CHAPTER-2

GENOME ORGANISATION

2.1 DNA Organisation in Chromosomes

DNA is known to store the genetic information through its nucleotide sequences which is organized into genes. However, the main question is that how these basic units of genetic function are organized into chromosomes and within the genome. It was found that genomic organization varies in organisms (among prokaryotes and eukaryotes) and has a major implication in the regulation of genetic expression. The chapter deals on the various ways DNA organisation in prokaryotes and eukaryotes, its organisation into the different level to form chromatin, which in turn is organized into chromosomes.

One of the advantages of DNA organisation in organisms is the ability to package an exceedingly long DNA molecule into a relatively small volume. For example, in bacteriophage λ, the 17 mm long DNA has to fit into phage head of the size 0.1 mm.

2.2 DNA Organisation in Prokaryotes

The chromosomes in prokaryotic system (like viruses and bacteria) are simple and less complicated. Prokaryotic genome consists of a single nucleic acid molecule and are largely devoid of associated proteins. Even their genetic information is relatively less than higher organism. For examples, 1000 genes in *E. coli* as compare to the 20,000 genes in human.

The chromosomes of **viruses** consist of a nucleic acid moleculeeither DNA or RNAthat can be either single or double stranded. They exist as circular structures (such as polyoma virus having dsDNA) or they linear molecules (ssDNA in □X174 bacteriophage ssDNA). It is interesting that bacteriophage lambda (λ), on the other hand, consist a linear dsDNA molecule prior to infection, but get circularise inside the host cell upon infection.

Similarly, **bacteria** also have circular and relatively simple of dsDNA, that form a compact structure referred to as the **nucleoid.***Escherichia coli*has circular chromosome ~1.2 mmin length. The chromosome was found to be associated with several types of DNA-binding proteins. Like HU and H1 proteins, having high content of positively charged amino acids which interact with phosphate groups in DNA. HU and H1 proteins has similar to histone but does not involved in compacting DNA in a similar way. In different to viruses, bacterial chromosome is not functionally inert, and they can be readily replicated and transcribed.

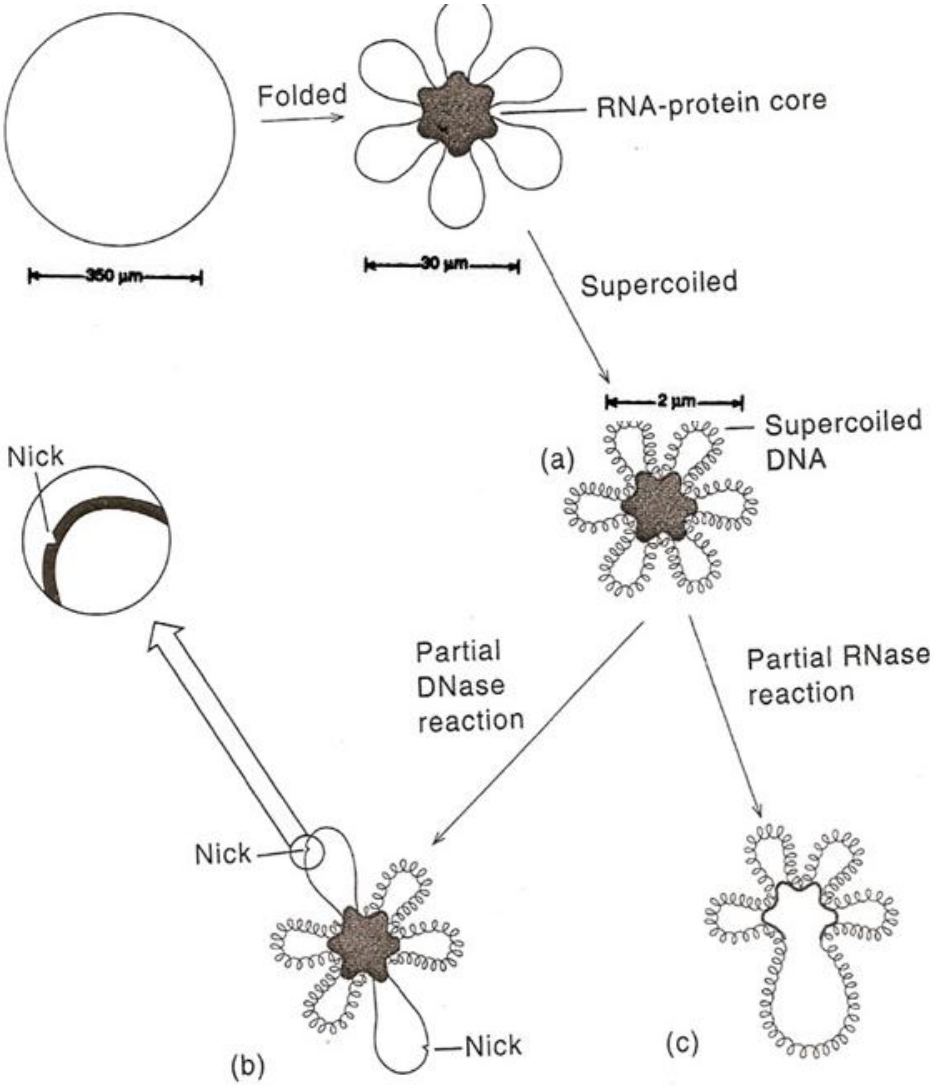


Figure to represents the packaging of DNA in prokaryotes

2.3 Overview: How Eukaryotic Genomes Work and Evolve

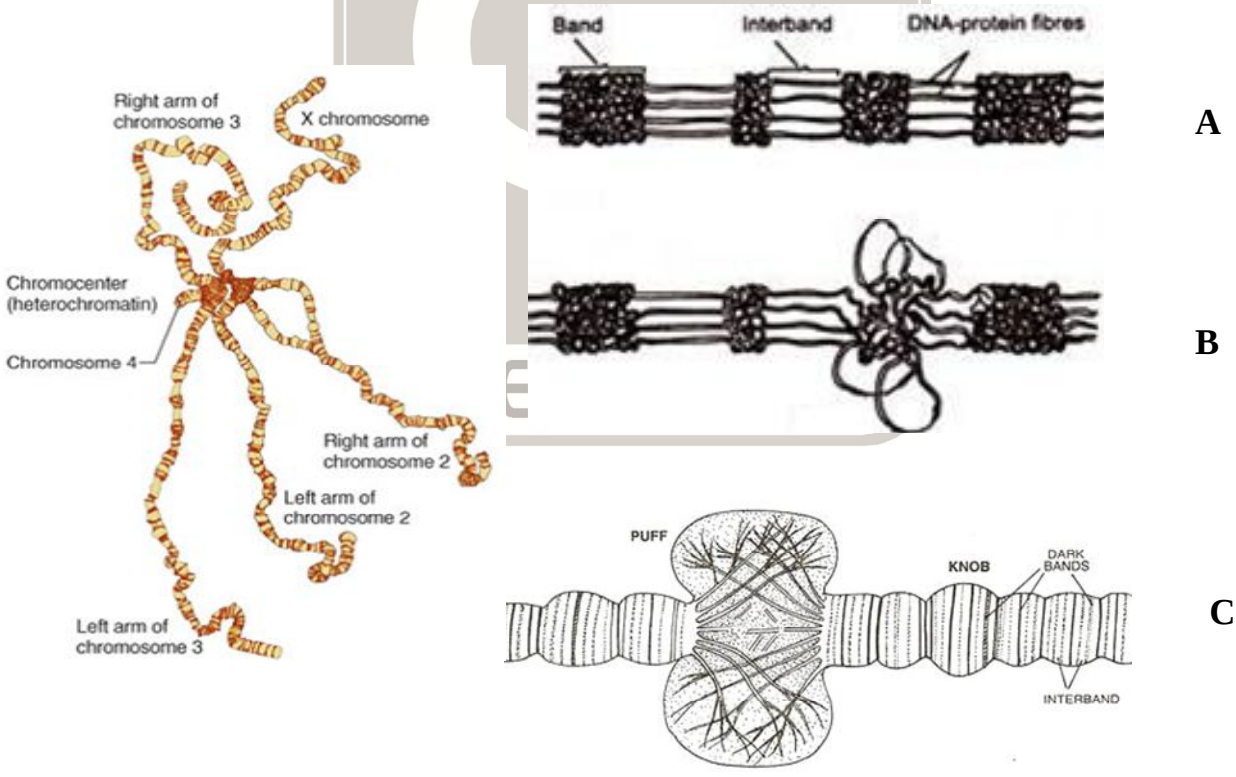
- Two features of eukaryotic genomes present a major information-processing challenge. First, the typical multicellular eukaryotic genome is much larger than that of a prokaryotic cell.
- Second, cell specialization limits the expression of many genes to specific cells.
- The estimated 25,000 genes in the human genome include an enormous amount of DNA that does not code for RNA or protein.
- This DNA is elaborately organized.
- Not only is the DNA associated with protein, but also this DNA-protein complex called chromatin is organized into higher structural levels than the DNA-protein complex in prokaryotes.

2.4 Polytene Chromosomes

In some flies (*Drosophila*), as well as in several species of protozoans (*Chironomus*) and plants giant **polytene chromosomes** are found in various tissues in the larvae. These included the larvae tissue of salivary, midgut, rectal, and Malpighian excretory tubules. First reported by E. G. Balbiani in 1881, the giant chromosomes are found in the interphase nuclei of dividing cells.

Following are the characteristics features of polytene chromosome,

- Large enough to be seen in the nuclei of interphase cells, ~200 to 600 mm long.
- exhibits a linear series of alternating bands (chromomere.) and interbands.
- Usually found in the **somatic cells** and polytene chromosomes represent paired homologs of chromosome.
- Endoduplication of the chromosome results their large size and distinctive in the nuclei. In this, the DNA of the paired homologs undergoes many rounds of replication (**endorelication**), but without strand separation or cytoplasmic division. Hence, composed of large numbers of identical DNA strands, having 1000 to 5000 DNA strands that remain in precise parallel alignment with one another
- Uncoiling of the chromosome regions results, a **puff**, having a high level of gene activity. Experiments using the radioactively labeled RNA precursors evidenced by their high rate of incorporation of radioactively labeled RNA precursors, hence maximum activity of the transcription that produces RNA.



EXAMPLE QUESTIONS

Q1. After salivary gland cells from *Drosophila* are isolated and cultured in the presence of radioactive thymidylic acid, autoradiography is performed, revealing polytene chromosomes. Which of the following is true for the actively transcribed regions of the chromosome?

a) No incorporation of radioactive thymidine in the regions.
b) Incorporation of radioactive thymidine in the condensed chromosome regions.
c) Heavy incorporation of radioactive thymidine in the regions.
d) Distribution of the radioactive thymidine grains is uniform along the chromosomes.

Explanation: radioactive thymidylic acid act as the substrate for the actively transcribed RNA. So heavy incorporation of radioactive thymidine will be observed in the actively transcribed regions of chromosome.

2.5 Lampbrush Chromosomes

The name of the chromosome come from the brushes used to clean kerosene lamp chimneys in the nineteenth century, so the chromosome is called **Lampbrush chromosomes.** First discovered in 1882 by Walther Flemming, Lampbrush chromosomes are characteristic of most vertebrate **oocytes**, as well as the **spermatocytes** of some insects. Therefore, they are meiotic chromosomes.

Following are the characteristics features of polytene chromosome,

- First observed in salamander oocytes by W. Flemming, and by J. Ruckert in shark oocytes, the chromosome is characteristics of meiotic chromosomes.
- Found in the diplotene stage of the first prophase of meiosis, where they are active in directing the metabolic activities of the developing cell.

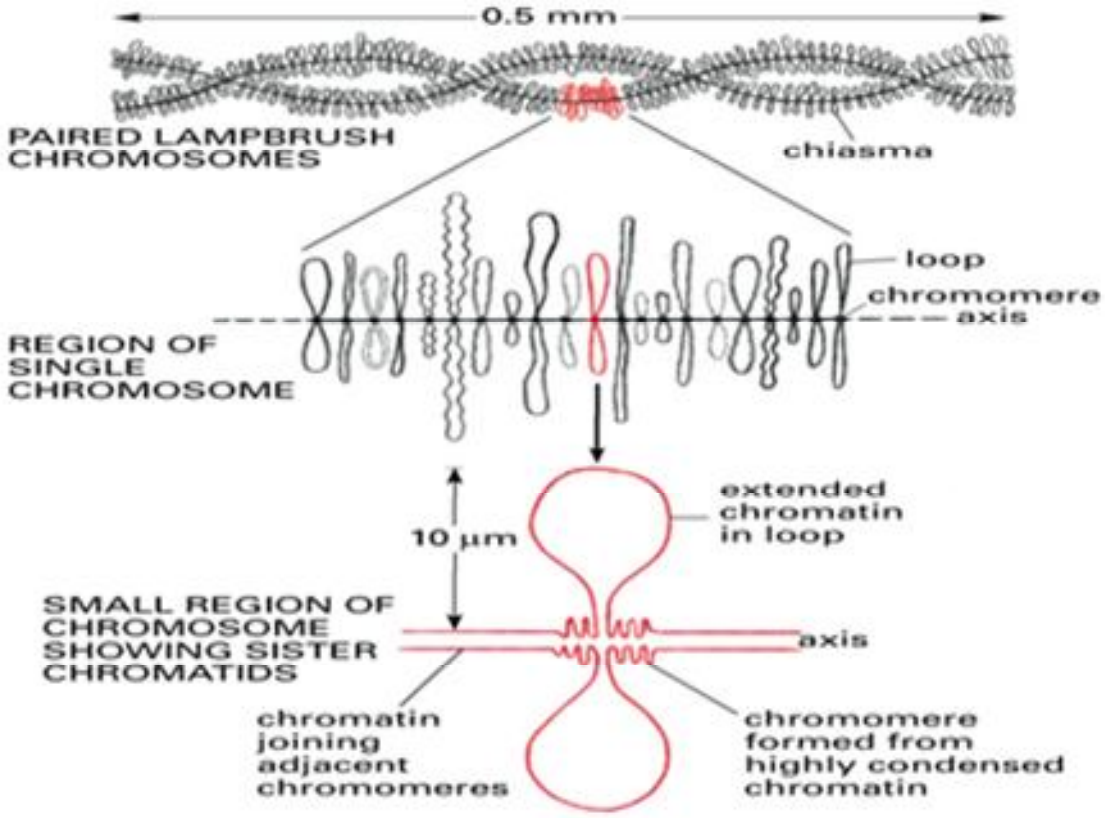


Figure 2-2: Structure and organisation of lambrush chromosome. The homologs pairs synapsed and form chiasmata. The linear axis each have condensed area, called as **chromomeres and** pair of lateral loops extends along the axes giving the chromosome its distinctive appearance of lambrush structure.(Bruce Alberts, 4th Ed. 2008)

- The homologs are seen as synapsed pairs held together by chiasmata (**Figure 3-2).** It is extended, uncoiled versions of the normal meiotic chromosomes (extended to lengths of 500 to 800 mm that are otherwise 15 to 20 µm in normal).
- The chromosome morphology consists of the linear axis each horizontal structure having a condensed area, called as **chromomeres.**Each chromomere results a pair of lateral loops, giving many adjacent pairs of loops along the axes giving the chromosome its distinctive appearance of lambrush structure.
- Each chromosomal loop is composed of one DNA double helix, while the central axis is made up of two DNA helices. This is consistent with the fact that each meiotic chromosome is composed of a pair of sister chromatids.
- The lampbrush loops is similar to puffs in polytene chromosomes. It represents DNA that has been unwound from the central chromomere axis during transcription. As a results studies using radioactive RNA precursors reveal maximum incorporation in the loops and are active in the synthesis of RNA.

Histone type	Functions	Content of lysine - arginine
H1	Linker proteins	Lysine rich
H2A	Core protein	Slightly Lysine rich
H2B	Core protein	Slightly Lysine rich
H3	Core protein	Arginine rich
H4	Core protein	Arginine rich

Table 2-1: Different types of Histone and their function in the nucleosome

2.6 DNA Organisation in Eukaryotes

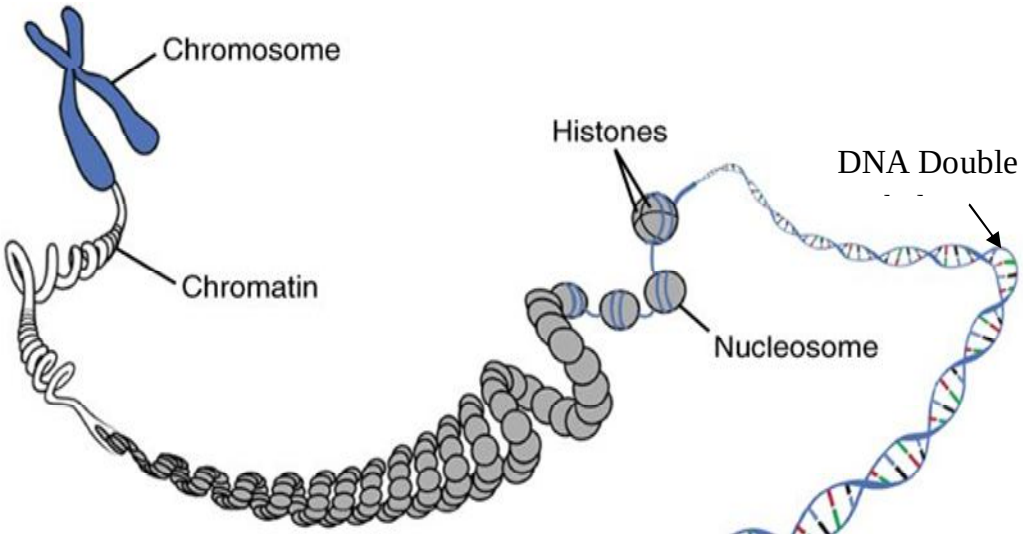
Eukaryotes has greater amount of DNA per chromosome in contrast to the small size in bacteria. In eukaryotic system, the chromosome is highly organised structure in which the DNA is packed with DNA binding protein in chromatin and then chromosome. The chromosome undergoes uncoil form (chromatin) at interphase and whereupon chromatin coils and condenses back into metaphase chromosome allowing to segregate into the daughter cells. This condensation represents a length contraction of some 10,000 times for each chromatin fiber.

2.6.1 Nucleosomes and Chromatin Structure

DNA in eukaryotes were found to be associated with the many BNA binding, forming the chromosomal DNA in all phases of the cell cycle. The associated proteins is oh two types

- **Histones:** these are positively charged proteins. It consists of large amounts of the positively charged amino acids **lysine** and **arginine**, which makes them possible to interact electrostatically to the negatively charged DNA.Thus, the histones have a crucial in the structural organisation of chromosomes.
- **Non-histone proteins:** these less positively charged. Does not have much role in the structural organisation of DNA, but rather have functional role in replication, transcription and translation of genes.

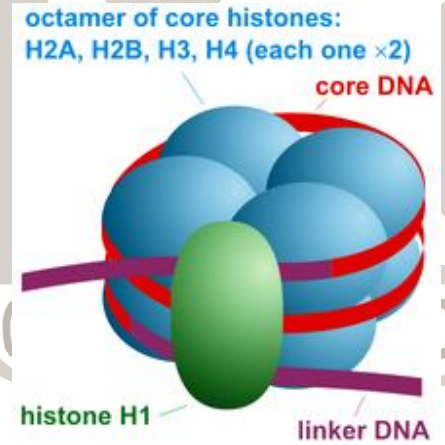
Characteristically, an eukaryotic chromosome has repetitive units of specialised structure called nucleosomes (DNA is wrapped with histones), forming linear array of chromatin structure. Then the uncoil and dispersed form of chromatin are condensed to chromosome that are visible in the metaphase stage of cell cycle as shown in the **Figure 3-3.**



(DifferenceBetween.com)

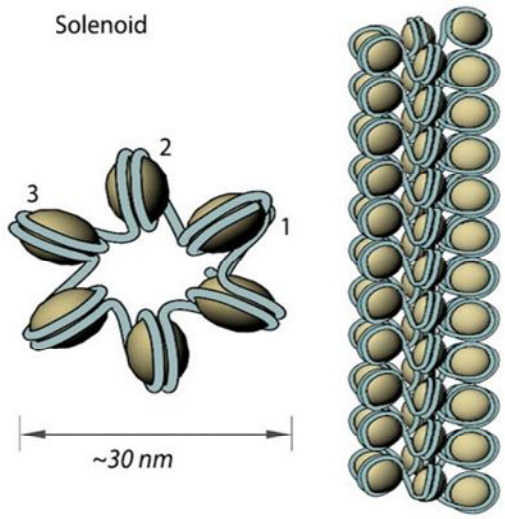
The basic model for chromatin structure in eukaryotes was put forward by John T. Finch, Aaron Klug, and others. The detailed of the model can be summarized as follows,

- **First level of organisation**: a 147-bp length DNA with of the 2 nm in diameter coils around an octamer of histones (two molecules of each of the H2A, H2B, H3 and H4) in a left-handed superhelix to form **nucleosomes**. The DNA completes ~1.7 turns per nucleosome and each nucleosome is ellipsoidal in shape and 11 nm at its longest point. First level of packing, reduced DNA helix to about one-third of its original length.



Structure of Nucleosome

- **Second level of organisation:** In this stage the nucleosomes are packed further to form 30-nm diameter solenoid structure. Here numerous nucleosomes coiled around and stacked upon one another. This provides a six-fold increase in compaction of the DNA. It is this structure that is characteristic of an uncoiled chromatin fiber in interphase of the cell cycle. Here histone H1 plays an important role in creating a second level of packing.



Solenoid structure of chromatin

- **Third level of organisation**: During the transition to the mitotic chromosome, further compaction leads to metaphase chromosome. The 30-nm structures are further folded into a series of looped domains, which further condense the chromatin fiber into a structure that is 300 nm in diameter. This forms chromosome arms that constitute a chromatid, one of the longitudinal subunits of the metaphase chromosome as summarized in the **figure 3-4**.

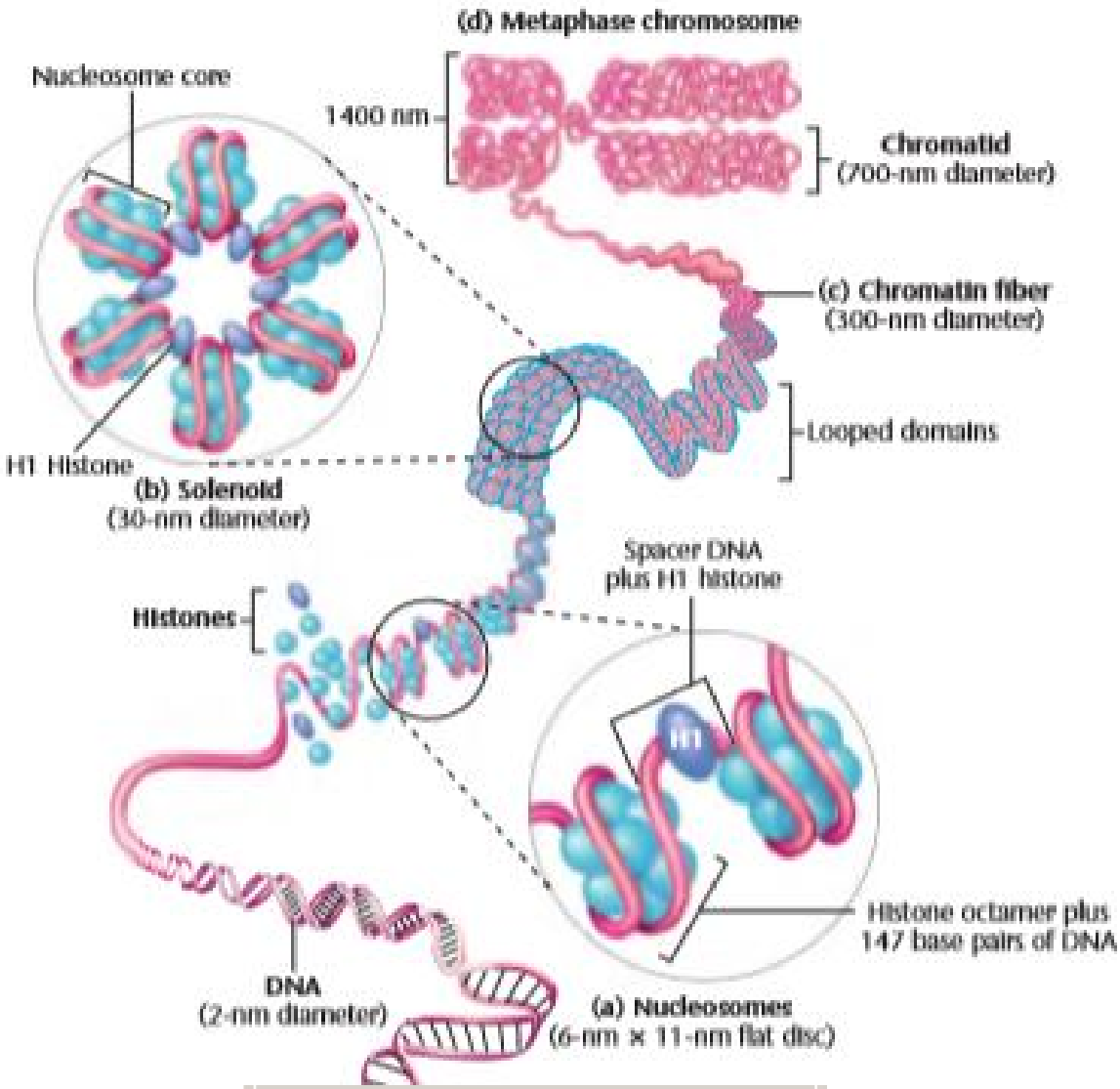
Some important evidences of nucleosome structure model

I. Endonucleases digestion of chromatin, using **micrococcal nuclease**results in DNA fragments that are ~200 bp in length or in multiple thereof. This leads to the conclusion that chromatin consists of repeating unit **nucleosome**. This structure protects the DNA from enzymatic cleavage, but not at the linker region of DNA between units.

II. Observations of chromatin under electron microscopy revealed that chromatin fibers are consist of linear arrays of spherical particles (**nucleosome**), resembling **beads on a string**. This confirm the repeating units as nucleosome.

III. Studies of the chemical association between histone molecules and DNA in the nucleosomes of chromatin confirm the H2A, H2B, H3, and H4 tetramers in two types. Thus, each repeating nucleosome unit consists of octamer (two of each tetramer) in association with about 200 base pairs of DNA.

IV. Extended nuclease treatment results in **nucleosome core particle** consisting of 147 bp of DNA. In this the nuclease removes the portion of DNA from 200 bp of DNA which is responsible for linking nucleosomes together, called **linker DNA** normally bound by histone, H1.



EXAMPLE QUESTIONS

Q1.Which of the following forms of chromatin will be present if histone Hl and all non-histone proteins are removed?

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(a) 30 nm fibre (b) 700 nm chromatin (c) Naked DNA (d) 10 nm bead-on-a-string

Explanation: In the nucleosome, 147-bp length DNA coils around an octamer of histones (H2A, H2B, H3 and H4) forming a ~10nm bead on a string thread like structure. Next, the linker protein, H1 further packed the nucleosome to form 30-nm diameter solenoid structure. Thus, if histone Hl and all non-histone proteins are removed chromatin structure of nucleosome will remain unaffected. Hence option D is correct.

2.7. Heterochromatin

Heterochromatic are the region in the chromosomes that are highly condensed and genetically inactive. Due to the chromatin compaction, the regions are not highly accessible to the transcription factors and other proteins, thus remain repressed. In most of the case the region has less gene contents, rather rich in non-coding sequence. Major heterochromatic regions in human are centromeres (involved in chromosome movement during cell division), the telomere (involved in maintenance of the chromosome's structural integrity). They stained deeply during interphase. **Position effect** is associated with heterochromatin, in whichwhen certain heterochromatic regions are translocated to a new site on the same or another chromosome, genetically active areas become inactive in the influence of the translocated heterochromatin that lie adjacent to it.

2.8 Euchromatin

These are the regions in the chromosomes that are lightly packed, a form of chromatin that is enriched in genes, and are often highly active. The region is known to have highly active transcription in the genome. In this this wrapping of DNA to the histones are loose and DNA is opened structure which are accessible to the Transcription factors. They appear as light-coloured bands when stained in G-banding, which is contrast to the heterochromatin which is darkly stained.

EXAMPLE QUESTIONS

Q1. Active chromatin is strongly characterized by which of the following histone modifications?

(JNU PhD LIFE SCIENCES_2015)

(a) H3- K9 trimethylation (b) **H3- K4 acetylation**

(c) H4- S1 phosphorylation (d) H4- K5 acetylation

Explanation: Acetylation of histones, H3 and H4 opened the chromatin structure, thus made possible to access by proteins and several transcription factor to promote gene activation. Hence option B is correct.

Q2. Activation of genes in euchromatic regions is an outcome of _____ of histone N-terminal tails **(DBT_BET_2009)**

(a) deacylation(b) methylation (c) **hyperacetylation** (d) phosphorylation

Explanation: correct option is C.

2.9 Repetitive DNA

The main distinguishing features of eukaryotic chromosomes from the prokaryotes is the presence of DNA sequence that are repetitive in addition to the single copies of unique genes. The majority such repetitive sequences do not encode protein, rather involves in chromatin remodelling and many other functions.

satellite DNA

Characteristics of eukaryotes, highly repetitive DNA with identical or nearly identical nucleotide sequence. It consists of short sequences repeated a large number of times.

Centromere

Also known as **primary constrictions region**, consist of the region with repetitive DNA sequences like AT. The region helps in separation of homologs during mitosis and meiosis. **CEN region**in centromere is the minimal region that supports the function of chromosomal segregation.

Telomere

DNA sequences in Telomeric region consist of short tandem repeats. This repetitive sequence helps in the stability and integrity of the chromosome. For example, ciliate *Tetrahymena* has more than 50 tandem repeats of the 5´-TTGGGG-3´. In humans, the sequence 5´-TTAGGGG-3´ is repeated many times.

Middle Repetitive Sequences: VNTRs and STRs

Variable number tandem repeats (VNTRs) represents repeating DNA sequences upto 15 to 100 bp long. VNTRs usually found within and between genes which are dispersed throughout the genome, sometimes called as **minisatellites.**VNTRs form the basis for the DNA fingerprinting, a forensic technique.This is because the number of tandem copies of each specific sequence at each location varies among individuals.

Short tandem repeats (STRs), is another tandemly repeated sequences that consists short repeats of di-, tri-, tetra-, and pentanucleotides, etc. Like VNTRs, STRs also dispersed throughout the genome and vary among individuals in the number of repeats present at any site. For example, in humans, dinucleotide $(CA)_n$ repeats is the most common microsatellite

Repetitive Transposed Sequences: SINEs and LINEs

This includes the repetitive DNA sequences that are interspersed individually throughout the genome (between and within genes), rather than being tandemly repeated. They can be either short or long, and many often have transposable sequences so that they are potentially mobile and move to different locations within the genome. This includes SINEs and LINEs.

SINEs are short interspersed elements, less than 500 bp long and may be upto 500,000 times in the human genome. The best characterized human SINEs is the ***Alu family***(a set of closely related sequences, recognized by the restriction endonuclease *Alu I*). In humans, it forms almost 5 percent of the entire genome. The interesting fact of Alu family is that they are transcribed into RNA, despite the uncertainty in their specific rolein human. Even Alu sequences has the potential to transpose within the genome.

LINEs:long interspersed elements (LINEs) represents is another class of repetitive transposable DNA sequence. LINEs are usually about 6 kb in length and in the human genome are present approximately 850,000 times. L1 family in humans is an example of LINEs. L1 members has

6400 bp long sequence and are present more than 500,000 times. The functionsof L1 is yet to be defined, but LINEs are referred to as retrotransposons.L1 DNA sequence is first transcribed into an RNA molecule, which further synthesise DNA complement with the help of reverse transcriptase (present within the L1 sequence). The new L1 copy then integrates into the DNA of the chromosome at a new site.

MULTIPLE CHOICE QUESTIONS

1. In the beads on a string model, the bead is made up of _____
a) 6 histone proteins
b) 8 histone proteins
c) 6 histone proteins and DNA
d) 8 histone proteins and DNA

2. Nucleosome is made up of _____
a) DNA, histone core protein
b) DNA, histone core protein, linker H1
c) RNA, histone core protein
d) RNA, histone core protein, linker H1

3. Histones have a high content of …….charged amino acids.
(a) negative (b) Positive
(c) Uncharged (d) acidic

4.Association of DNA and histone is mediated by_____
a) Covalent bonding
b) Hydrogen bonding
c) Hydrophobic bonding
d) Vander Waals interactions

5. Which of the following statements about the human genome is correct?
a**)** About 1.6% of the genome encodes protein sequences.
b) All non-protein coding sequences in the genome are believed to be 'junk' DNA with no function.
c) Introns are the sections of protein-coding genes that actually encode amino acid sequences.
d) The human genome is believed to contain approximately 50,000 protein coding genes,

6. Approximately what proportion of the human genome is made up of repetitive DNA sequences?
a) 1% b) 15% c) 50% d) 90%

MULTIPLE SELECTIVE QUESTIONS

7. With respect to the “tails” of the histone molecules which of the following is true?
a) It is the N – terminal extension
b)It Lacks defined structure
c) Required for the association of nucleosome
d) It is sites for extensive modifications
a) A, B, C b) B, C, D c) A, B, D d) all

8.Which of the following is true of histones?
a) Histones are basic proteins.
b) Histones are found in animal chromatin but in not in plant cells.
c) The amino acid sequences of histone proteins are very similar in different organisms.
d)All histones form part of the nucleosome core particles in chromatin.
a) A, B b) B, C c) A, D d) A, C

9. The DNA in a eukaryotic chromosome is many times longer than the chromosome itself. How many times longer is the DNA in a human chromosome than the length of the chromosome?
a) 10 x b)100 x c)10,000 x d)1,000 x

10. A large part of the human genome has in the past been regarded as 'junk' DNA. Which of the following statements about 'junk' DNA is correct according to our current understanding?
a) About 1.6% of the human genome has no known function and has been described as 'junk' DNA.
b) Bacteria have no junk DNA.
c) There are no genes in junk DNA.
d) MicroRNA genes do not code for proteins.
a)A, B b) B, C c) A, B, C d) only D

ANSWER KEY

1.B	2.B	3.B	4.B	5.A	6.C
7.D	8.D	9.C	10.D		