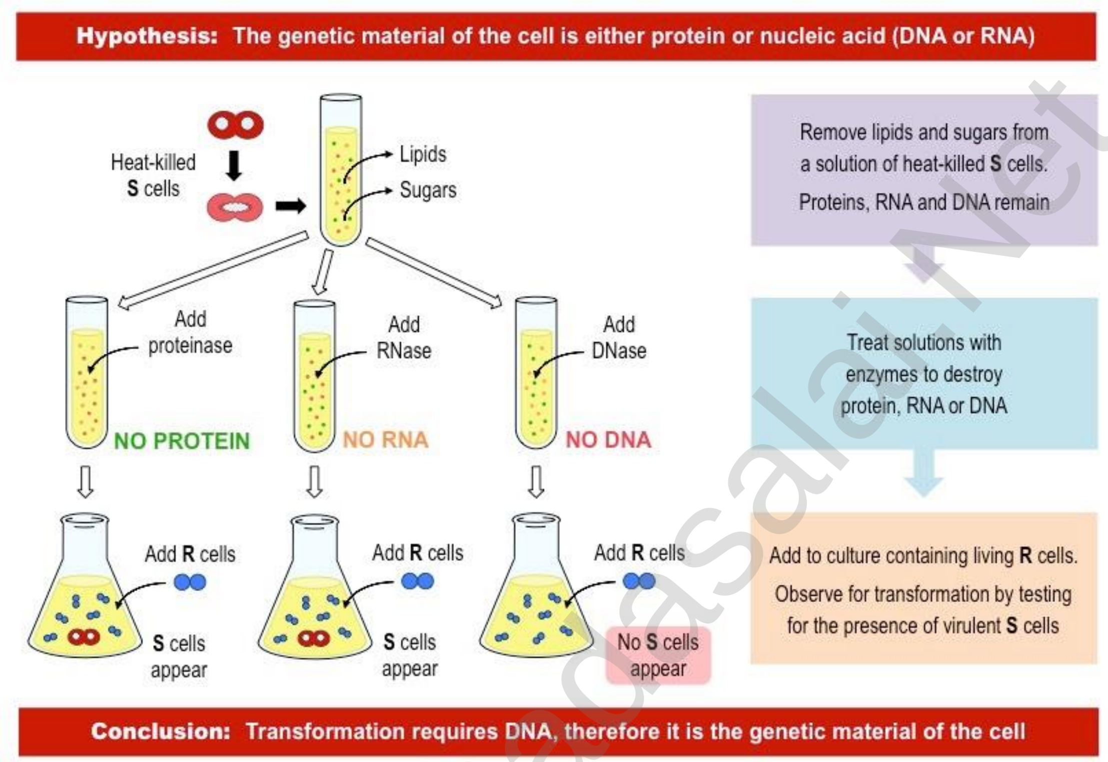
1. **S- strain ⟶ inject into mice ⟶ mice dies**
2. **R- strain ⟶ inject into mice ⟶ mice lives**
3. **S- strain (heat killed) ⟶ inject into mice ⟶ mice lives**
4. **S- strain(heat killed) + R- strain ⟶ inject into mice ⟶ mice dies**

Conclusion:

Based on the observation, Griffith concluded that R strain bacteria had been transformed by S strain bacteria. The R strain inherited some 'transforming principle' from the heat-killed S strain bacteria which made them virulent. And he assumed this transforming principle as genetic material.

- Later, **Oswald Avery, Colin Macleod and Maclyn McCarty** in 1944 repeated Griffith's experiments in an 'in vitro' system in order to identify the nature of the transforming substance responsible for converting a non-virulent strain into virulent strain.

- They observed that the DNA, RNA and proteins isolated from the heat-killed S-strain when added to R-strain *changed their surface character from rough to smooth* and also made them pathogenic
- But when the extract was treated with DNase (an enzyme which destroys DNA) the transforming ability was lost.
- RNase (an enzyme which destroys RNA) and proteases (an enzyme which destroys protein) did not affect the transformation.

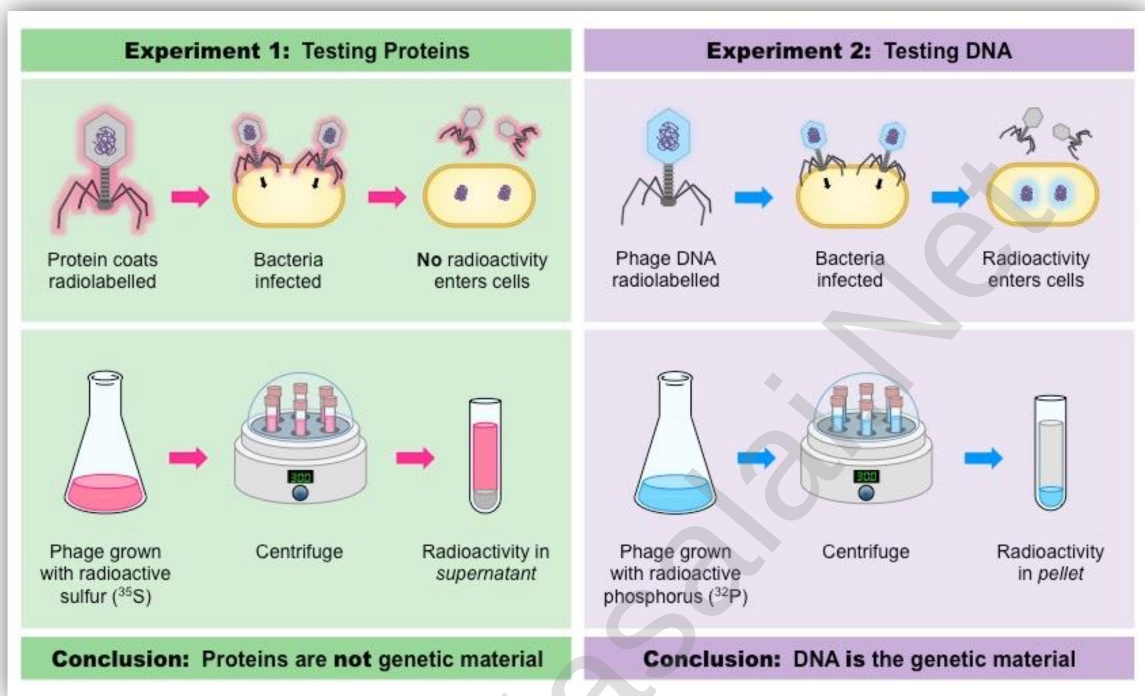


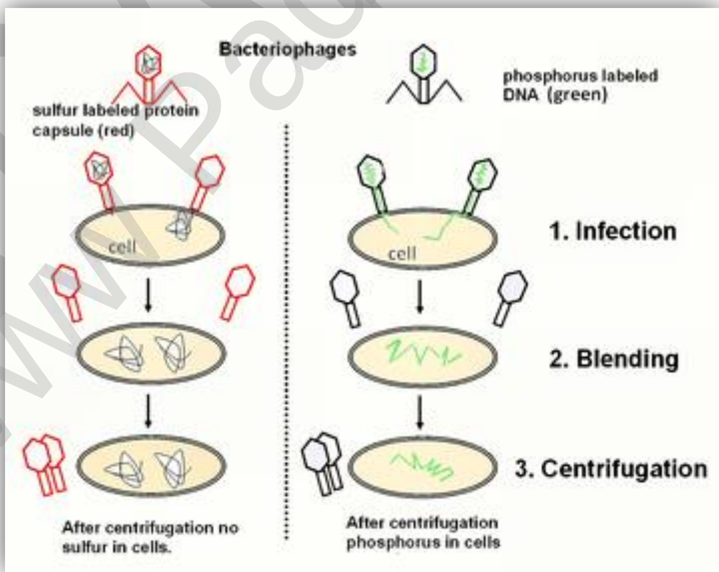
- Digestion with DNase inhibited transformation suggesting that the DNA caused the transformation.
- These experiments suggested that **DNA** and not proteins is the **genetic material**.

The phenomenon, by which DNA isolated from one type of cell (S – strain), when introduced into another type (R-strain), is able to retain some of the properties of the S - strain is referred to as transformation.

DNA is the genetic material

Hershey–Chase experiment





- In 1952, Alfred Hershey and Martha Chase conducted a series of experiments to prove that DNA was the genetic material
- Viruses (T2 bacteriophage) were grown in one of two isotopic mediums in order to radioactively label a specific viral component
- Viruses grown in radioactive sulfur (^{35}S) had radiolabelled proteins (sulfur is present in proteins but not DNA)

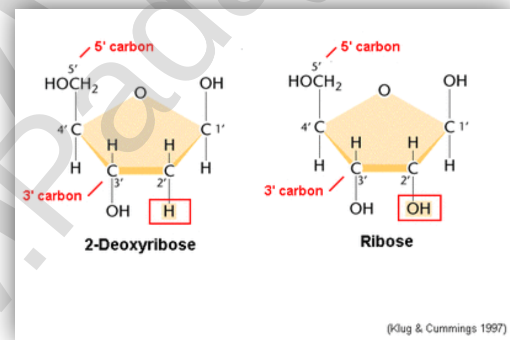
- Viruses grown in radioactive phosphorus (^{32}P) had radiolabeled DNA (phosphorus is present in DNA but not proteins)
- The viruses were then allowed to infect a bacterium (E. coli) and then the virus and bacteria were separated via centrifugation
- The larger bacteria formed a solid pellet while the smaller viruses remained in the supernatant.
- The bacterial pellet was found to be radioactive when infected by the ^{32}P–viruses (DNA) but not the ^{35}S–viruses (protein).
- This demonstrated that DNA, not protein, was the genetic material because DNA was transferred to the bacteria.

Chemistry of Nucleic Acids

- Each nucleotide subunit is composed of three parts:
  - ✓ a nitrogenous base,
  - ✓ a five carbon sugar (pentose) and
  - ✓ a phosphate group.

Pentose sugar:

- The sugars found in nucleic acids are pentose sugars; a pentose sugar has five carbon atoms.
- There are two types of nucleic acids depending on the type of pentose sugar.
- Those containing deoxyribose sugar are called **Deoxyribo Nucleic Acid** (DNA)
- Those with ribose sugar are known as **Ribonucleic Acid** (RNA).

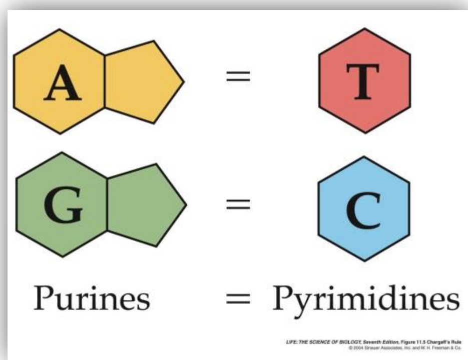


- The only difference between these two sugars is that there is one oxygen atom less in deoxyribose.

Nitrogenous bases:

- These bases are nitrogen containing molecules having the chemical properties that accepts H^+ ion or proton in solution.
- DNA and RNA both have four bases (two purines and two pyrimidines) in their nucleotide chain.
- Two of the bases, Adenine (A) and Guanine (G) have double carbon‑nitrogen ring structures and are called purines.
- The bases, Thymine (T), Cytosine (C) and Uracil (U) have single ring structure and these are called pyrimidines.

- Thymine is unique for DNA, while Uracil is unique for RNA.

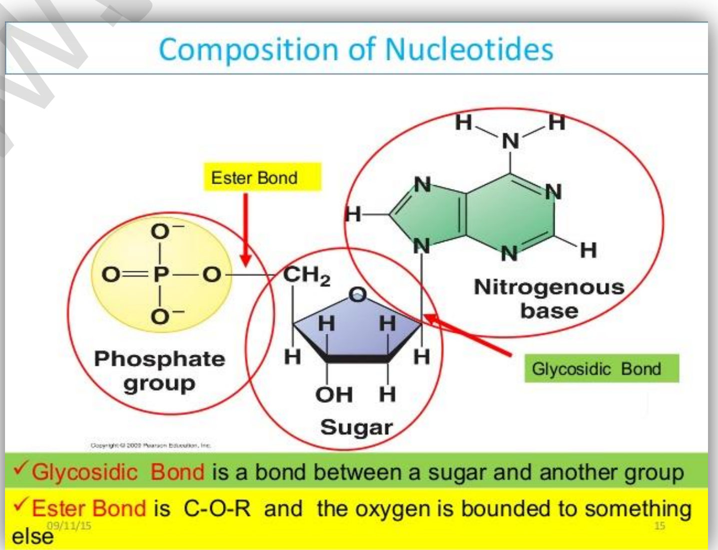


<u>The phosphate functional group</u>:

- It is derived from phosphoric acid (H3PO4), has three active OH- groups of which two are involved in strand formation.
- The phosphate functional group (PO4) gives DNA and RNA the property of an acid (a substance that releases an H+ ion or proton in solution) at physiological pH, hence the name **nucleic acid**.
- The bonds that are formed from phosphates are esters.
- The oxygen atom of the phosphate group is negatively charged after the formation of the <u>phosphodiester bonds</u>.
- This negatively charged phosphate ensures the retention of nucleic acid within the cell or nuclear membrane.

<u>Nucleoside and nucleotide :</u>

- In a nucleoside, *the nitrogenous base is bound to either ribose or deoxyribose* via a <u>glycosidic bond</u> at carbon 1' position.



- A single nucleotide is made up of three components:
  - a nitrogenous base,

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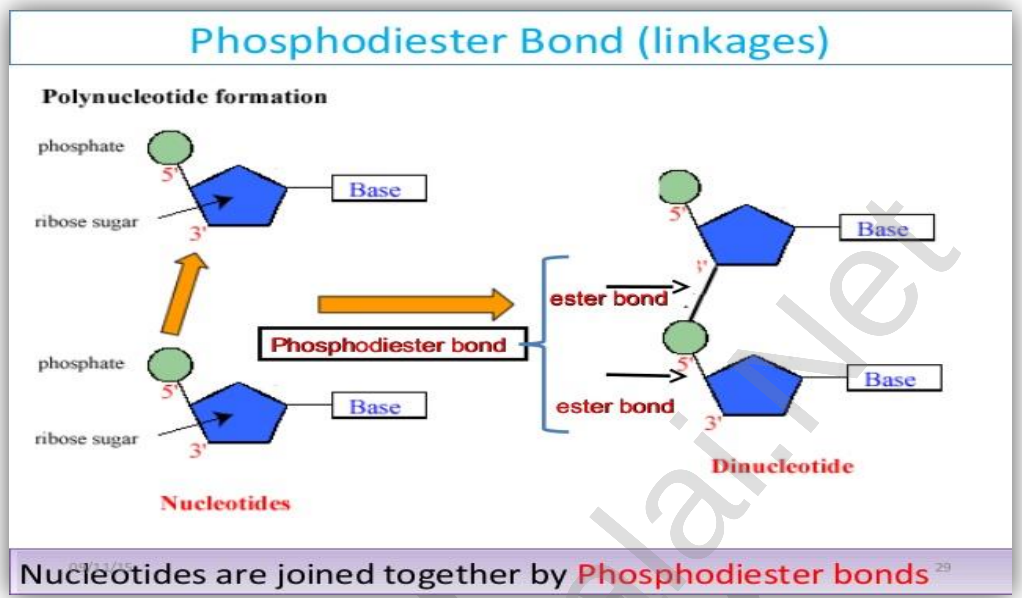
- a five-carbon sugar (pentose),
- one phosphate group with all three joined.

- The hydroxyl group on the 3' carbon of a sugar of one nucleotide forms a phosphodiester bond with the phosphate of another nucleotide.



Chargaff's rule:

Erwin Chargaff proposed that

- Adenine pairs with Thymine (A = T) with two hydrogen bonds
- Guanine pairs with Cytosine (G ≡ C) with three hydrogen bonds.
- The ratios between Adenine with Thymine and Guanine with Cytosine are constant and equal.

DNA structure:

- Based on the X - ray diffraction analysis of **Maurice Wilkins** and **Rosalind Franklin**, the
double helix model for DNA was proposed by **James Watson** and **Francis Crick in 1953**.
- The highlight was the base pairing between the two strands of the polynucleotide chain.
- This proposition was based on the observations of Erwin Chargaff.

RNA world

- **Fraenkel-Conrat and Singer** (1957) first demonstrated that RNA is the genetic material in TMV (Tobacco Mosaic Virus); they also separated RNA from the protein of TMV.
- **Leslie Orgel, Francis Brick and Carl Woese** independently proposed the 'RNA world' as the first stage in the evolution of life.

- The term 'RNA world' first used by **Walter Gilbert** in 1986:
- RNA as the first genetic material on earth.
- RNA has the ability to act as both genetic material(viruses) and catalyst (as ribozyme).
- RNA being a catalyst was reactive and hence unstable.
- This led to evolution of a more stable form of DNA, with certain chemical modifications.
- Some RNA molecules function as gene regulators by binding to DNA and affect gene expression.
- Andrew Fire and Craig Mellow were of the opinion that RNA is an active ingredient in the chemistry of life.

Properties of genetic material:

A molecule that can act as a genetic material should have the following properties:

Property	DNA	RNA
Stability	• Complementary double strands, if seperated can coil back • Chemically more stable. • Less reactive • Presence of thymine confers additional stability.	• Highly reactive in nature – presence of 2' –OH group makes RNA liable and easily degradable. • RNA is catalytic.
Information storage	• Stable and stores genetic information. • Depends on RNA for protein synthesis.	• Directly codes for genetic information. • Transfers genetic information.
Self replication	• Undergoes duplication.	• Undergoes duplication.
Variation through mutation	• Undergoes mutation.	• Mutates faster- being unstable.

- The above properties indicates that both RNA and DNA can function as a genetic material. DNA is more stable, and is preferred for storage of genetic information.

Genophore:

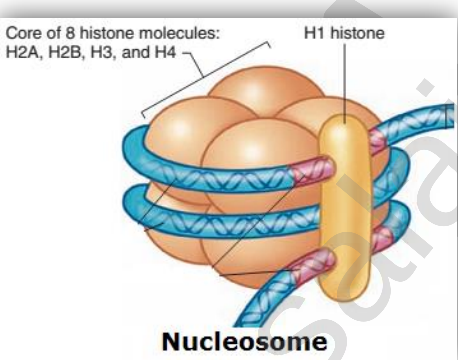
- In prokaryotes such as E. coli though they do not have defined nucleus, the DNA is not scattered throughout the cell.
- DNA (being negatively charged) is held with some proteins (that have positive charges) in a

region called the nucleoid.

- The DNA as a nucleoid is organized into large loops held by protein. DNA of prokaryotes is almost circular and lacks chromatin organization, hence termed **genophore**.

Nucleosomes:

- In eukaryotes, this organization is much more complex.
- Kornberg proposed a model for the nucleosome, in which 2 molecules of the four histone proteins H2A, H2B, H3 and H4 are organized to form a unit of eight molecules called histone **octamere**.
- The negatively charged DNA is wrapped around the positively charged histone octamere to form a structure called **nucleosome**.



- A typical nucleosome contains 200 bp of DNA helix.
- Neighbouring nucleosomes are connected by linker DNA (H1) that is exposed to enzymes.
- The DNA makes two complete turns around the histone octameres which are sealed off by an H1 molecule.
- Chromatin lacking H1 has a beads-on-a-string appearance in which DNA enters and leaves the nucleosomes at random places.
- H1 of one nucleosome can interact with H1 of the neighbouring nucleosomes resulting in the further folding of the fibre.
- The chromatin fiber in interphase - mitotic chromosomes have a diameter that vary between 200-300 nm and represents inactive chromatin.
- 30 nm fibre arises from the folding of nucleosome, chains into a solenoid structure having six nucleosomes per turn.(stabilized by interaction between different H1 molecules)
- DNA is a **solenoid** and packed about 40 folds.
- Additional set of proteins are required for packing of chromatin at higher
- level and are referred to as **non-histone chromosomal proteins (NHC).**
- In a typical nucleus, some regions of chromatin are loosely packed (lightly stained) and are referred to as **euchromatin**. Euchromatin is transcriptionally active
- The chromatin that is tightly packed (stained darkly) is called **heterochromatin**. Heterochromatin is transcriptionally inactive.