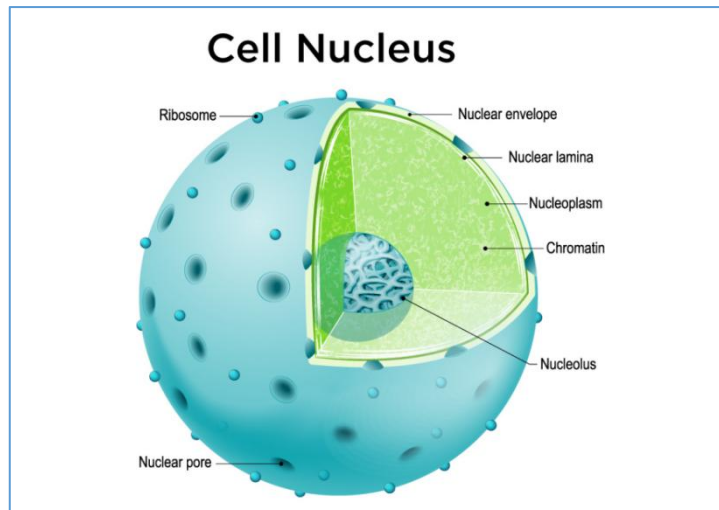


SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

DEFINITION OF NUCLEUS : Nucleus is the double membrane bound cell organelle, the centre for genetic inheritance, DNA replication, transcription & RNA processing i.e. all the stages of gene expression of eukaryotes except translation which takes place within cytoplasm. It controls the various functions of the cell & heredity.

PARTS OF A NUCLEUS: A nucleus may be described as having three important parts, namely, **nuclear membrane** or **nuclear envelope**, **nucleolus** and **chromosomes**. The fluids, in which nucleolus and chromosomes are present and which is enclosed in nuclear membrane, is called **nucleoplasm**.



STRUCTURE OF NUCLEUS

NUCLEAR ENVELOPE OR KARYOTHECA :

An envelope like double layered structure forms around the nuclear contents that provides the structural framework of the nucleus and separates the contents of the nucleus from the cytoplasm is known as nuclear envelope or karyotheca. It consists of following structures:

- Nuclear membranes
- Nuclear lamins
- Nuclear pore complexes

NUCLEAR MEMBRANES:

A double lipid bilayer membrane (each unit membrane is 7.5 nm in diameter) that encloses the nucleus, encases the genetic material in eukaryotic cells and separates the contents of the nucleus from the cytoplasm.

There are some transmembrane proteins which link the outer nuclear membrane with elements of the cytoskeleton. Nuclear membrane consists of following structures:

OUTER MEMBRANE:

- The outer layer is continuous with the endoplasmic reticulum and is similar to endoplasmic reticulum. Ribosomes are attached to its cytoplasmic surface and are engaged in protein synthesis. These proteins are transported to the perinuclear space.
- Enriched in membrane proteins that bind the cytoskeleton and lacking the proteins that give the ER its tubular organization.

SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

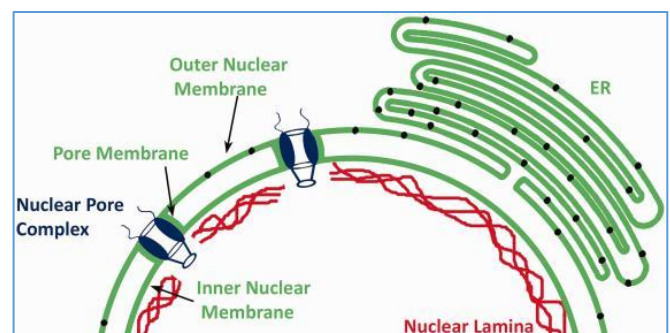
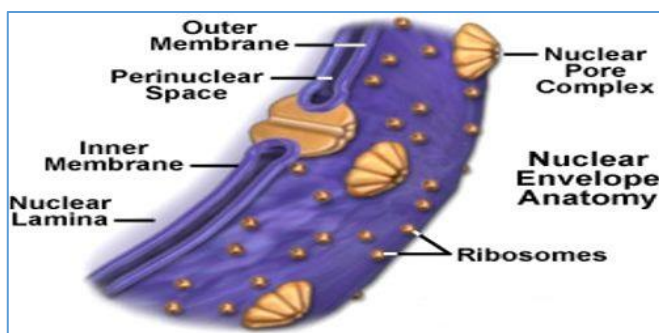
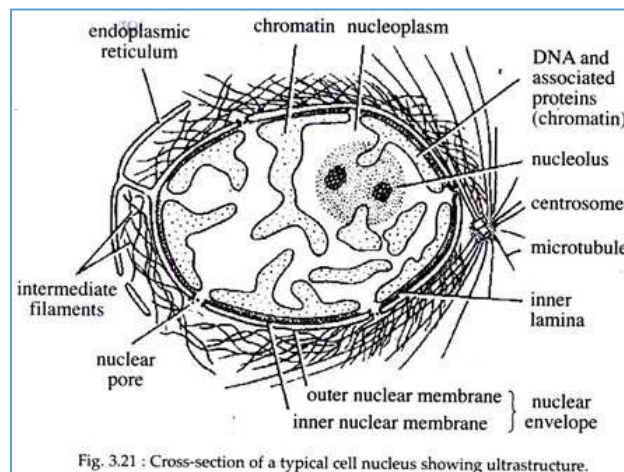
- Outline of nuclear envelope is interrupted by pores which appear circular in surface view. These pores provide direct contact between nucleus and cytoplasm and allow import and export of protein and RNA.

PERINUCLEAR SPACE:

- The space between the two membranes is known as the perinuclear space. It varies from regular 15 nm wide spaces to irregular cavities of several hundred times wide.
- It contains fibres, crystalline deposits, lipid droplets and electron dense materials.

INNER MEMBRANE:

- The inner nuclear membrane is lined by the nuclear lamina, which is composed of A, B and C type lamins. Inherited abnormalities in these proteins (lamins) cause more than a dozen human diseases (e.g., Hutchinson Gilford progeria, with symptoms of old age appearing in young children).
- Inner membrane contains proteins that rearrange the nucleus and girdle the genetic material in position.
- It also provides structural support to the nucleus, assist in DNA repair, controls cell cycle and DNA replication.



NUCLEAR LAMINA:

A fibrous meshwork of intermediate filaments present on the nuclear side of the inner membrane is known as nuclear lamina or nuclear cortex.

- 30 to 100 nm in thickness and made up of 60 or 80 kDa fibrous proteins called lamins along with associated proteins.

SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

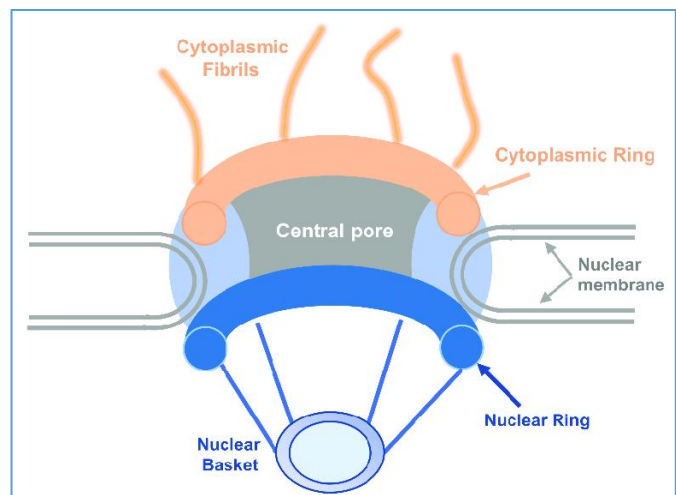
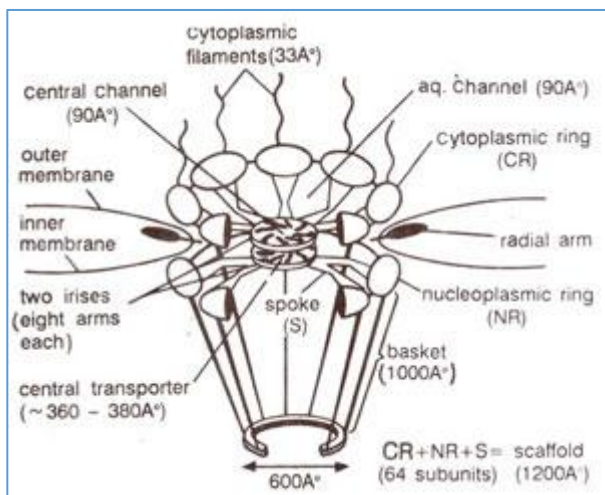
NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

- The lamins form dimers that have a rod like domain and two globular heads at one end. The dimers associate into filaments that have a diameter and repeating structure similar to those of cytoplasmic filaments.
- The nuclear lamina-associated membrane proteins are either integral or peripheral membrane proteins. The most important are lamina associated polypeptides 1 and 2 (LAP1, LAP2), Lamin B-receptor (LBR), Olfm1 and MAN1. They mediate the attachment of the nuclear lamina to the nuclear envelope.
- Lamina provides structural support to the nucleus.

NUCLEAR PORE COMPLEX (NPC) :

The large protein complexes that are embedded throughout the nuclear envelope and perforate the nuclear envelope of all eukaryotes known as nuclear pore complexes (NPCs). These are circular openings where the outer nuclear membrane is fused with the inner nuclear membrane.

- The nuclear pore is a large complex structure of 125 million daltons or 30 times the size of a eukaryotic ribosome. The pore is 100-150 nm in diameter and 50-70 nm in thickness.
- It consists of four separate elements:
 - i. the **scaffold**, which includes majority of the pore,
 - ii. the central hub or **transporter**, which carries out active transport (both import and export) of proteins and RNAs,
 - iii. short **thick filaments** attached to the cytoplasmic side of the pore and
 - iv. a **basket** attached to the nucleoplasmic side of the pore.



- The scaffold is a stack of three closely apposed rings, namely the **cytoplasmic ring**, the **nucleoplasmic ring** and a **central ring** of thick spokes. Each ring has an eight-fold symmetry. The spokes of the central ring are attached to the transporter on the inner side, and to the nucleoplasmic and cytoplasmic ring on the outer side. Interspersed between the spokes are **aqueous channels**, which allow diffusion of proteins and metabolites between the nucleus and the cytoplasm.
- The transporter is a proteinaceous ring, consists of two **irises** of eight arms each. The two irises are assumed to be stacked atop one another and open sequentially, each like the diaphragm of a camera, to let a nuclear protein or RNA pass through from the nucleus to the cytoplasm.
- On the cytoplasmic side of the pore, **thick fibres** extend into the cytoplasm.
- On the nuclear side, a large **basket** like structure is found, which consists of eight filaments, extending from the nucleoplasmic ring of the pore and meeting a smaller ring within the nucleus.
- This basket may play an important role in RNA export.
- Each NPC contains approximately 30 unique proteins called Nup (nuclear pore proteins or nucleoporins), which are often classified into following three groups: i. **transmembrane Nups**,

SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

anchoring the NPC in the nuclear envelope, ii. **FG Nups** with FG (phe-gly) repeats and iii. **Structural Nups**, which make a scaffold for interaction with transmembrane Nups and FG Nups.

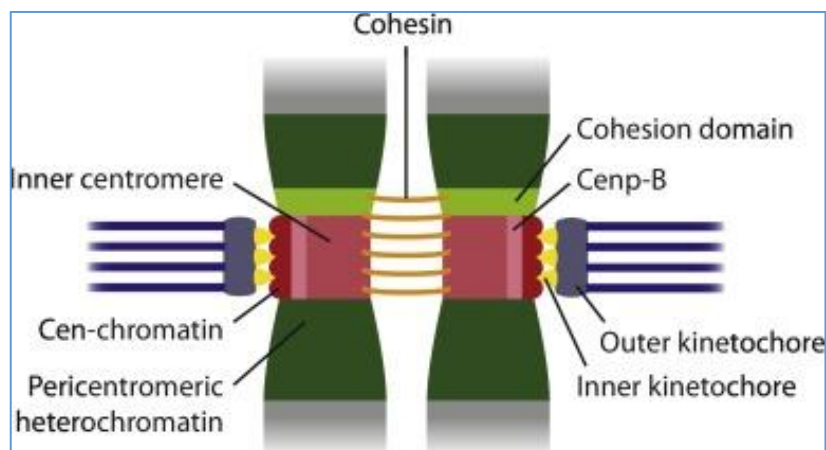
- NPC is a highly selective bidirectional transporter, which allows not only passive diffusion of small molecules, but also allows selective exchange of macromolecules between the cytoplasm and the nucleus.

KINETOCHORE AND CENTROMERIC DNA

Kinetochores and centromeres are not synonyms, as may sometimes be misunderstood. Infact, kinetochores are the macromolecular protein complexes that govern chromosome movement by binding spindle microtubules during mitosis and meiosis. Centromeres are the specific chromosomal regions that serve as the specific platform on which kinetochores assemble.

Each chromosome has two kinetochores, one on each sister chromatid, facing in opposite directions during mitosis and meiosis II, but facing same direction in meiosis I. This kinetochores are the sites for binding of kinetochores microtubules, which assist in the movement of chromosomes during anaphase.

The DNA sequences representing the centromere contain the information specifying assembly of a kinetochore. This centromeric DNA sequences have been isolated and characterised in a number of organisms but, unlike the telomeric sequences, have not been found to be conserved.



CENTROMERIC DNA IN BUDDING YEAST : In budding yeast, each of the 17 chromosomes contains a different centromere repeat sequence about 125 base pairs long, although there are substantial regions of homology between centromeres of different chromosomes. These centromeres in budding yeast, each bind to a single microtubule and can move chromosomes that are 0.26-3.0 megabases in size. Budding yeast contains three centromeric DNA sequence elements (CDEI, CDEII, CDEIII). In the centromere of the budding yeast, there are also found centromeric proteins, called centromere DNA-binding factors.

CENTROMERIC DNA IN FISSION YEAST, MAMMALS AND HIGHER PLANTS : Centromeres of fission yeast, mammals and higher plants are much more complex than those from budding yeast, and are called **regional centromeres**. They include both unique and repetitive DNA sequences, several kilobases or megabases in length. For instance, in mammals, centromeres consist of many different and much longer DNA sequences, that are both unique and repetitive in nature. Several of these sequences form much larger kinetochores, each of them binding 30 or 40 microtubules instead of one in budding yeast. It is believed that much of the repetitive DNA sequence (called satellite DNA) in mammalian centromeres is required for the special chromatin organization at the centromere. However, it is shown that these tandemly repeated centromeric DNA families are poorly conserved between species.

SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

The best studied centromeric satellite sequence is alpha-satellite DNA or alphoid DNA that is found in all human and primate centromeres. The basic units of alpha-satellite are diverged, and consist of 171bp long monomers (arranged in tandem manner) that are organized into higher order repeat units (making up to millions of base pairs).

CHROMATIN AND LEVELS OF ITS PACKAGING

CHROMATIN

- DNA is the genetic material in most of the organisms, This DNA with histone and non-histone protein constitutes the chromatin. The chromatin organizes into chromosome during cell division and is composed of two chromatids.
- Chromatin is highly condensed and wrapped around nuclear proteins in order to fit inside the nucleus.
- Exists as less condensed euchromatin and more condensed heterochromatin form which typically not transcribed. Chromatin looks like a beads on a string, the beads are the nucleosomes.

CHROMATIN ORGANIZATION OR PACKAGING

Histones are the primary proteins of chromatin which acts as anchors and around which DNA strands are wound. The manner of coiling and folding of DNA was a matter of debate and dozens of models were available for this purpose; of them only two stand out and are important.

FOLDED FIBRE MODEL:

- A popular model is the folded fibre model, proposed by E.J. Dupraw in 1965. According to it, the bulk of the chromosome is visualized to be composed of a tightly folded fibre which has a rather homogeneous diameter of 200 to 300 angstrom.
- This folded fibre is supposed to contain the DNA histone helix (30 a diameter) in a supercoiled condition.

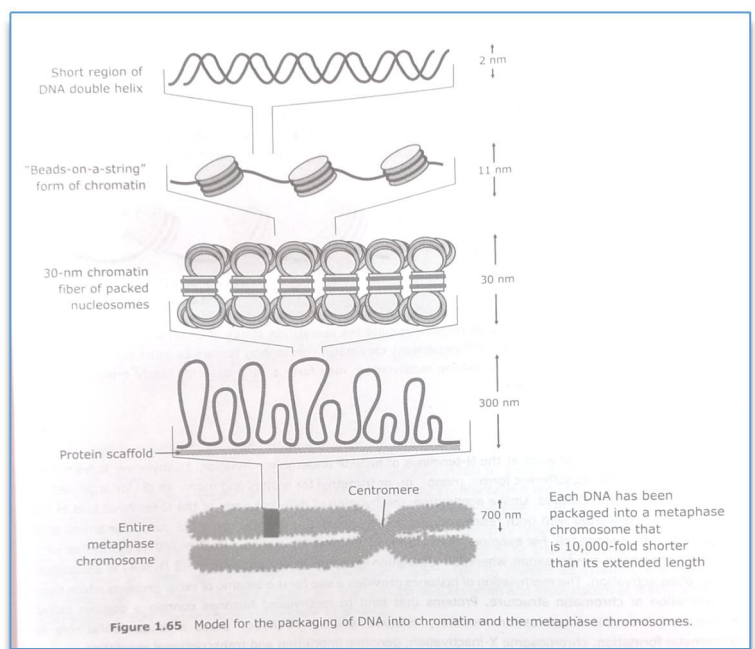
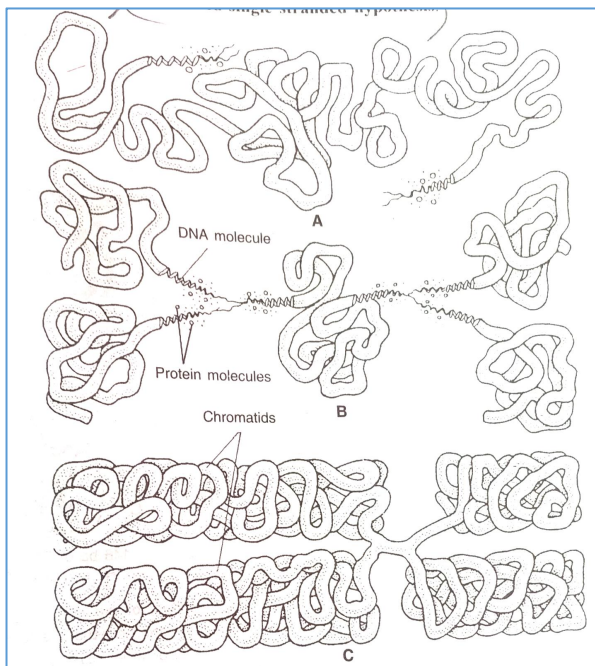


Figure 1.65 Model for the packaging of DNA into chromatin and the metaphase chromosomes.

SEMESTER 1 CORE COURSE - 1 : CELL BIOLOGY UNIT 5 : NUCLEUS

NUCLEAR ENVELOPE, NUCLEAR PORE COMPLEX (TRANSPORT NOT INCLUDED), KINETOCHORE AND CENTROMERIC DNA; CHROMATIN AND LEVELS OF ITS PACKAGING. EUCHROMATIN & HETEROCHROMATIN, POSITION EFFECT VARIATION. CHROMATIN REMODELLING COMPLEX.

NUCLEOSOME AND SOLENOID MODEL OF CHROMATIN:

- It is most significant and universally accepted one, which was proposed by R.D Kornberg (1974) and confirmed and christened by P. Oudet et al., (1975).
- In the folded fibre model, it was proposed that the histones were bound on the outside of the DNA coils (i.e., histone shell around DNA), the nucleosome model has proposed the opposite (i.e., histone particle with DNA round it). In other words, the earlier theory that basic chromatin fibre had DNA core surrounded by histones was incorrect.
- The nucleosome is a bead like structure of chromatin fibres. The nucleosome contains ~200 bp of DNA, organized by an octamer of small, basic proteins called histones.
- The nucleosome provides the first level of organization, giving a packing ratio of 6.
- The second level of organization is the coiling of the series of nucleosomes into a helical array to constitute the fibre of diameter 30 nm (interphase chromatin). In chromatin this brings the packing ratio of DNA to ~40.
- The final packing ratio is determined by the third level of organization, the packaging of the 30 nm fibre. This gives an overall packing ratio of ~1000 in euchromatin.
- The nucleosome contains ~200 bp of DNA associated with a histone octamer that consists of two copies each of H2A, H2B, H3 and H4. These are known as the core histones. Histones H3 and H4 are among the most conserved proteins. The H2A and H2B show appreciable species-specific variation in sequence. Histone H1 proteins show appreciable variation between tissues and between species.
- The length of DNA per nucleosome varies for individual tissues in a range from 154-260 bp.
- The histone octamer has a kernel of a H3₂-H4₂ tetramer associated with two H2A-H2B dimers. Each histone is extensively interdigitated with its partner.
- Histone octamers are not conserved during replication, but H2A-H2B dimers and H3₂-H4₂ tetramers are conserved.