Chromatin

- **Genome**
  - **The total genetic material of the cell.**
  - **Amount varies species to species.**
  - **In general, Eukaryotes has much more DNA than Prokaryotes.**
  - **The number of expressed genes in eukaryotes is about 2-10 times more than that prokaryotes.**

- **Junk DNA**
- **Chromosome**
  - **Highly condensed form**
  - **long threadlike structure.**
- **Prokaryotes have their genome in a single chromosome. Eukaryotes have many Chromosomes.**

➢ Structure of Chromosome

- Total genome of an *E.coli* consist of about 1100um long circular DNA and cell diameter is 1-2um.

- DNA is present in a highly coil and condensed form.

- Nucleoid

- The bacterial nucleoid is the folded form of the genome.

- Structure can be maintained in the absence of ionic detergents and in presence of high concentration of cations such as polyamines or 1.0 M Salt.

➢ Eukaryotic Chromosome

- The chromosomes in eukaryotes are compact structure.

- The structure of chromosomes is maintained in a nucleoprotein form where the DNA is present in a complexed form with a number of proteins.

- Different chromosomes of a cell have different size and shape. E.g. 46 chromosomes in human, 44 homologues pairs and two are sex chromosomes and Heterologous.

- Largest of the human chromosomes has about 85mm of DNA.

- Size is only 0.5umx10um.

- This mean that there is about 10^4 fold condensation of DNA.

➢ Eukaryotic Chromosome

- This complex nature of chromosomes represent a number of possibilities.

i. **The entire DNA present in a chromosome may consist of a number of small DNA molecules. These molecules may be running parallel to each other thus making a compact structure. This type of arrangement will be known as multineme or multistrand.**

ii. **There is only one large of DNA present which is starting from one end and continues to other end of the entire genome which is present as a long stretch. This will be the mononeme model of DNA arrangement.**

iii. **The DNA may be multineme, however, the multiple DNA molecules present in the chromosomes may be joined either from end to end or may be present in any other molecular arrangement.**

➢ Eukaryotic Chromosome

iv. There is a mononeme DNA which is present in a highly coiled form.

In fact, total DNA of a chromosome is present in form of giant molecule of DNA that extends from one end of chromosome through centrosome all way to other end of chromosome. It is present in a highly coiled configuration. Thus the possibility number of iv is the actual scenario within cell.

➢ Lamp brush chromosomes

- **A special chromosome structure is present during prophase I of oogenesis in amphibians and many other eukaryotic species.**
- **Chromosome is about 800um long**
- **These have a central axis region of highly condensed chromatid with many lateral loops of DNA arranged in a paired manner. The loop represent the DNA which is transcriptionally active.**
- **The integrity of central axis and loop is DNA dependent and Dnase treatment fragment it .Rnase and Proteases remove surrounding matrix but do not destroy the continuity of the structure.**
- **The loops have a filament of 20A^{o} which is same as diameter of dsDNA. Loops are unineme.**

➢ The chromatin structure

- The coiling of DNA in the chromosome is in a form of precise and highly organized structure.

- Chromatin is made of complex of DNA with a number of chromosomal proteins and other chromosomal constituents isolated from nuclei. These may contain a small amount of RNA also.

➢ The proteins of chromatin

a. The basic proteins: These proteins have a net positive charge at neutral pH. Present in all animal and plant cells, these basic proteins are referred as the histones. Histones are present in almost all eukaryotic cells and only a few eukaryotic cells which include the sperms of certain species are known exceptions where histones are not present. The histones may have upto 20-30% Arg and Lys. These are present in almost equal ratio with the DNA.

➢ The proteins of chromatin

- **The qualitative analysis of histones on acrylamide gels have revealed five different classes of proteins in this fraction. H1, H2a, H2b, H3 and H4 histones.**
- **These are present in molar ratio of 1:2:2:2:2.**
- **The histones play an important role in maintaining the structure of chromatin.**
- **The histones molecules get complexed with DNA and help it in getting packaged into chromatin.**
- **The acidic proteins: The acidic proteins are heterogeneous in nature, varying in composition and structure from cell to cell and are commonly known as the non-histone proteins.**

➢ The organization of chromatin

- **The electron microscopic studies of chromatin has revealed that it is made up of ellipsoidal beads of 110x60A^{o} which are joined by a thin thread at regular interval.**
- **The digestion of chromatin with Dnase has revealed that a region of 146bp of DNA remains nuclease resistant. Further analysis revealed that upon partial digestion under sub optimal condition, the integral multiplies of smallest fragment were obtained .**
- **This suggest that these is a repeated structure of nuclease resistant form present in chromatin. This structure is referred as the nucleosome.**
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➢ The organization of chromatin

- **The nucleosome are basic units of chromatin structure.**
- **These nucleosomes are organized in bead like structure. A number of nucleosome are joined together by nuclease sensitive interbred thread or the linker DNA.**
- **The nuclease treatment and other studies have shown that about 200bp of DNA in each nucleosome is protected by partial digestion with nuclease.**
- **However, on extended digestion, only about 146bp DNA fragment is protected. This DNA fragment is wound around a histone octamer made of 2molecules each of H2a, H2b, H3 and H4. The DNA is wound as around the histone core.**

➢ The organization of chromatin

- **The complete chromatin subunit is made of many repeats of the structure consisted of the nucleosome core associated with a linker DNA, one molecule of H1 histone and some non histone proteins.**
- **The length of linker DNA varies from 8 to 114 bp between different organisms. Its length varies from cell to cell within the same individual also. Similarly the H1 molecule may not be evenly distributed in chromatin. Their ratio vis a vis the DNA vary at different loci of the chromatin.**
- **H1 histones helps in stabilizing the complete nucleosome which has two complete turns of supercoil DNA on histone octamer. The length of DNA in two turns is 166 bp. The H1 histone may involved in high degree of organization of chromatin which is about 300A^{o} chromatin fiber.**

- **The second degree of organization of chromatin: the 300A^{o} fibres.**

- The electron microscopy of isolated metaphase chromosomes revealed that nucleosome are further organized in a highly coiled, lumpy fibres.

- Average diameter of about 300A^{o}.

- **Third degree of chromatin organization**

- **The maximum degree of condensation of chromatin is observed during metaphase. The function of these structure is probably to package the giant DNA molecules in form which can be segregated into daughter nuclei. This condensation of DNA take place with the help of non histones proteins. The histones doe not participate in its formation. This result in formation of scaffold structure.**

➢ Euchromatin and Heterochromatin

- **Chromosomes were stained using Fuelgen reaction, two different types of chromatin structures can be seen.**
- **A dense structure which receives very intense staining was made of very tightly packed 300Aº fibers. Another region which receives relatively weak staining is made up of less tightly packed DNA.**
- **The deeply dense region is referred as heterochromatin. It has been found that this region is genetically inactive and remains highly condensed throughout the entire life of cell .Its structure does not vary during different stages of cell cycle. This region is not transcribed and greatly enriched in highly repetitive tandemely arranged DNA sequences.**

➢ Euchromatin and Heterochromatin

- **The lightly stained region is referred as euchromatin. This is not visible during the interphase under light microscope. Majority of known genes are present within this region and the region is actively transcribed.**
- **They paly an important role in regulation of gene expression.**

➢ The Satellite DNA

- **Eukaryotic DNA is banded, multiple bands are seen. The predominant band represents the majority of the DNA.**
- **Besides, one to several small bands are also obtained. The bands which represent distinct but minor species of DNA are referred as the satellite DNA.**
- **These satellite DNAs represent the species of DNA which is basically composed of the repeats of short sequence of small length.**
- **In higher eukaryotes, the repetitive DNA may constitute a substantial portion of the genome, upto 20-50% of total DNA.**
- **Some of the small sequences may be repeated upto 10^6 times.**

➢ The Satellite DNA

- **In Drosophila virilis, 3 bands of satellite DNA are present. These bands represent the multiple repeats of following three sequences.**
- **1. A C A A A C T**
- **2. A T A A A C T**
- **3. A C A A A T T**

- **Similar repeats are present in crab species which have 97% A:T rich region.**
- **In higher eukaryotes, the length of repeats may much larger.**
- **These satellite DNA are present within heterochromatin region.**
- **Selfish sequences**

➢ The Satellite DNA

- **Many of these sequences act as a transposable element which can integrates at various regions of genome.**
- **The DNA of Alu family and L1 element family of primates represent such sequences.**
- **Alu family are repeat of 300bp.**
- **Present in high number upto 500, 000 – 1000,000 copies, in human genome represent upto 5% of total DNA.**

➢ Types of Chromosomes

- **The four types of Chromosomes in animal cells are classified by the position of the centromere.**

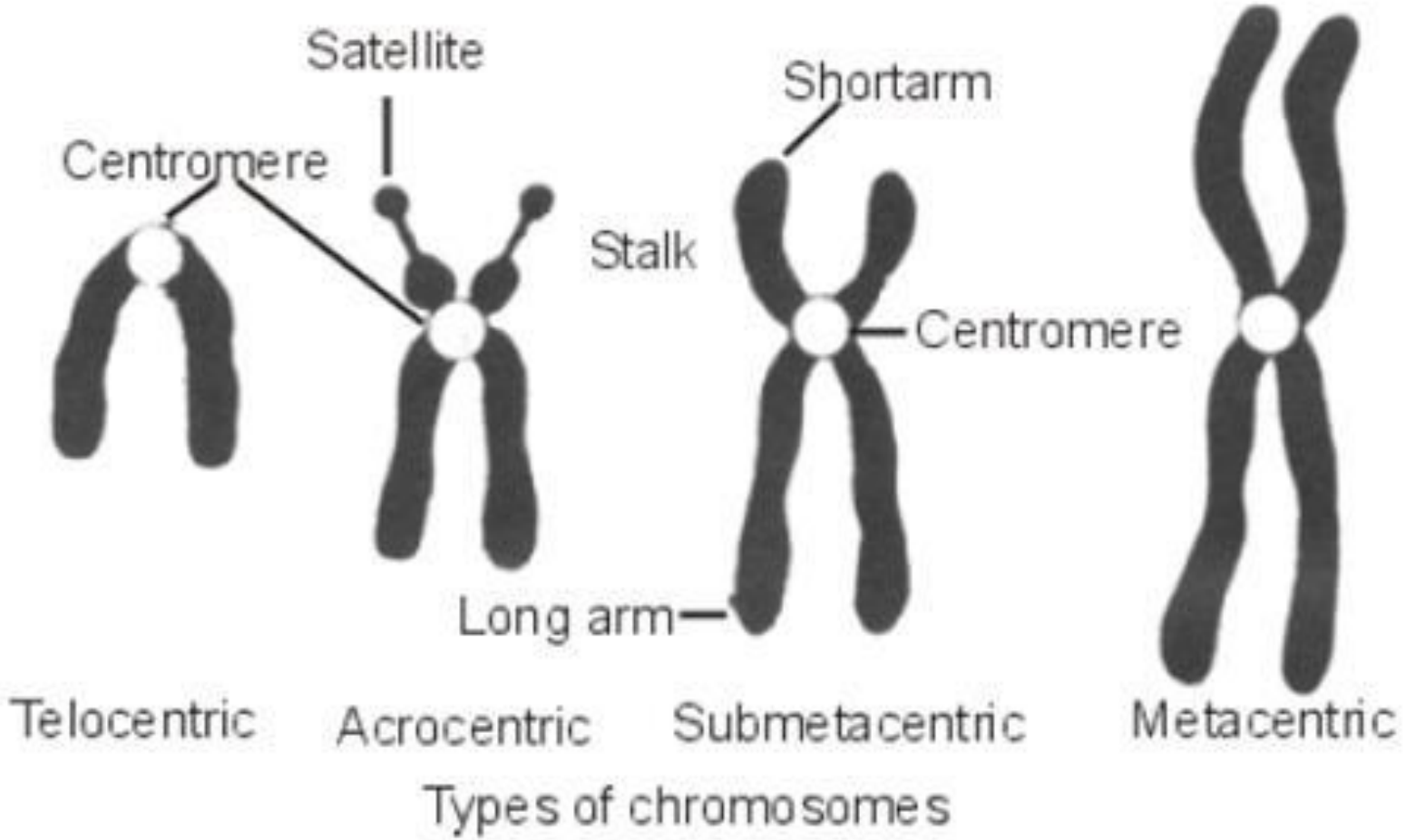
- **Metacentric Chromosomes**
- **Metacentric chromosomes have the centromere in the center. Such that both sections are of equal length. Human chromosome 1 and 3.**

- **Submetacentric Chromosomes**
- **Centromere slightly offset from the center leading to a slight asymmetry in the length of the two sections. Human Chromosomes 4 through 12;**

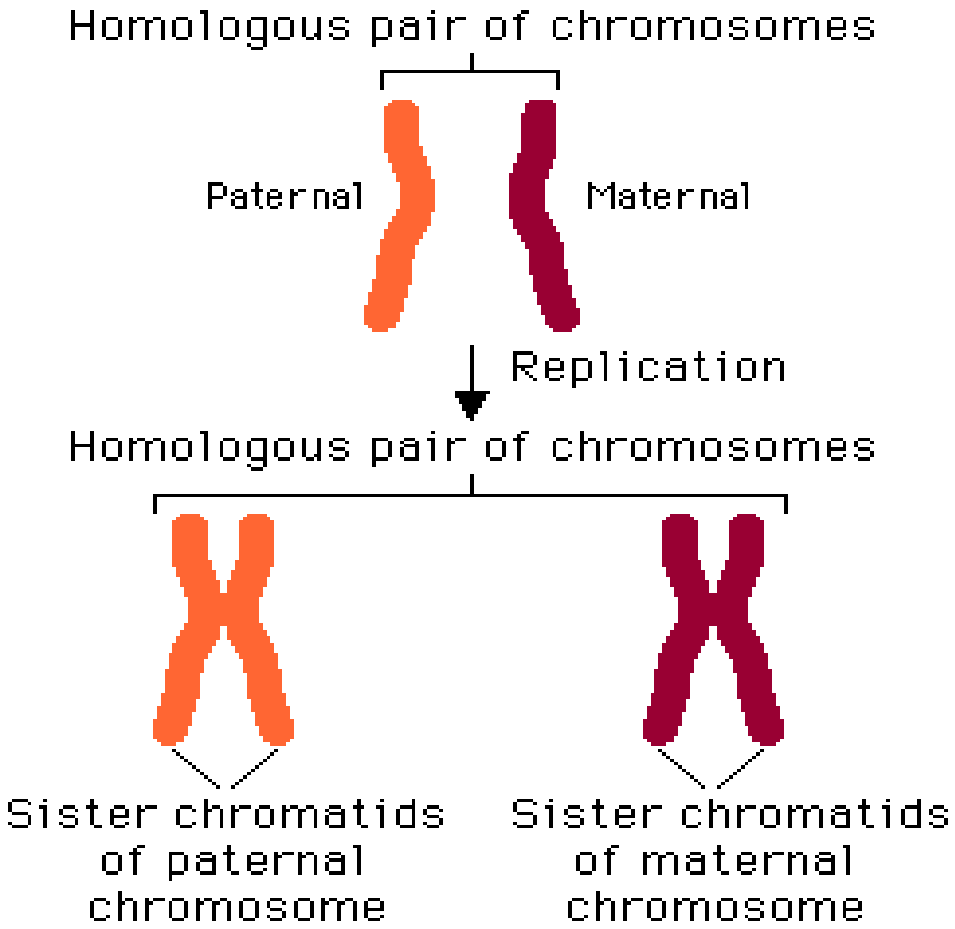
➢ Acrocentric Chromosomes

- **Centromere which is severely offset from the center leading to one very long and one very short section. Human chromosomes 13, 15, 21 and 22.**

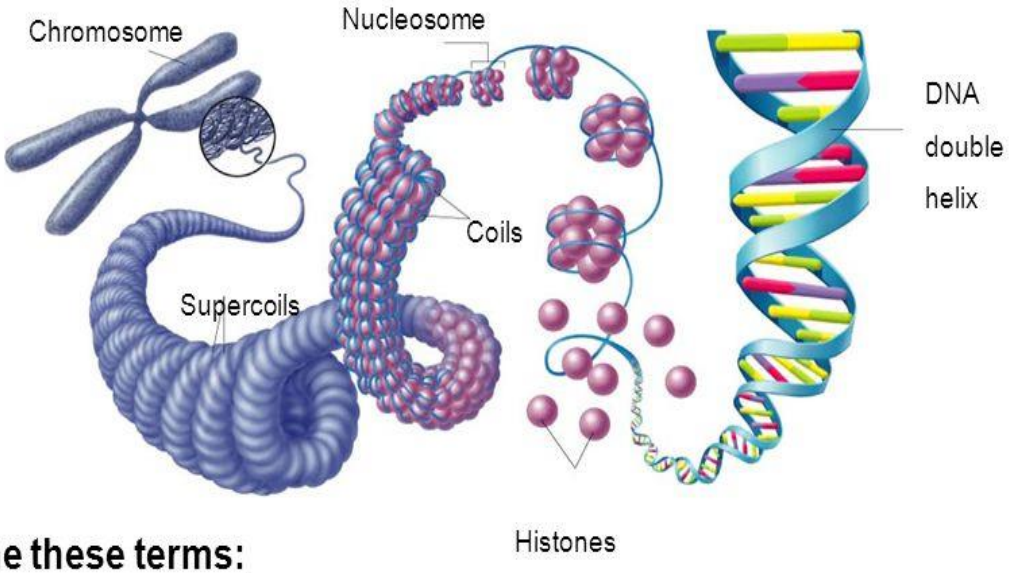
- **Telocentric Chromosomes**
- **Centromere at the very end of the chromosome. Human do not possess telocentric chromosomes but they are found in other species such as mice.**



Types of chromosomes


Homologous pair of chromosomes
Paternal
Maternal
Replication
Homologous pair of chromosomes
Sister chromatids of paternal chromosome
Sister chromatids of maternal chromosome

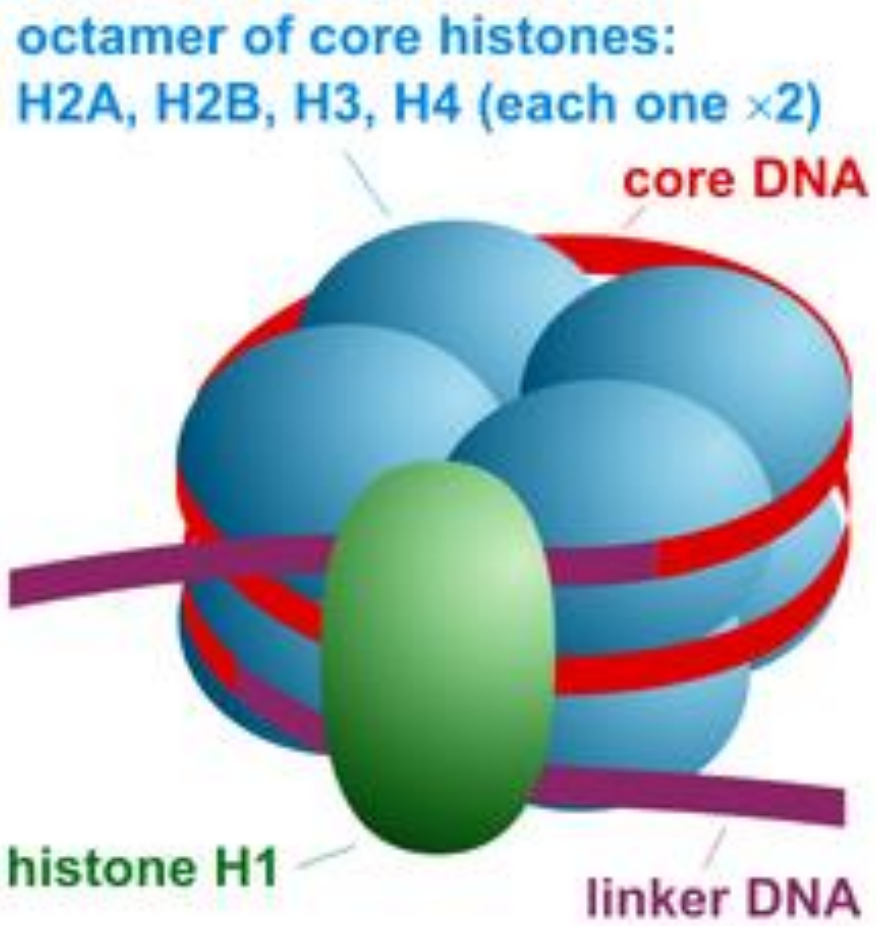
Organizational Level of Genetics

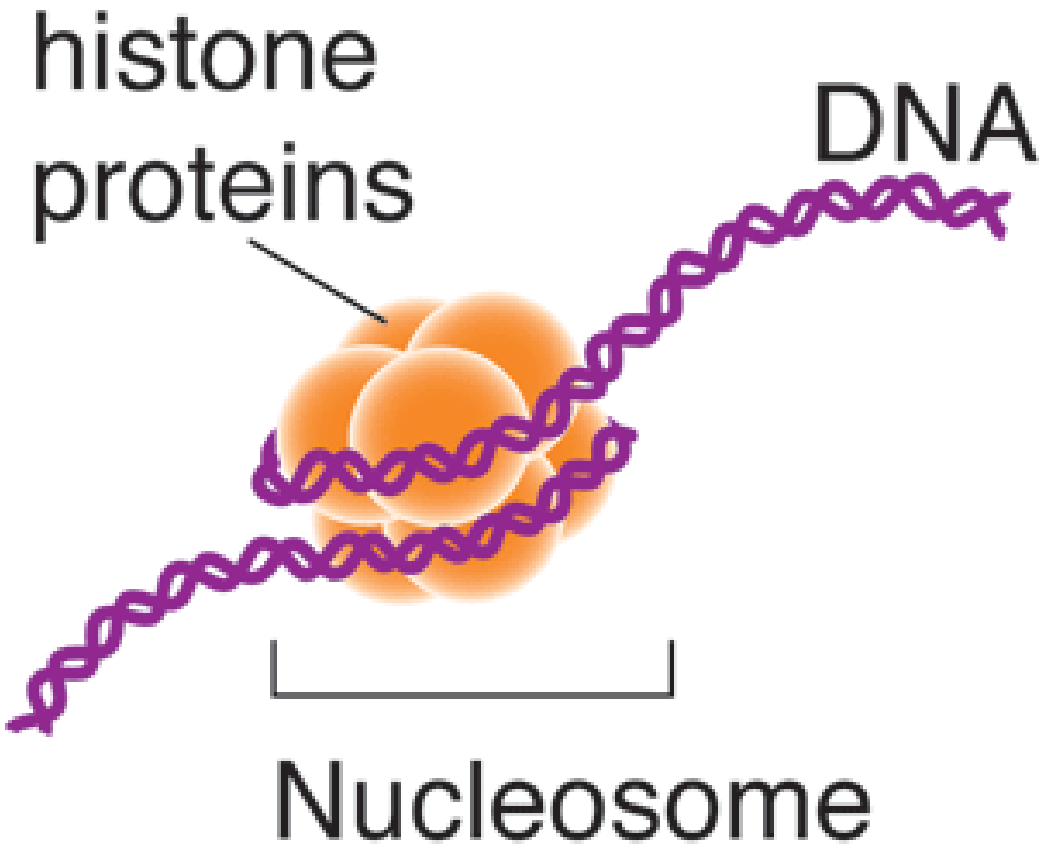


Define these terms:

Histones-

Nucleosome-


octamer of core histones:
H2A, H2B, H3, H4 (each one ×2)
core DNA
histone H1
linker DNA


histone
proteins
DNA
Nucleosome

Chromatin structure

- **Chromatin**= DNA+ Protein
- Basic unit of chromatin is nucleosome
- Chromatin structure is dynamic