## SEM- IV

## MBIO CC 408. MICROBIAL GENETICS

# UNIT 1. Genome Organization

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# Genome

- It is the **sum total of all genetic material** of an organism.
- The nature of genome may be DNA ( in all eukaryotes and prokaryotes; some viruses) or RNA (viruses).
- **C-value** is the amount of DNA present in genome of a species.
- **C-value paradox-** DNA content in lower eukaryotes like yeast is related to the complexity of organisms i.e., with increasing complexity, DNA content increases. But in higher eukaryotes there is lack of correlation between organism's complexity and genome size known as C-value paradox.
- For eg. human are genetically more complex than amphibians but cells of later contains 30 times more DNA than human cells.

| Organism | Genome size (Mb) | Estimated genes | % single copy |
|---|---|---|---|
| *Escherishia coli* | 4.6 | 4,405 | 92 -99 |
| *Saccharomyces cerevisiae* (Yeast) | 12 .1 | 6,200 | 90 |
| *Drosophila melanogaster* (Fruit fly) | 180 | 13,600 | 60 |
| *Homo Sapiens* (Human) | 3,300 | 30,000 | 64 |
| *Arabidopsis thaliana* | 125 | 25,500 | 80 |

# Types of genome sequences

| Types | | Nature | Examples/Location |
|---|---|---|---|
| I. **Non repetitive DNA** | | Unique sequences present in only one copy of haploid genome | In Prokaryotes **(all DNA non -repetitive)**<br>In lower eukaryotes (mostly non repetitive)<br>In higher eukaryotes (upto half DNA is non repetitive) |
| **II. Repetitive DNA** | | DNA sequence present in more than one copy in a haploid genome | |
| | **II a. Moderately repetitive DNA** | Relatively short sequences repeated 10-1000 times in genome | Sequence for r_RNA, t RNA, Transosable elements |
| | **II b. Highly repetitive DNA** | Very short sequences (less than100bp) repeated many times tandemly in large clusters (more than 100kb to several Mb)in genome.<br>those repetitive DNA that renatures more rapidly | |
| | **Satellite DNA** | Repeat size 5-171bp | At Centromere |
| | **Minisatellite DNA** | Repeat size 10-100 bp | At or close Telomere |
| | **Microsatellite DNA** | Repeat size 2-13 bp | dispersed throughout chromosome |

# Genome organization in Prokaryotes: An overview

- Each bacterial chromosome is made by a single circular DNA molecule (rarely linear) without basic proteins.
- *Borrelia burgdorferi* B31, for example, has a linear chromosome of 911 kb, carrying 853 genes.
- The genetic material can be seen as a fairly compact clump (or series of clumps) that occupies about a third of the volume of the cell called **NUCLEOID**.
- The DNA of these loops is not found in the extended form of a free duplex, but instead is compacted by association with proteins.
- The nucleoid has 400 independent **negatively supercoiled domains**. The average density of supercoiling is 1 turn/100 bp.
- Each domain consists of a loop of DNA, the ends of which are secured in some (unknown) way that does not allow rotational events to propagate from one domain to another.
- They may also have additional genes DNA molecules called **plasmids**, coding for properties such as antibiotic resistance, or the ability to utilize complex compounds such as toluene as a carbon source.

# *Escherichia coli* Genome

- Consists of single main chromosome and plasmids.
- Main chromosome is **circular ds DNA**.
- 88% genome encodes proteins or RNA, 115 involved in gene regulatory function and less than 1% consists of repetitive DNA sequences.
- The contour length of the circular DNA present in the chromosome of the *Escherichia coli* is about 1500µm (1.5mm). However, diameter an *E. coli* cell is only 1 to 2 µm.
- This large DNA molecule present in each bacterium must exist in a highly condensed (folded or supercoiled) configuration.
- **The folded genome-** in this condensed state, the single large chromosome of *E. coli* is folded into 50 to 100 independently **negatively supercoiled domains or loops**. This state is comparable in size to the nucleoid in vivo and is the functional state of a bacterial chromosome.
- These domains are further held together in **a scaffold of RNA and protein**, and the entire nucleoid is attached to cell membrane.
- Attachment of chromosome to the cell membrane helps in its segregation after DNA replication during cell division. Bacteria lack the histone proteins that are found bound to DNA and that form a nucleosomes of eukaryotic chromosomes.

- Many sets of genes on the *E. coli* chromosome are organized into operons. An operon is a set of functionally related genes that are controlled by a single promoter and that are all transcribed at the same time.
- It is also quite common for bacterial species to possess extrachromosomal genetic elements called plasmids. These are small, circular DNA molecules which, when present, vary in number from one to about thirty identical copies per cell. Plasmids include the fertility factor, as well as plasmids that carry drug-resistance genes.

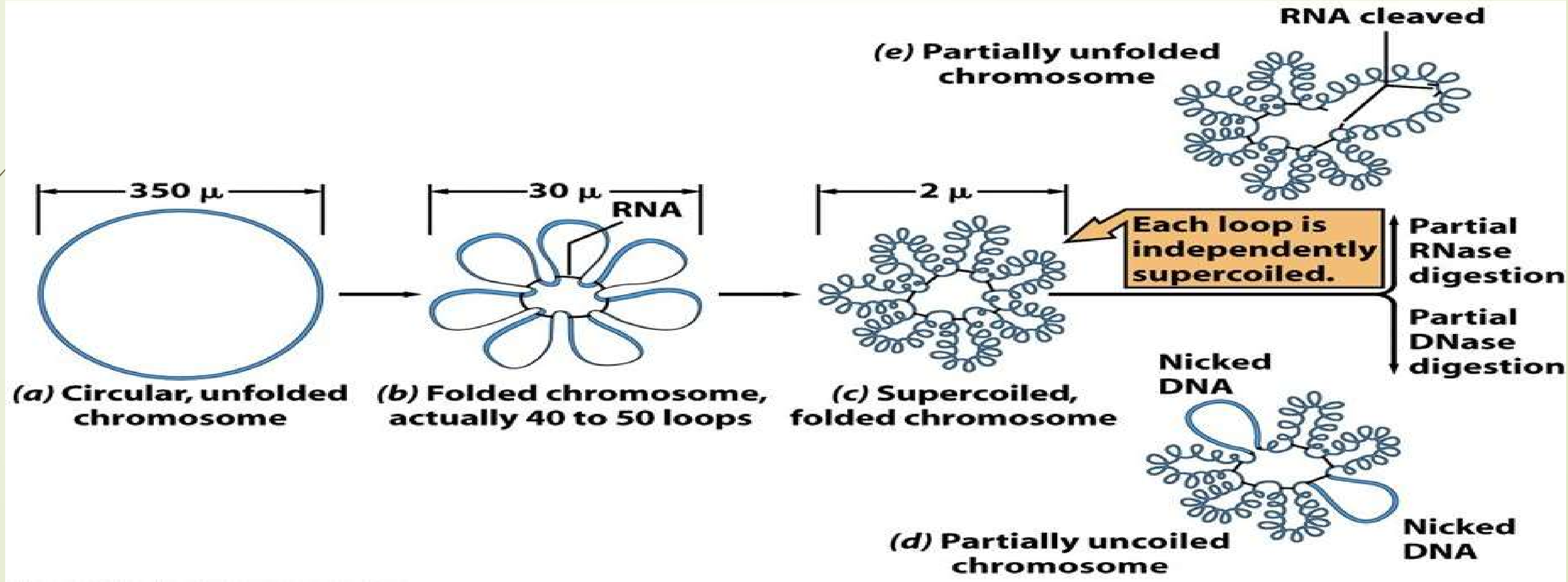



**Fig. Structure of functional state of *E.coli* chromosome**

# Genome organization in Eukaryotes

- Each eukaryotic chromosome is made by linear ds DNA molecule.
- Eukaryotes have from 2 – 15 times as many genes as *E. coli*.
- This many times the amount of DNA is packaged in several chromosomes and each chromosome is present in 2 (diploid) or more (polyploidy) copies.
- Contour length of *E. coli* chromosome is about 1.5 mm, while haploid chromosome complement ( genome) of human contains about 1000 mm DNA (i.e.2000 mm in diploid per diploid cell).
- This 1000 mm DNA is divided among 23 chromosomes of variable size and shape, each chromosome containing from15-85 mm of DNA.
- How this 85 mm (i.e., 85,000 μm) of DNA in the largest human chromosome get condensed into a mitotic metaphase chromosome structure that is about 0.5 μm in diameter and 10 μm in length???
- This DNA molecule somehow gets packaged into a metaphase structure that is about 0.5 μm in diameter and about 10 μm in length—a condensation of almost $10^4$-fold in length from the naked DNA molecule to the metaphase chromosome.

How does this condensation occur?

**Before that let's have a view of chromatin composition……**

# Chemical composition of eukaryotic chromosome

- During interphase of diving cells and G0 of non dividing cells, DNA exist as nucleoprotein complex called Chromatin, less condensed structure.
- While during M-Phase it gets condensed into chromosome. Metaphase chromosome stage is highly condensed DNA and so shortest and thickest.

| Constituent | Types | Content | Property |
|---|---|---|---|
| Nucleic Acid (50%) | DNA | 40-50% | Polyanionic (-vely charged) due to presence of phosphate group |
| | RNA | 1-10% | Polyanionic (-vely charged) due to presence of phosphate group |
| Proteins (50%) | Histones | 40% | • Basic proteins (20-30% lysine and arginine)<br>• Acts as polycationic<br>• Play key role in chromatin structutre i.e. DNA packaging<br>• Its chemical modification regulates gene expression |
| | Non Histones | 10% | • Acidic proteins (Negatively charged)<br>• its composition varies widely among different cell types of the same organism.<br>• Do not play central roles in the packaging of DNA into chromosomes.<br>• Play role in regulating the expression of specific genes |

# Nucleosome Model

- Proposed by R.D. Kornberg and J.O. Thomas in 1974.
- According to this theory,
- ✓ Nucleosome was considered as **repeating unit** (subunit) of chromatin.
- ✓ Adjacent nucleosomes are joined by Linker DNA
- ✓ Each nucleosome is associated with one histone H1

**NUCLEOSOME**

- The term nucleosome was given by P. Outdet in 1975
- NUCLEOSOME = 2(H2A H2B, H3, H4) +200 bp DNA + H1
- It is a octamer of histones and 200 bpDNA
- 2[H3, H4] tetramer makes a central kernel of nucleosome
- (H2A-H2B) dimer are placed at opposite faces of tetramer.

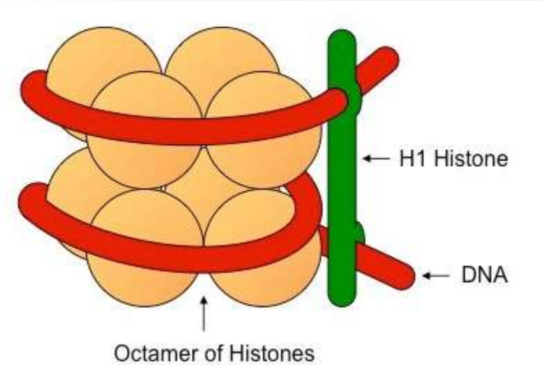



**Fig. A Nucleosome**

| | DNA | Turns of DNA | Histones |
|---|---|---|---|
| Nucleosome Core particle | invariant length of 146 bp | 1 ¾ turns | 2(H2A H2B, H3, H4) |
| Linker DNA | variant length depending upon species and cell type 8-114 bp | | |
| Nucleosome | variant length of 154-260bp | 2 full turn stabilized by H1 | 2(H2A H2B, H3, H4) + H1 |

# Three Levels of DNA packaging in eukaryotic chromosomes

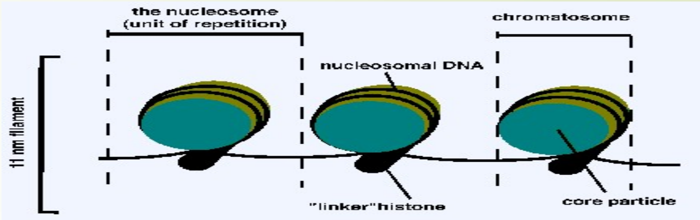
- Higher level of chromosome is achieved by folding & again folding of chromatin at 3 levels:

**Level I. Extended nucleosome (Beads-on-a-string structure** )

- It is first level of condensation that Involves packaging of DNA

into nucleosome to produce 11nm diameter interphase

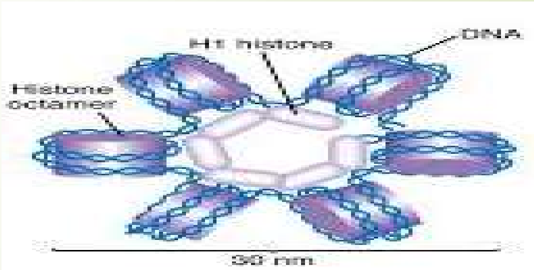
(S- subphase) chromatin fibre




- Involves octamer of histones H2A, H2B, H3 and H4

**Level II. Solenoid Structure**

- Involves further folding or supercoiling of 11nm nucleosome fibre into 30 nm chromatin fibre, each turn consisting of 6 nucleosmes.

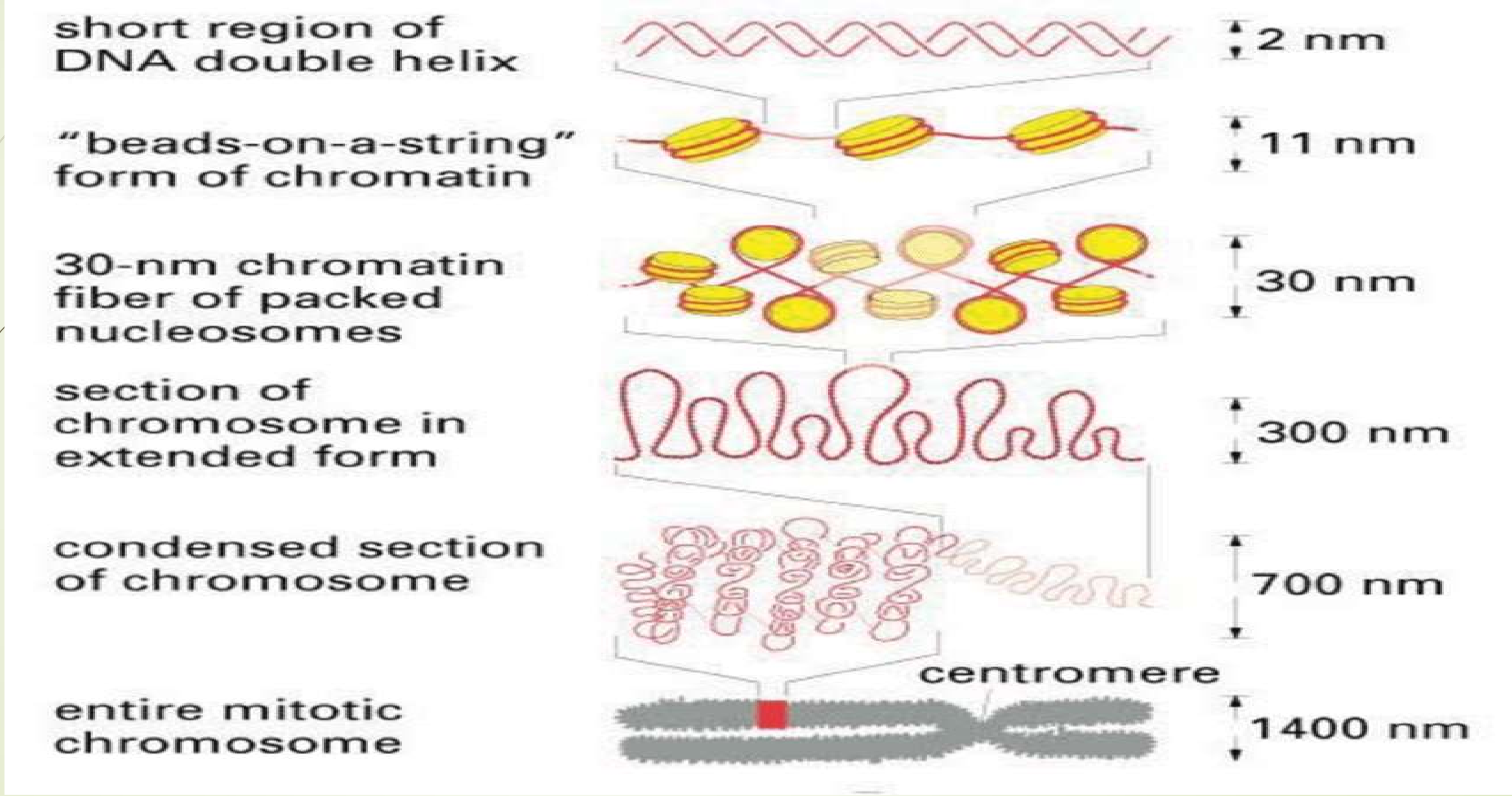



- Involves HI
- G2 of interphase.

**Level III. Loops, domain and scaffold in chromatin**

- 3rd and last level of condensation
- Involve non-histone chromosomal proteins that form a scaffold that is involved in condensing the 30-nm chromatin fiber into the tightly packed metaphase chromosomes.
- This third level of condensation appears to involve the separation of segments of the giant DNA molecules present in eukaryotic chromosomes into independently supercoiled domains or loops. The mechanism by which this third level of condensation occurs is not known.

## Hierarchy of chromatin organization



# THANK YOU