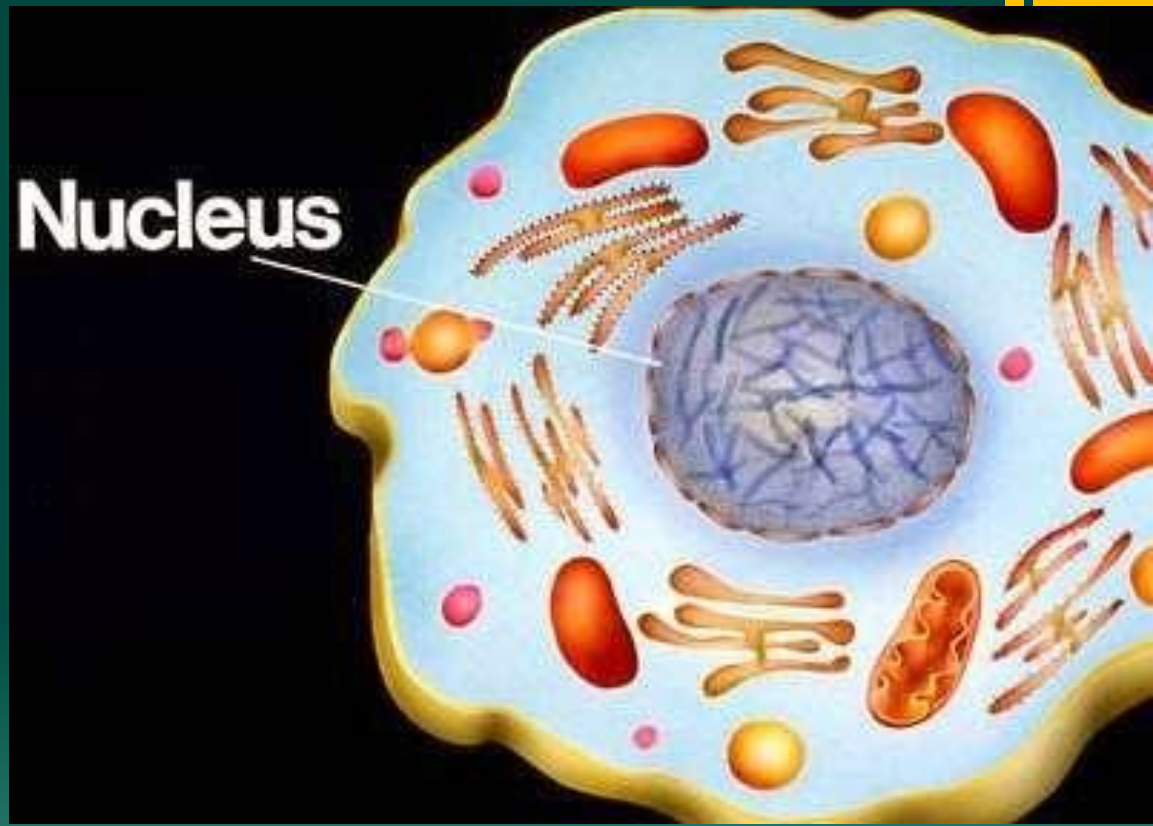
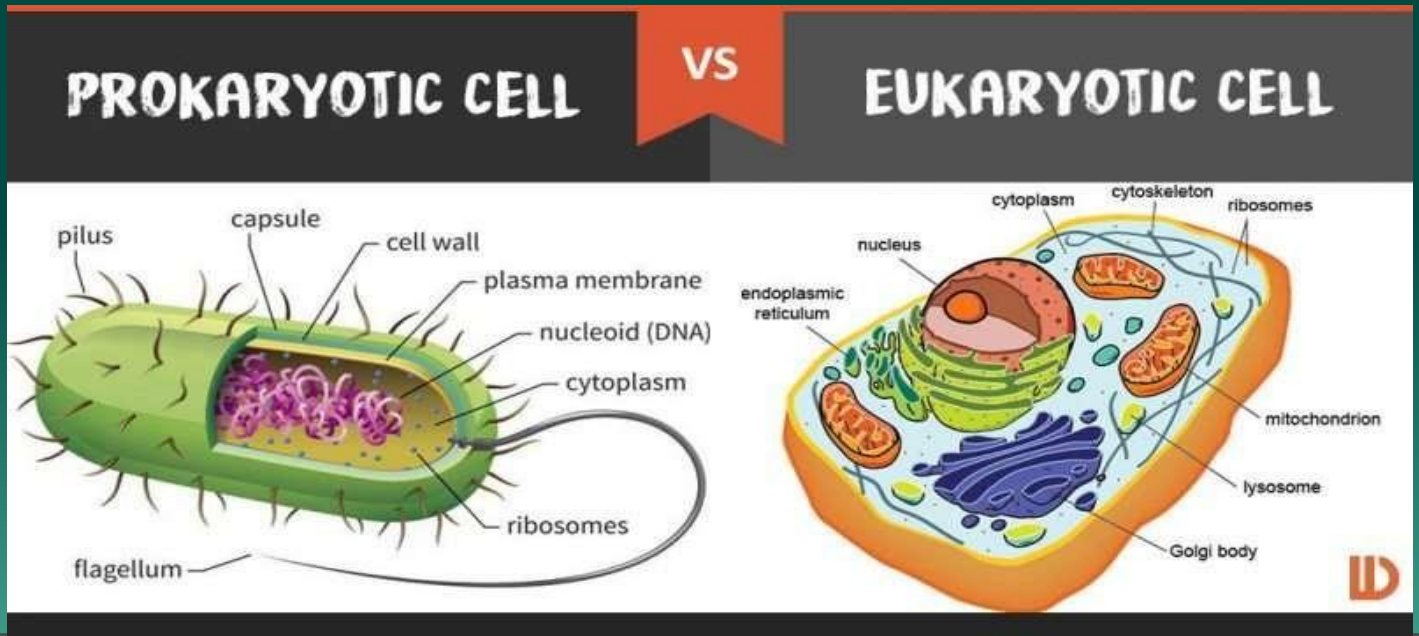
MUNMUN CHATTERJEE

VIJAYGARH JYOTISH RAY COLLEGE



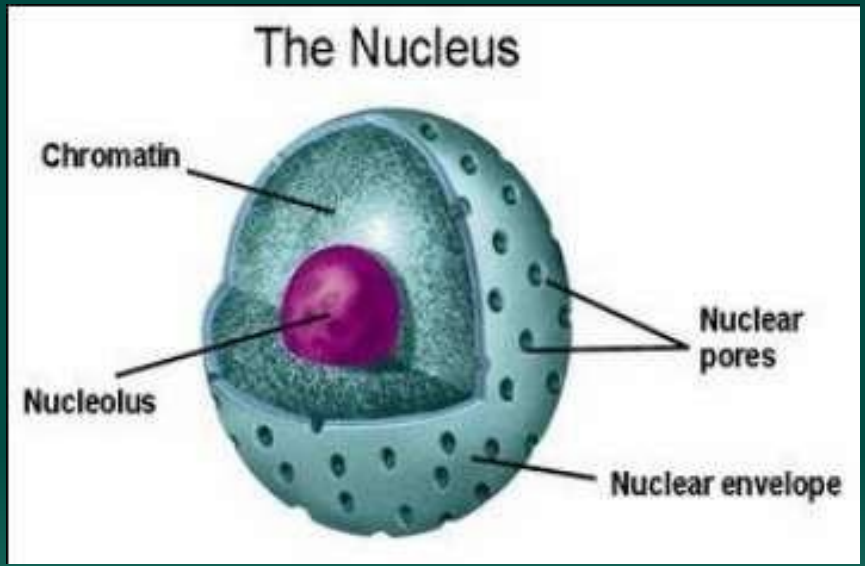
Nucleus

- The presence of a nucleus is the principal feature that distinguishes eukaryotic from prokaryotic cells.
- The nucleus serves both as the repository of genetic information and as the cell's control center.
- DNA replication, transcription, and RNA processing all take place within the nucleus, with only the final stage of gene expression (translation) localized to the cytoplasm.



Nucleus

- Nuclear envelope
- Nucleolus
- Nucleoplasm
- Chromatin reticulum



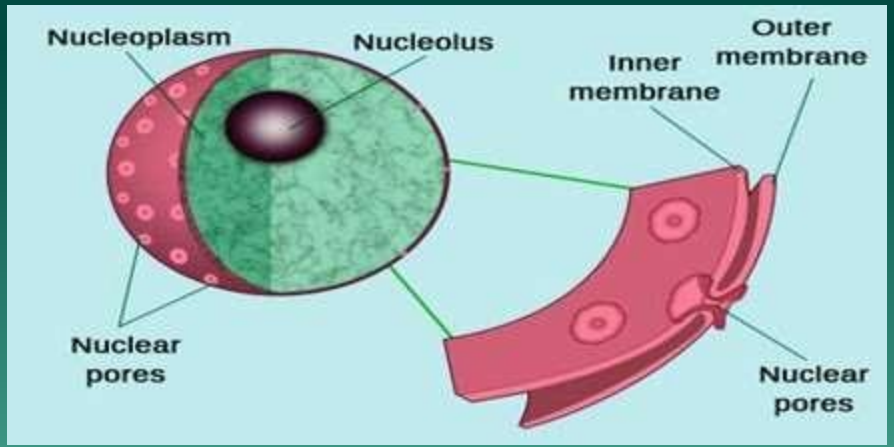
Nuclear envelope

Nuclear envelope

- The nuclear envelope separates the contents of the nucleus from the cytoplasm and provides the structural framework of the nucleus.

- The two envelope membranes act as barriers that prevent the free passage of molecules between nucleus and cytoplasm thus maintaining the nucleus as a distinct biochemical compartment.

- The nuclear pore complexes allow regulated exchange of molecules between nucleus and cytoplasm.



Structure of nuclear envelope

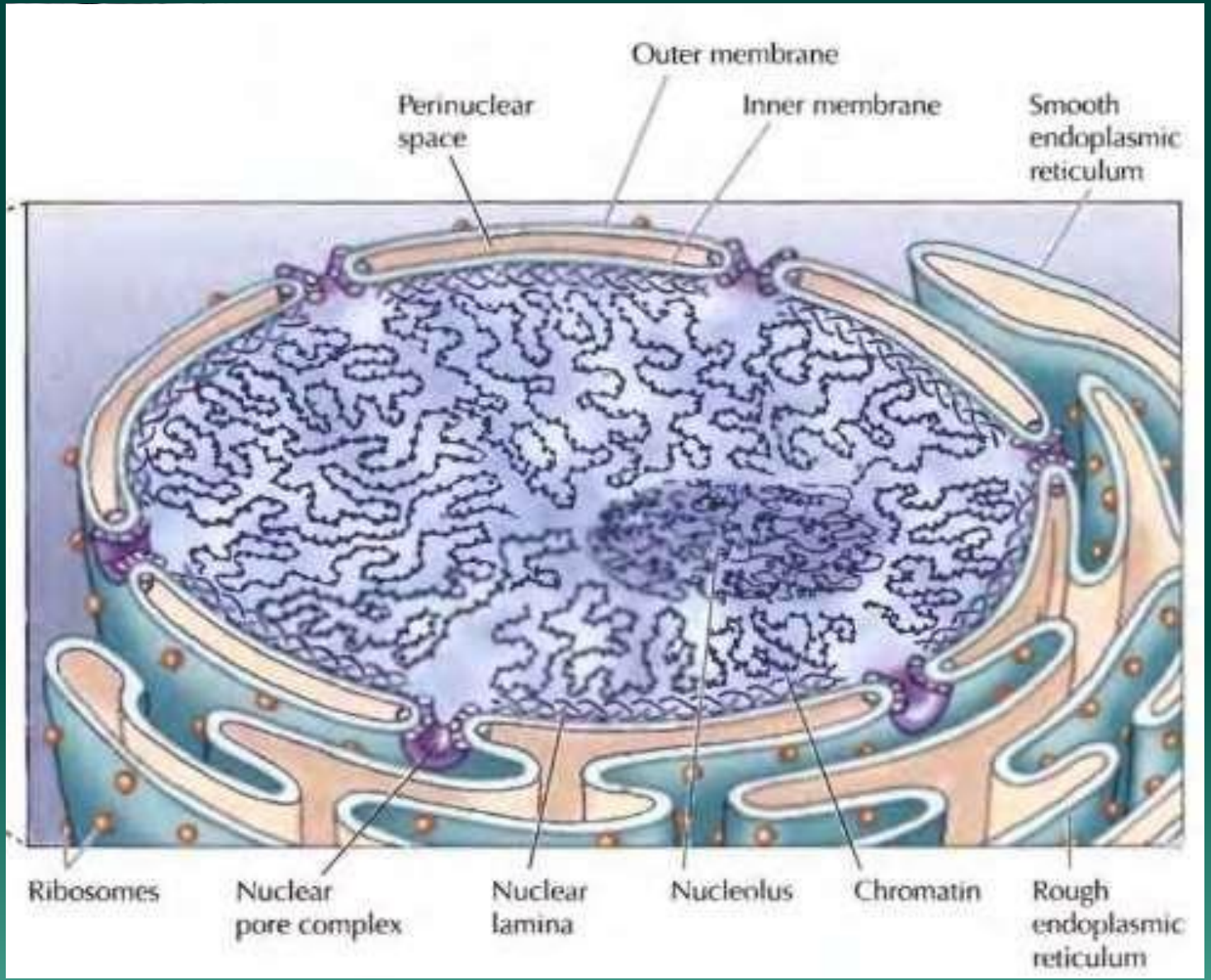
- The nuclear envelope has a complex structure consisting of two nuclear membranes, an underlying nuclear lamina, and nuclear pore complexes.

- The nucleus is surrounded by a system of two concentric membranes, called the inner and outer nuclear membranes. Both the membranes shows unit membrane model, i.e. they are made up of phospholipid bilayer.

- The outer nuclear membrane is continuous with the endoplasmic reticulum, so the space between the inner and outer nuclear membranes is directly connected with the lumen of the endoplasmic reticulum. The outer membrane also has ribosomes bound to its cytoplasmic surface.

- The outer and inner membranes are separated by a space, called perinuclear space which is 10-50 nm wide.

- The inner nuclear membrane carries proteins that are specific to the nucleus, such as those that bind the nuclear lamina.


Outer membrane
Perinuclear space
Inner membrane
Smooth endoplasmic reticulum
Ribosomes
Nuclear pore complex
Nuclear lamina
Nucleolus
Chromatin
Rough endoplasmic reticulum

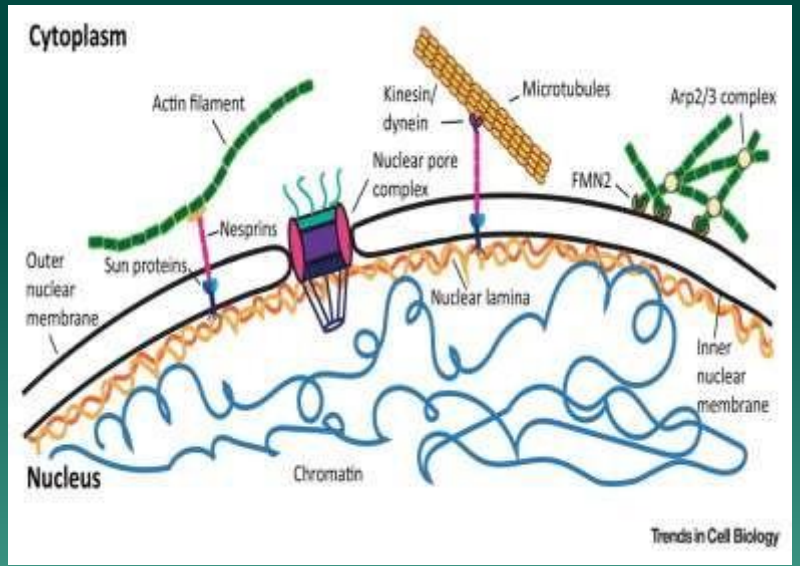
Permeability

- The nuclear membranes are permeable to small non-polar molecules (like O_2, CO_2, etc.). Other molecules can not pass.

- The inner and outer nuclear membranes are joined at nuclear pore complexes, the sole channels through which small polar molecules, macromolecules (proteins, RNA, etc.) or ions (Ca^{++}, K^{+}, etc.) pass through the nuclear envelope.

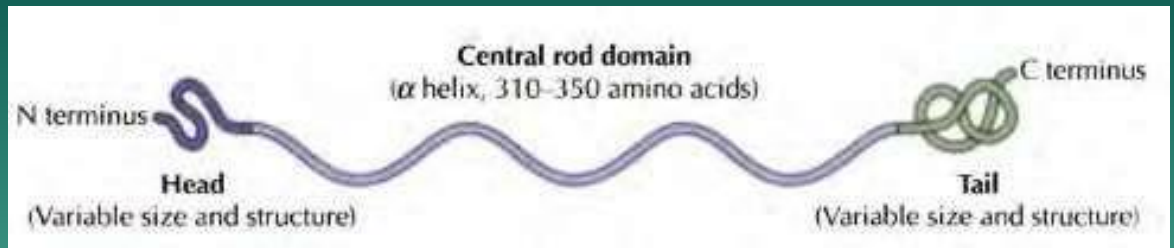
Nuclear lamina

- Underlying the inner nuclear membrane is the nuclear lamina, a fibrous meshwork that provides structural support to the nucleus.
- The nuclear lamina is composed of 60 to 80 kd fibrous proteins called lamins.
- Lamins are a class of intermediate filament proteins. Mammalian cells have three lamin genes, designated A, B and C, which code for at least seven distinct proteins.
- A-type and C-type lamins are inside, next to the nucleoplasm, forming the lamina network, but are not associated with the inner nuclear membrane.
- B-type lamins are near the inner nuclear membrane and may bind to integral proteins inside this membrane. Specific lipids (prenyl groups) are attached to the end of the polypeptide chains of B-type lamins which helps in attaching the B-type lamins to the nuclear membrane.

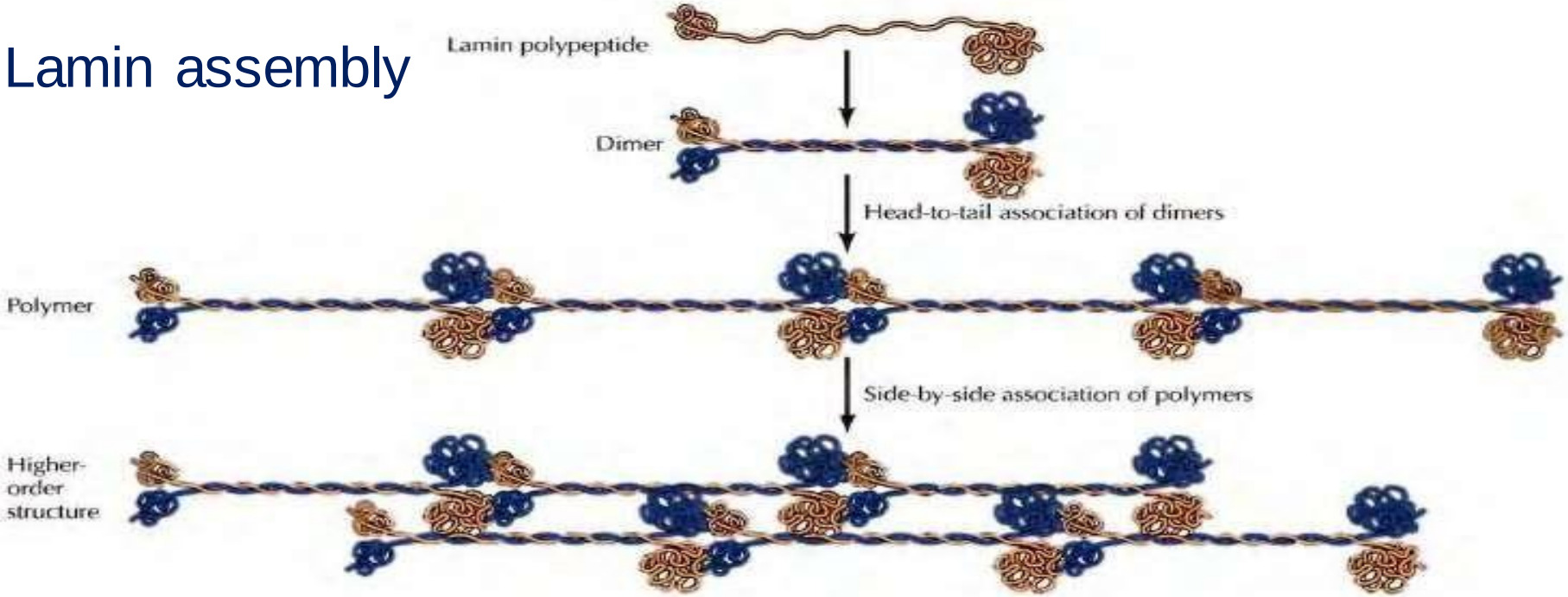


Intermediate filaments

- Intermediate filaments have diameters between 8-11 nm, which is intermediate between the diameters of actin filaments (about 7 nm) and microtubules (about 25 nm).
- They play a structural role by providing mechanical strength to cells and tissues.
- More than 65 different intermediate filament proteins have been identified.
- All of the intermediate filament proteins have a central α-helical rod domain of approximately 310 amino acids (350 amino acids in the nuclear lamins).
- This central rod domain is flanked by amino- and carboxy-terminal domains, which vary among the different intermediate filament proteins in size, sequence, and secondary structure.
- They are apolar structures.



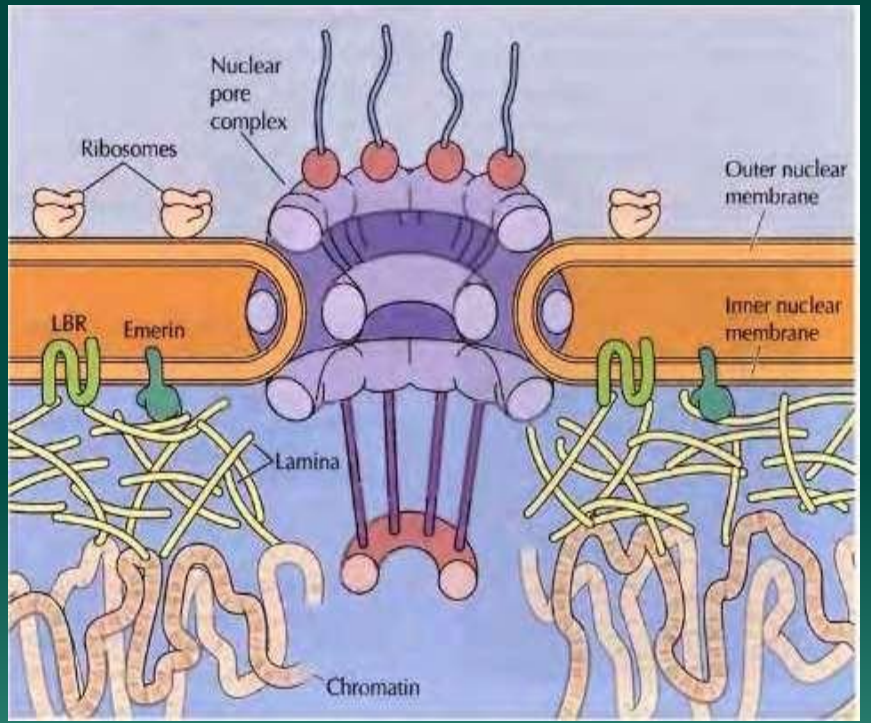
Lamin assembly



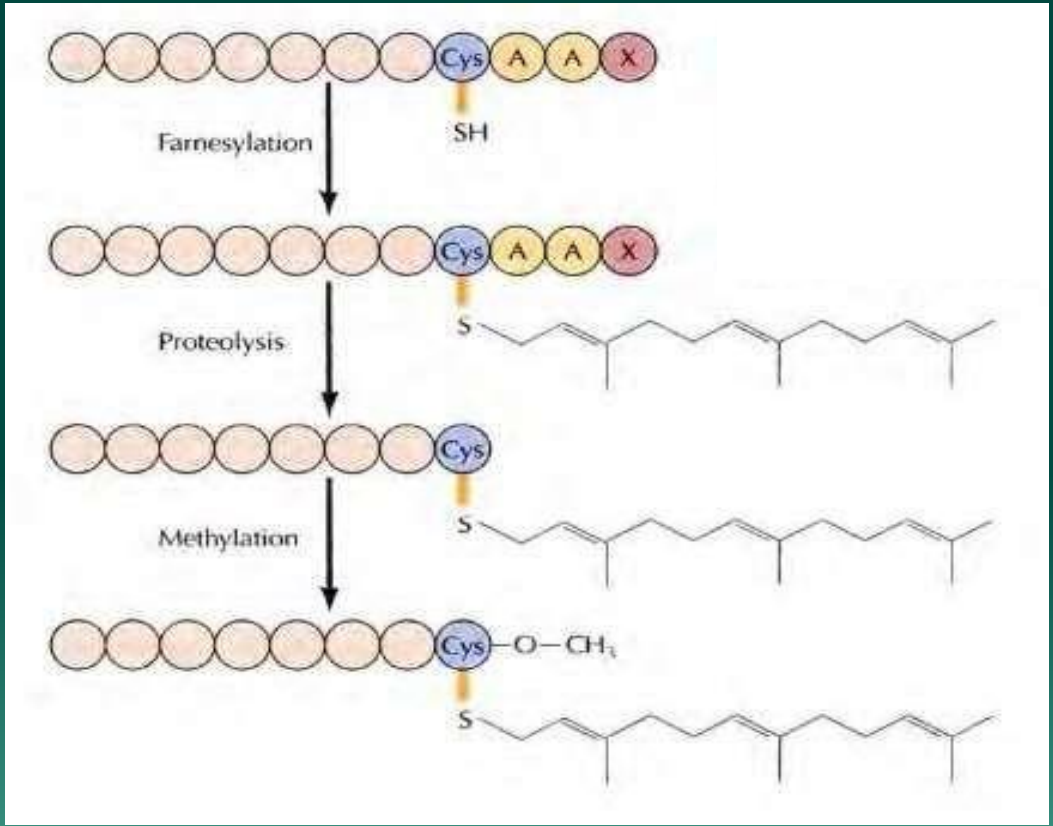
- The lamins associate with each other to form higher order structures, filaments.
- The lamin polypeptides form dimers in which the central α-helical regions of two polypeptide chains are wound around each other, and form a structure called a coiled coil.
- Further assembly may involve the head-to-tail association of dimers to form linear polymers and the side-by-side association of polymers to form higher order structures.

Lamin assembly

- The association of lamins with the inner nuclear membrane is facilitated by the posttranslational addition of lipid, in particular, prenylation of C-terminal cysteine residues.
- In addition, the lamins bind to specific inner nuclear membrane proteins such as emerin and the lamin B receptor, mediating their attachment to the nuclear envelope and localizing and organizing them within the nucleus.
- The nuclear lamina also binds to chromatin through histones H2A and H2B as well as other chromatin proteins.



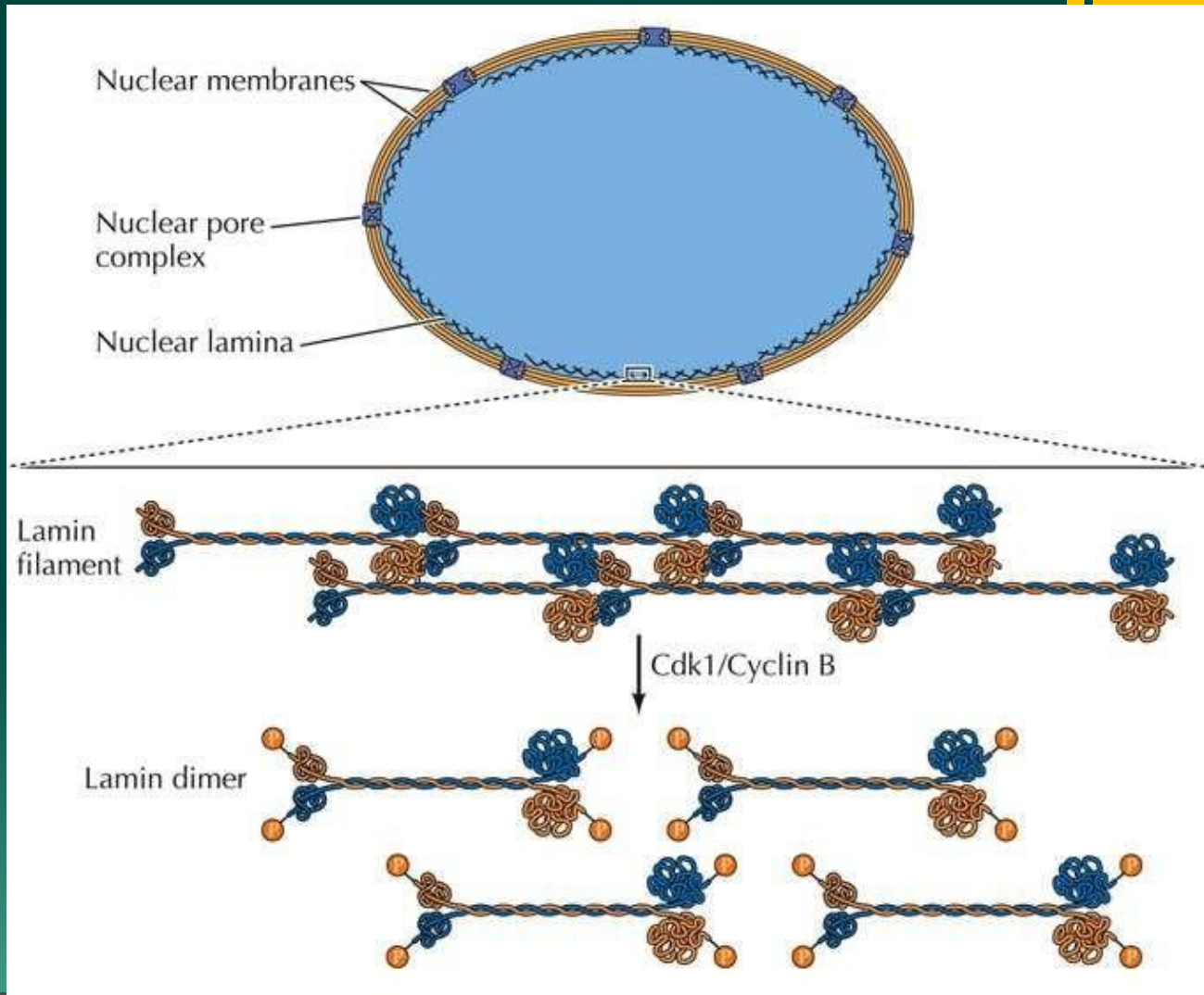
Prenylation of nuclear lamins



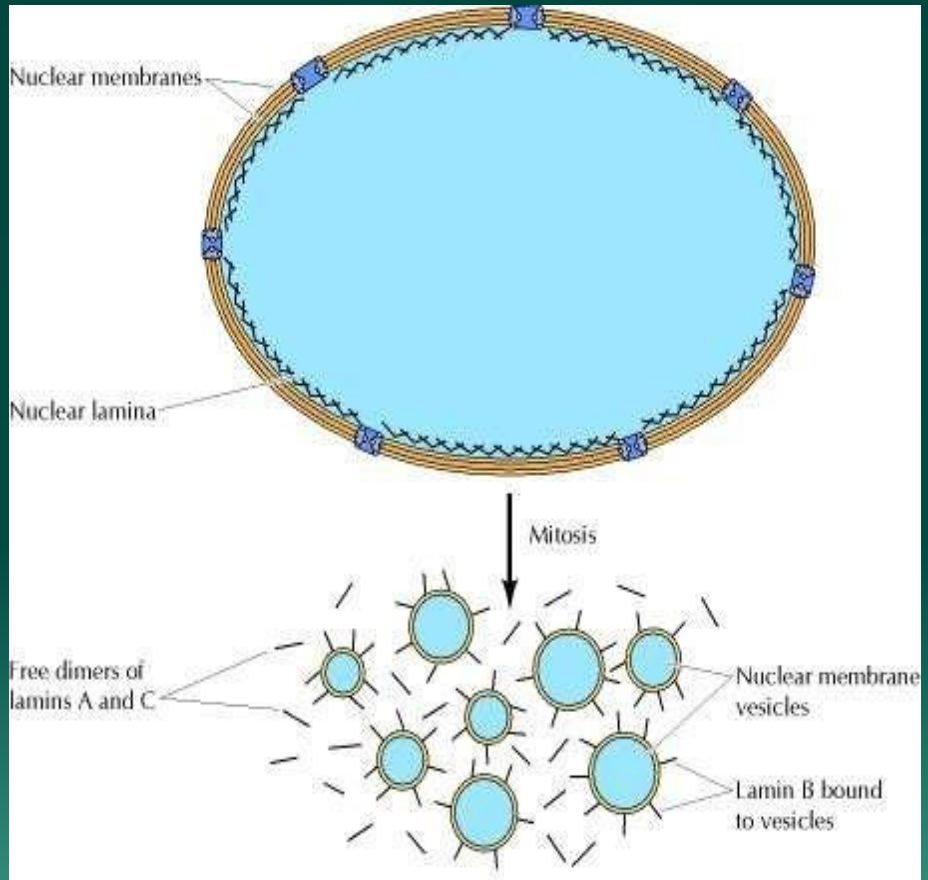
- These proteins terminate with a cysteine residue (Cys) followed by two aliphatic amino acids (A) and any other amino acid (X) at the C terminus.
- The first step in their modification is addition of the 15-carbon farnesyl group to the side chain of cysteine (farnesylation).
- This step is followed by proteolytic removal of the three C-terminal amino acids and methylation of the cysteine, which is now at the C terminus.

Disappearance of nuclear envelope

- Lamins play a role in assembly and disassembly of nuclear envelope during cell division.
- At the end of prophase, a phosphate group attaches to the cystein residue of the lamin polypeptide. This phosphorylation triggers disassembly of lamina and breakdown of nuclear envelope into vesicles. Phosphorylation is catalyzed by the Cdc2 protein kinase.
- Dephosphorylation reverse these changes and allows the nucleus to re-form.

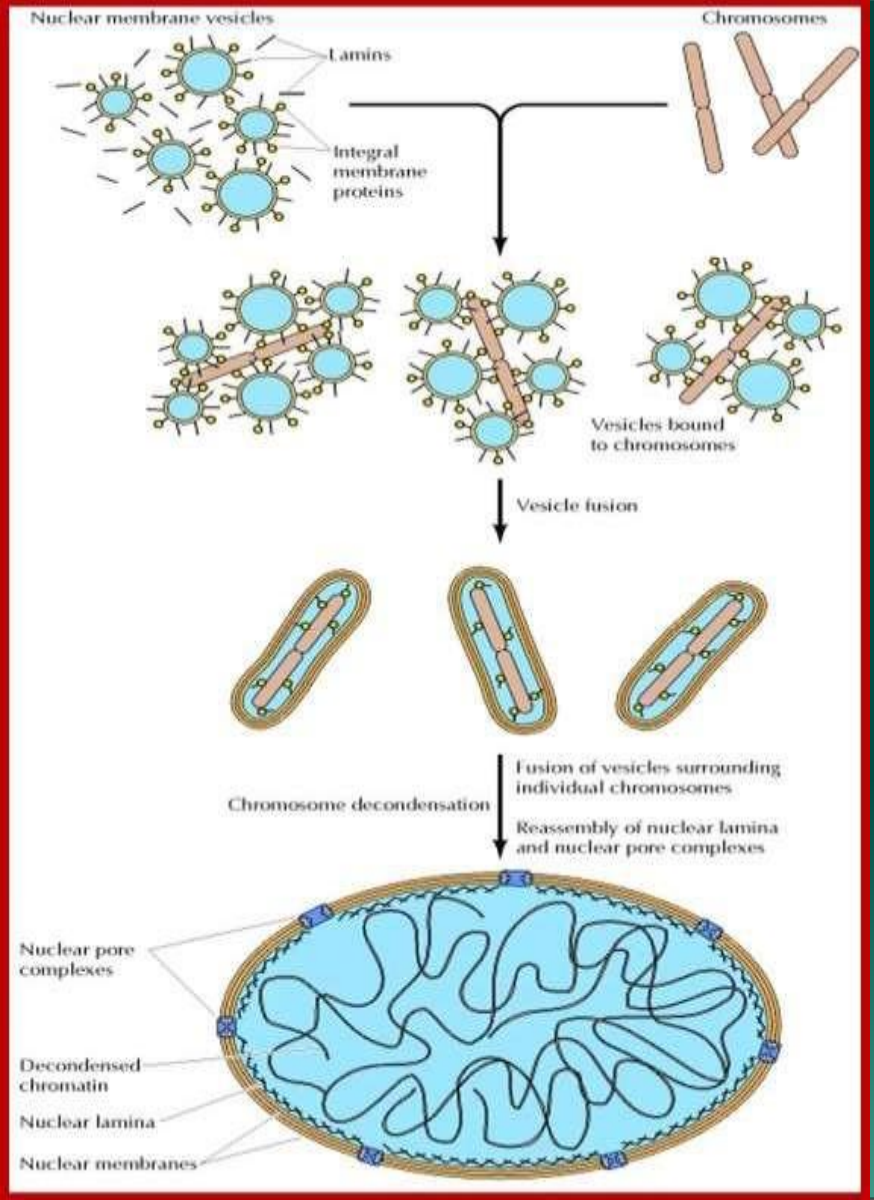


- Disassembly of nuclear envelope involves changes in all its 3 components.
- The nuclear membranes are fragmented into vesicles, nuclear pore complexes dissociates and nuclear lamina depolymerizes into individual lamin dimers.
- The B-type lamins remain associated with the vesicles, but A- and C-type lamins dissociate from the nuclear membrane and are released into the cytosol as free dimers.
- The nuclear pore complexes appear to dissociate into subunits and phosphorylation of one or more nuclear pore proteins may play a role in this disassembly.
- Integral nuclear membrane proteins are also phosphorylated during cell division.



Reappearance of nuclear envelope

- During the completion of mitosis (telophase), two daughter nuclei form around the separated sets of daughter chromosomes.
- The progression from metaphase to anaphase involves the activation of a ubiquitin-mediated proteolysis system that inactivates Cdc2 by degrading its regulatory subunit, Cyclin B.
- Inactivation of Cdc2 leads to dephosphorylation of the proteins that were phosphorylated at the initiation of mitosis, resulting in exit from mitosis and the reformation of interphase nuclei.



Reappearance of nuclear envelope

- The initial step in reformation of nuclear envelope is the binding of the vesicles formed during nuclear membrane breakdown to the surface of chromosomes.
- These vesicles then fuse to form a double membrane around the chromosomes.
- This is followed by reassembly of the nuclear pore complexes, reformation of nuclear lamina and chromosome de-condensation.
- The double membranes surrounding the chromosomes then fuse to with each other to form a complete nuclear membrane.

Functions of lamins

- Provides structural support and maintains integrity of nuclear envelope.
- Plays important role in assembly and disassembly of nuclear envelope during cell division.
- Serves as site for chromosome end attachment.

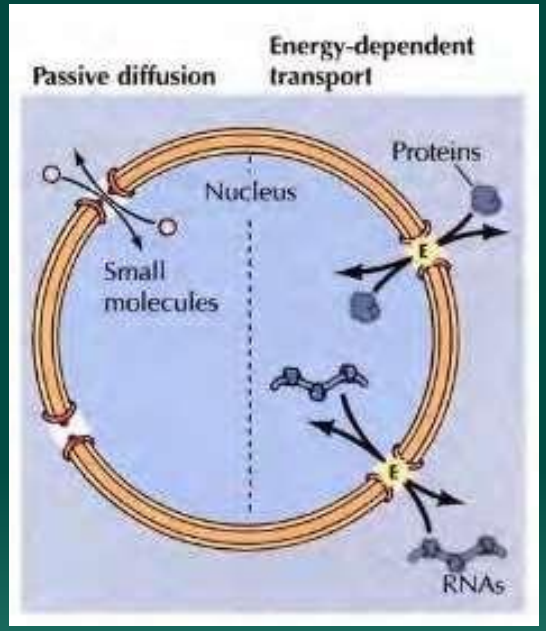
Diseases related to lamin defect

- Defects in lamin gene causes some diseases.
- *LMN*1 defect causes muscular dystrophy which results in production of fragile nucleus in muscle cells.
- Defect in *lamin* gene also causes a disease called progerias, i.e. premature aging. An individual looks like an aged person at an age of 12 – 14 years. This disease can also result from defects in the telomerase enzyme.

Nuclear pore complex

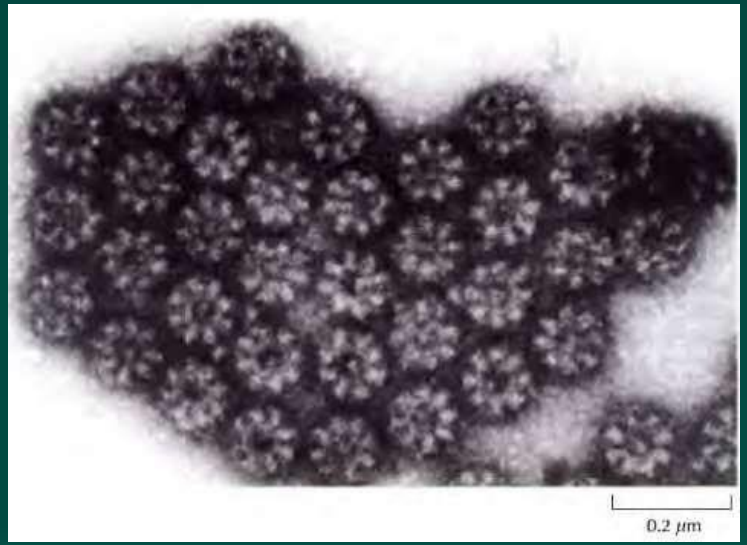
Nuclear Pore Complex

- The nuclear pore complexes are the only channels through which small polar molecules, ions, and macromolecules (proteins and RNAs) can travel between the nucleus and the cytoplasm.
- The nuclear pore complex is an extremely large structure with a diameter of about 120 nm and an estimated molecular mass of approximately 125 million dalton -about 30 times the size of a ribosome.
- By controlling the traffic of molecules between the nucleus and the cytoplasm, the nuclear pore complex plays a fundamental role in the physiology of all eukaryotic cells.
- Nucleoplasmin is the basic constituent of nuclear pore complex.



Structure under electron microscope

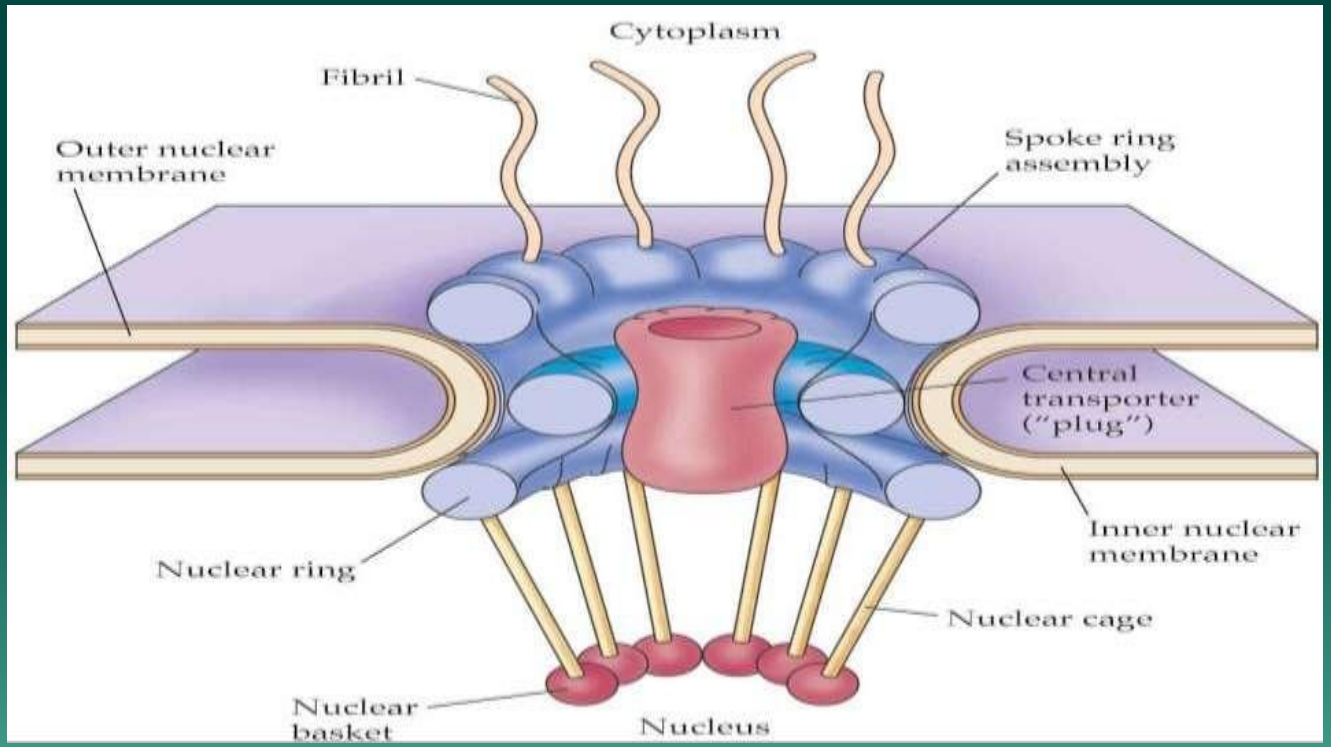
- Visualization of nuclear pore complexes by electron microscopy reveals a structure with eightfold symmetry organized around a large central channel.
- The complex consists of 8 ring-like structures at the periphery and one ring at the center.
- From the central ring, 8 spoke like structures arise and the structure resembles the spokes of a bicycle tire.



- Under higher resolution, the pore complex shows 2 concentric rings superimposed upon one another.
- Each ring consists of 8 individual granules called annuli.
- The outer ring remains attached to the outer nuclear membrane while the inner ring remains attached to the inner nuclear membrane.

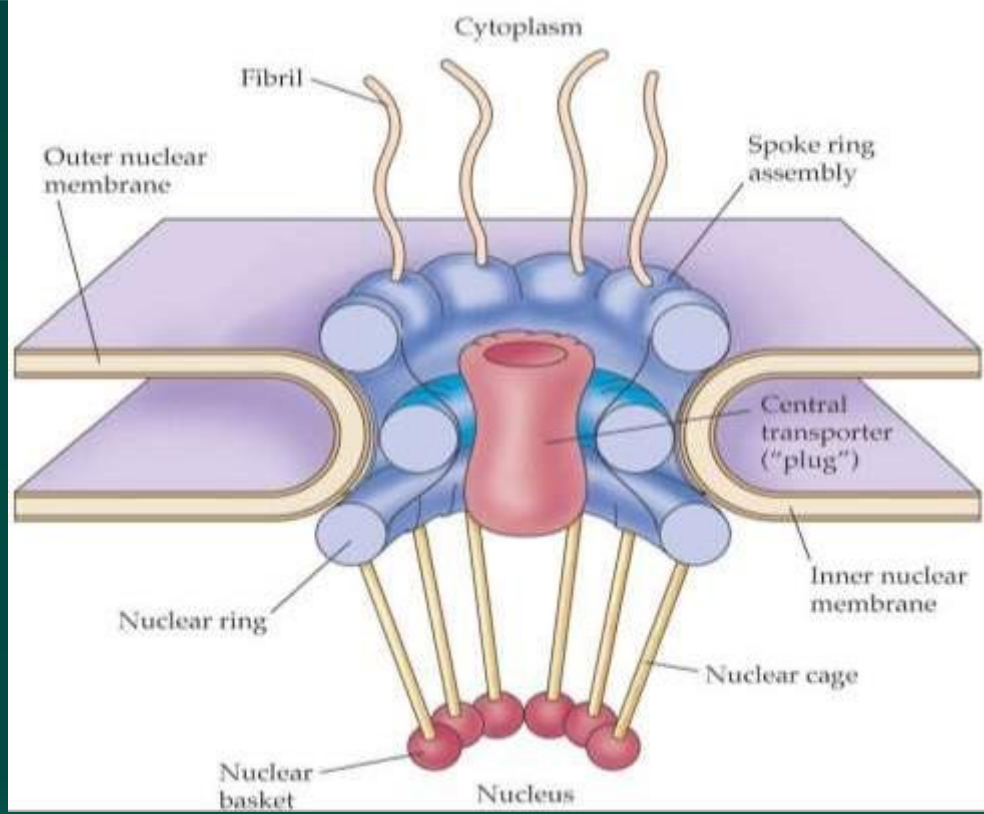
3D structure

- Detailed structural studies, including computer-based image analysis, have led to the development of three-dimensional models of the nuclear pore complex.
- The nuclear pore complex consists of 3 concentric rings superimposed upon one another.



3D structure

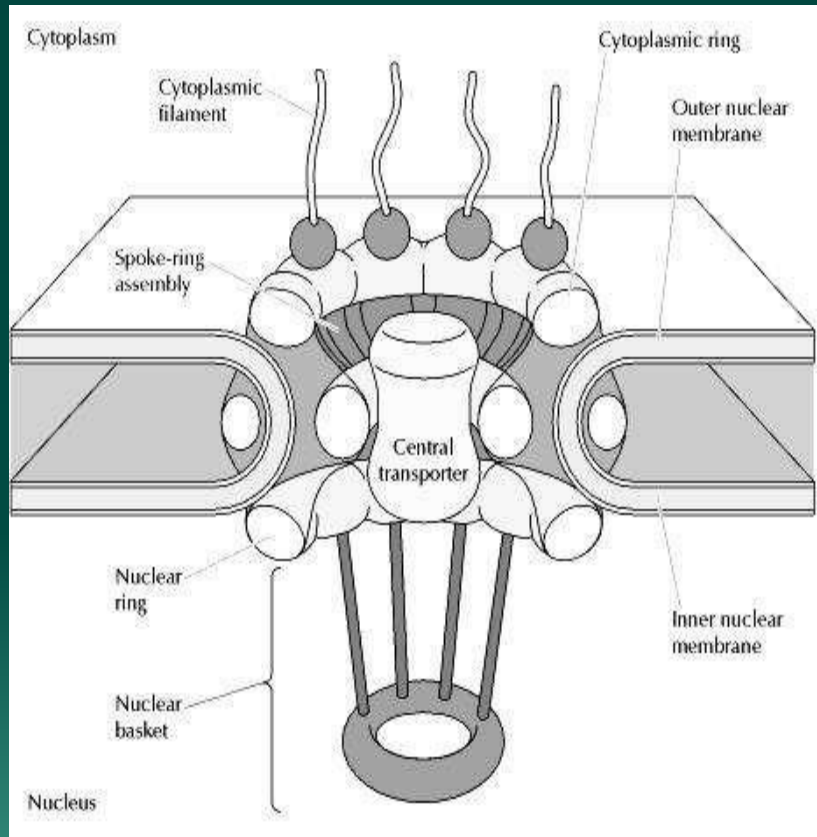
- The outer most ring is the cytoplasmic ring which lies towards the cytoplasm and remains attached to the outer nuclear membrane.
- The inner most ring is the nucleoplasmic ring which lies towards the nucleoplasm and remains attached to the inner nuclear membrane.
- Each of these two rings are composed of 8 annuli.



- 8 protein filaments extends from the cytoplasmic ring towards the cytoplasm.
- 8 protein filaments also projects from the nucleoplasmic ring towards the nucleoplasm where they form a nuclear basket.
- These filaments send signals to open the pore complex to the central transporter.

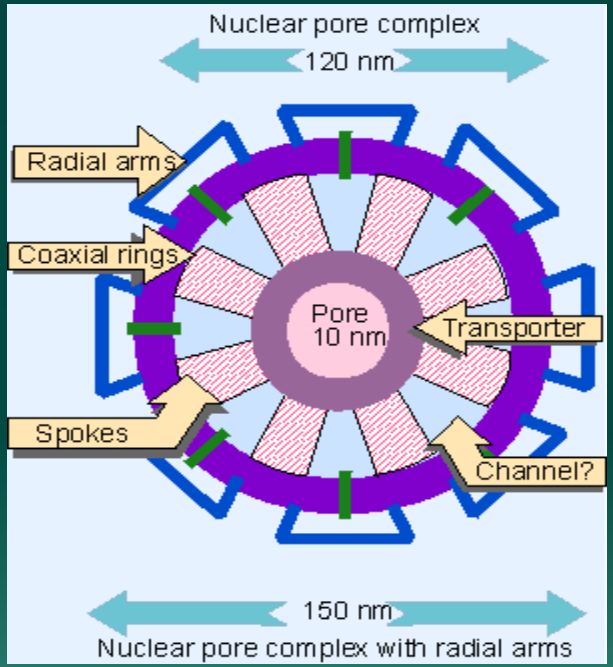
3D structure

- The central ring is composed of the spoke-ring assembly.
- It consists of an assembly of 8 spokes around a central channel.
- Each of these rings bifurcates and attaches itself to the cytoplasmic as well as the nucleoplasmic rings.
- This spoke-ring assembly is anchored within the nuclear envelope at sites of fusion between the outer and inner nuclear membranes.
- The outsides of the cytoplasmic and nucleoplasmic rings are connected to radial arms. The interior is connected to spokes that project towards the transporter that contains the central pore.
- The 8 radial arms outside the ring may be responsible for anchoring the pore complex in the nuclear envelope; they penetrate the membrane.



3D structure

- The central channel formed by the spoke-ring assembly is approximately 10 nm in diameter and can open upto at least 25 nm, which is wide enough to accommodate the macromolecules.
- The central channel often appears to contain a structure called the central plug, the nature of which is unclear.
- The assembly of spoke-ring complex creates 8 smaller channels between the spokes which have diameter of about 10 nm. They form open aqueous channels through which small molecules diffuse and continuity of cytoplasm and nucleus is maintained.
- This model of nuclear pore complex resembles the structure of a waste paper basket and hence is called ‘Basket model’.



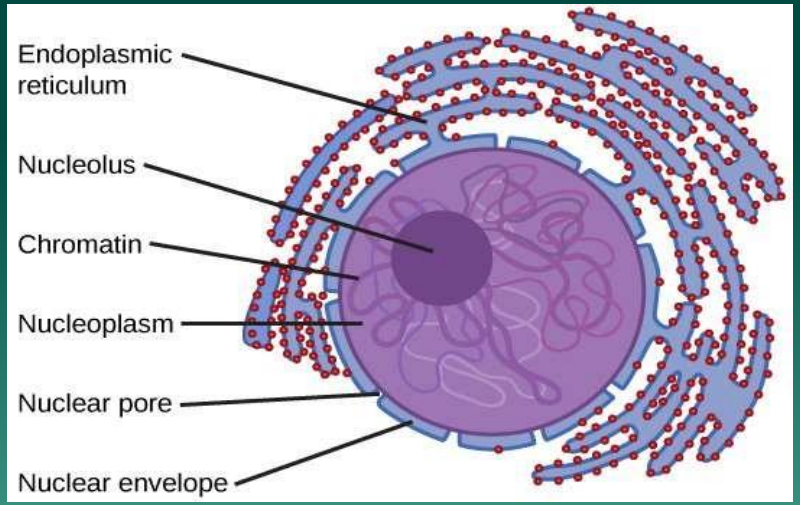
Transport through nuclear pore complex

- Depending on their sizes, molecules can travel through the nuclear pore complex.
- Small molecules (ions, nucleotides, and other small molecules) with molecular mass **<20-40 kD** pass rapidly across the nuclear envelope in either directions. They **diffuse passively** through open aqueous channels in the pore complex. The diameter of these channels is approximately 10 nm.
- Proteins having molecular mass **<50 kD** can pass through the central pore (**passive diffusion**).
- Most proteins and RNAs, however, are **>50kD** and are unable to pass through these open channels. Instead, these macromolecules pass through the central pore by an **active process** in which appropriate proteins and RNAs are recognized and selectively transported in a specific direction (nucleus to cytoplasm or cytoplasm to nucleus).
- Proteins require **signals** to be transported through the pore. The **NILS and NES** consist of short sequences that are necessary and sufficient for proteins to be transported through the pores into or out of the nucleus.

Nucleolus

Nucleolus

- The most prominent nuclear body is the nucleolus, which is the site of rRNA transcription and processing as well as aspects of ribosome assembly.
- It is a sub-organelle of the nucleus, which itself is an organelle.
- The nucleolus is roughly spherical and is surrounded by a layer of condensed chromatin. *No membrane separates the nucleolus from the rest of the nucleus*.
- The nucleolus is visible in the interphase (G1 and G2) of the cell cycle. On the onset of division, it disappears and again reappears at the end of the division. It is not a permanent structure of the cell.
- Existence of nucleolus was first reported by Fontana in 1781.



Structure under light microscope

- Under light microscope, nucleolus is a dense body of variable size and shape.
- It is a highly refringent body.
- It is feulgen-negative after fixation, indicating absence of DNA and stains with pyronin and absorbs UV light at 260nm which indicates presence of RNA components.
- It may be surrounded by a ring of feulgen-positive chromatin which represents the heterochromatic regions of nucleolar chromosome.
- The 3 main components of isolated nucleolar fractions are:
  - DNA
  - RNA
  - Protein
- Chemically nucleolus is made up of ribonucleoprotein fibrils (RNP fibrils), i.e. RNA+Proteins.

Structure under electron microscope

- Under electron microscope, nucleolus shows some electron opaque and some electron transparent zones. The granular and fibrilar zones are electron opaque.

- The following components can be distinguished:

  - **Granular component:** Contains ribonucleoprotein granules of 15-18 nm diameter, usually distributed towards the periphery (*pars granulosa*). Although the nucleolus is not bound by any membrane, due to presence of the granular components the border with the surrounding chromatin and nucleoplasm is usually distinct.

  - **Fibrillar component:** It includes a fibrillar region of a meshwork of filaments of 4-6 nm diameter containing RNA and a considerable amount of protein (*pars fibrosa*). The fibrillar component includes:
    - Fibrillar center
    - Dense fibrillar component

Structure under electron microscope

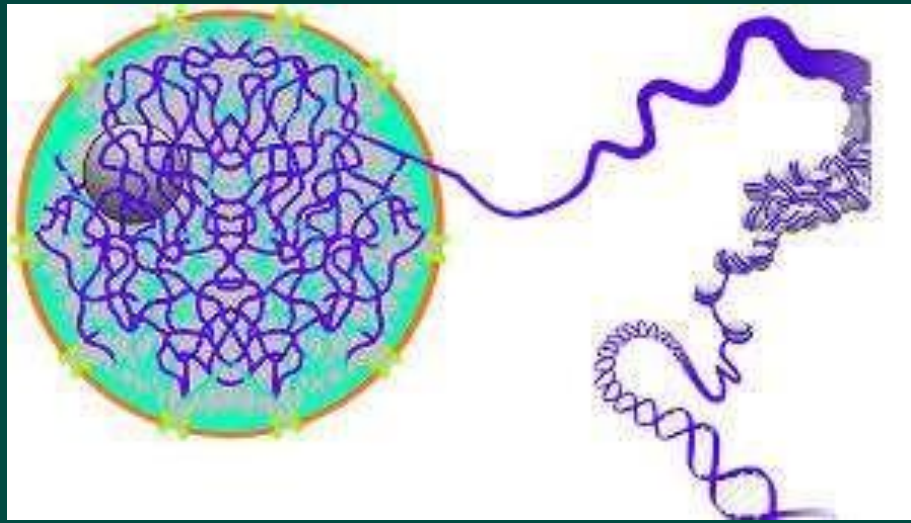
- **Fibrillar center:** Made up of a network of fine (4-5 nm thick) fibrils. The fibrillar center is typically globular with diameter ranging from 50 – 1000nm. Cells with lower cellular activity usually has fewer fibrillar center than others.

- **Dense fibrillar component:** Made up of fine (3-5 nm thick) fibrils which are densely packed. It usually surround the fibrillar component when they are present in a meshwork. The amount of dense fibrillar component roughly reflects the nucleolar engagement in ribosomal biogenesis. The are now considered as sites where rDNA cistrons are located.

- **DNA binding proteins:** Composed of DNA binding proteins which extends into the interstices of nucleolonema (*pars chromosoma*).

- **Matrix:** It is a heterogeneous proteinaceous matrix.

- **Nucleolonema:** it is a course thread composed either of *pars fibrosa* or *pars granulosa* or both. The ribonucleoproteins involved are the precursors of cytoplasmic ribosomes.



MOLECULAR ORGANIZATION OF CHROMATIN

Chromosomes and chromatin

- The genomes of eukaryotes are composed of multiple chromosomes, each containing a linear molecule of DNA.
- The DNA of eukaryotic cells is tightly bound to small basic proteins (histones) that package the DNA in an orderly way in the cell nucleus.
- For example, the total extended length of DNA in a human cell is nearly 2 meters, but this DNA must fit into a nucleus with a diameter of only 5 to 10 μm
- The degree of compaction of DNA, i.e. how many turns of DNA is required to accommodate it into the cell is denoted by an index called Packaging ratio.

$$\text{Packaging ratio} = \frac{\text{Length of DNA molecule}}{\text{Length of the unit that accommodates DNA}}$$

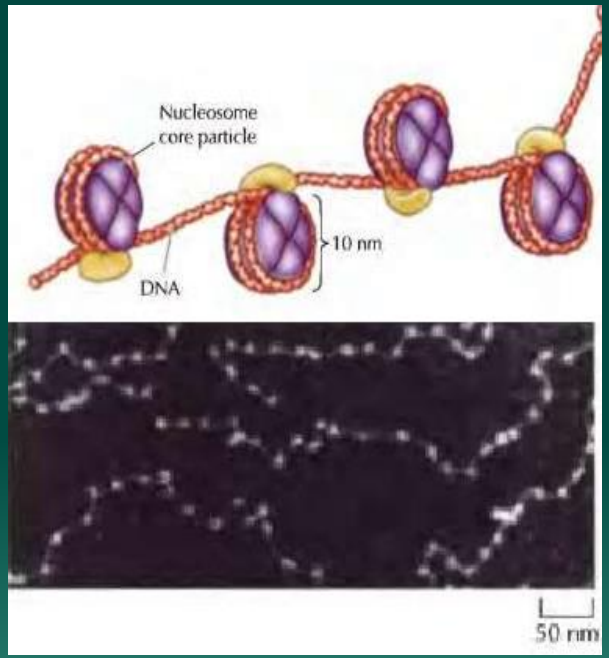
Chromatin

- The complexes between eukaryotic DNA and proteins are called chromatin.
- The major constituents of eukaryotic chromatins are DNA, RNA, basic proteins called histones and non-histone proteins.
- The contents of RNA and non-histone proteins is variable but DNA and histones are always present in a fixed ratio of 1:1.
- Non-histone proteins are heterogenous.
- Histones are small basic proteins containing a high proportion of basic amino acids (arginine and lysine) that facilitate binding to the negatively charged DNA molecule.
-
- The 4 major histones – H2A, H2B, H3 and H4 – are among the most conserved proteins known. These 4 'core histones' are present in equimolar amounts.
- Histone H1 is not conserved and is rather loosely associated with the chromatin. It is involved in maintaining higher order folding of chromatin.

DNA PACKAGING

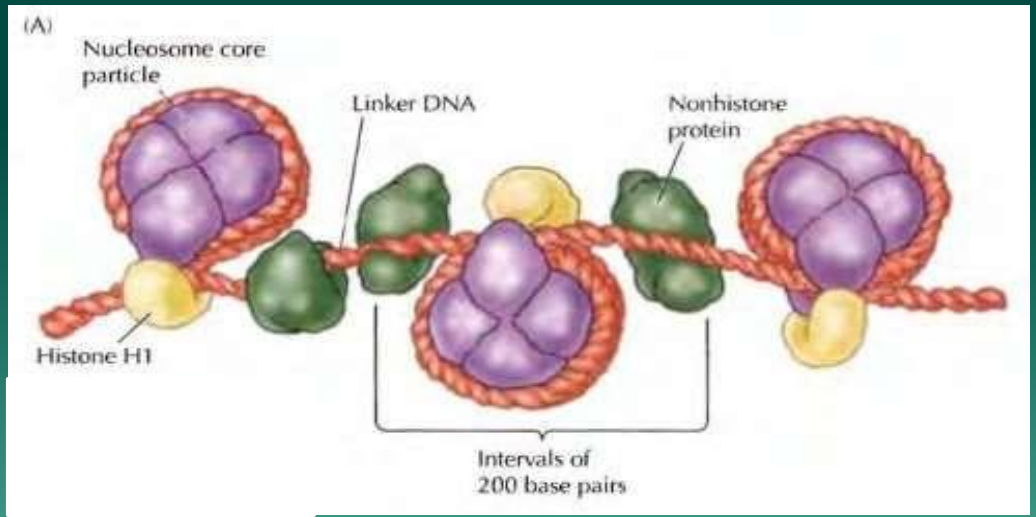
Nucleosome model: 1st stage of DNA compaction

- The basic structural unit of chromatin, the nucleosome, was described by Roger Kornberg in 1974. Two types of experiments led to his proposals:
  - Digestion of chromatin by micrococcal enzyme (which digests DNA)
  - Electron microscopic study
- When interphase was isolated and observed under electron microscope, it revealed a structure containing huge number of beads on a string of DNA.
- This is the 'beads on a string configuration' proposed by Kornberg. It represents the 10 nm chromatin fiber.
- Each bead represented a nucleosome.



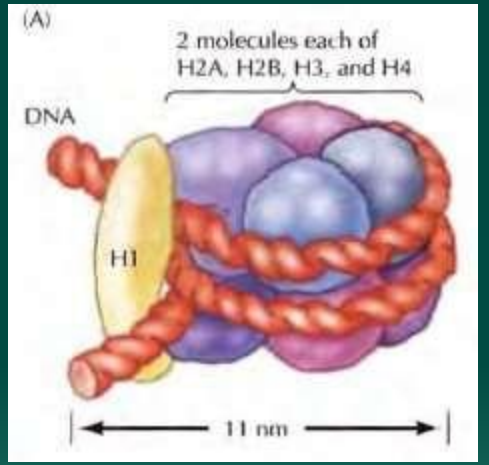
Nucleosome model: 1st stage of DNA compaction

- Nucleosome is flat disc-shaped particle, 11 nm in diameter and 5.7 nm in height.
- Neighboring nucleosomes are connected by linker DNA.
- The DNA coils 1 ¾th turn around each nucleosome and these turns are sealed off by an H1 (histone) molecule.
- After leaving a nucleosome and before entering the next nucleosome, the length of the linker DNA varies from 8 – 114 bp (avg. 50 bp).



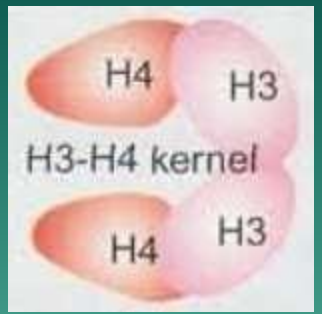
Nucleosome model: 1st stage of DNA compaction

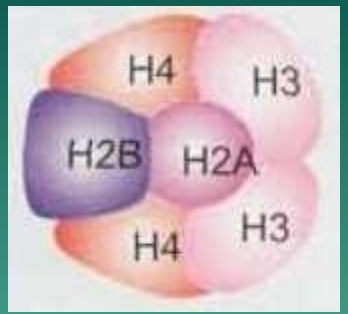
- Studies of digestion of chromatin by micrococcal nuclease showed that the DNA was cut into multiples of a unit size which was 200 bp. This 200 bp fragment is called mononucleosome.
- The nucleosome model proposes that the 4 core histones – H2A, H2B, H3 and H4 – are arranged in octamers containing 2 copies of each of them, every 200 bp of DNA.
- This histone octamer is coiled by DNA from outside.
- If digestion by micrococcal enzyme continues further, 166 bp fragments are formed. These are called trimmed nucleosomes or chromatosomes. It accommodates 166 bp DNA wrapped around H2A, H2B, H3 and H4 and sealed by H1.
- Further digestion yields nucleosome core particles which has 147 bp DNA along with H2A, H2B, H3 and H4.

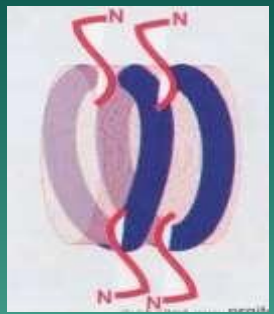


Nucleosome model: 1st stage of DNA compaction

- Two molecules of each of H3 and H4 associate with each other to form a tetramer with a horse-shoe shape. This is the 'kernel structure' of the nucleosome.
- The H2A-H2B dimers remain associated with this kernel structure – one on the upper side and one below the kernel. This constitutes the structure of core nucleosome.
- Several tail-like structures arise from the histones of the nucleosome core. These are histone tails which plays important functions.
- The histone tails interact with those of its neighboring nucleosome cores and thus helps in compaction. Histone tail modification plays crucial role in controlling gene activity.
- The DNA of a 10 nm fiber has a packing that is about 6-fold.

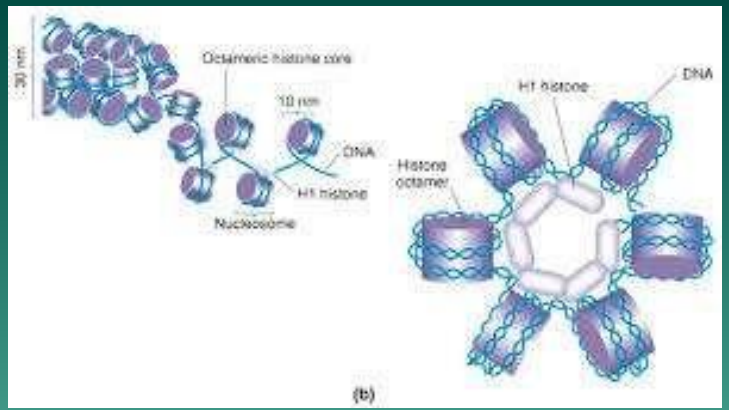






Solenoid model: 2nd stage of DNA compaction

- The ‘solenoid model’ was proposed by Klug.
- This 30 nm fiber of chromatin probably arises from the folding of the nucleosome chain in a solenoid structure having about 6 nucleosomes per turn.
- This represents a model of higher level packaging to explain the chromatin compaction during metaphase.
- The whole structure is stabilized by interactions between H1 molecules in the neighboring nucleosomes. The H1 molecules can interact with each other because they are located in the ‘central hole’ of the solenoid.
- H1 is thought to play a fundamental role in this condensation because solenoids can not be formed when H1-depleted chromatin is treated in the same way.
- The DNA of a 30 nm solenoid has a packing that is about 40-fold.

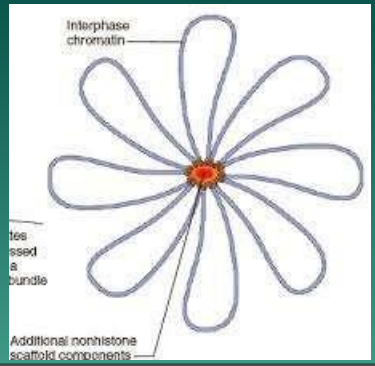


Histone depleted structure

- When metaphase chromosomes are treated with dextran sulphate (a polyanion), it starts to bind the cations and the histones are separated from the complex.
- This is the ‘histone depleted structure’.
- It consists of a pair of central axis (scaffold) made up of non-histone proteins from which a number of DNA loops generate. Each non-histone axis bearing DNA loops from a single DNA molecule corresponds to one chromatid of the chromosome.
- The axis was discovered by Laemii.

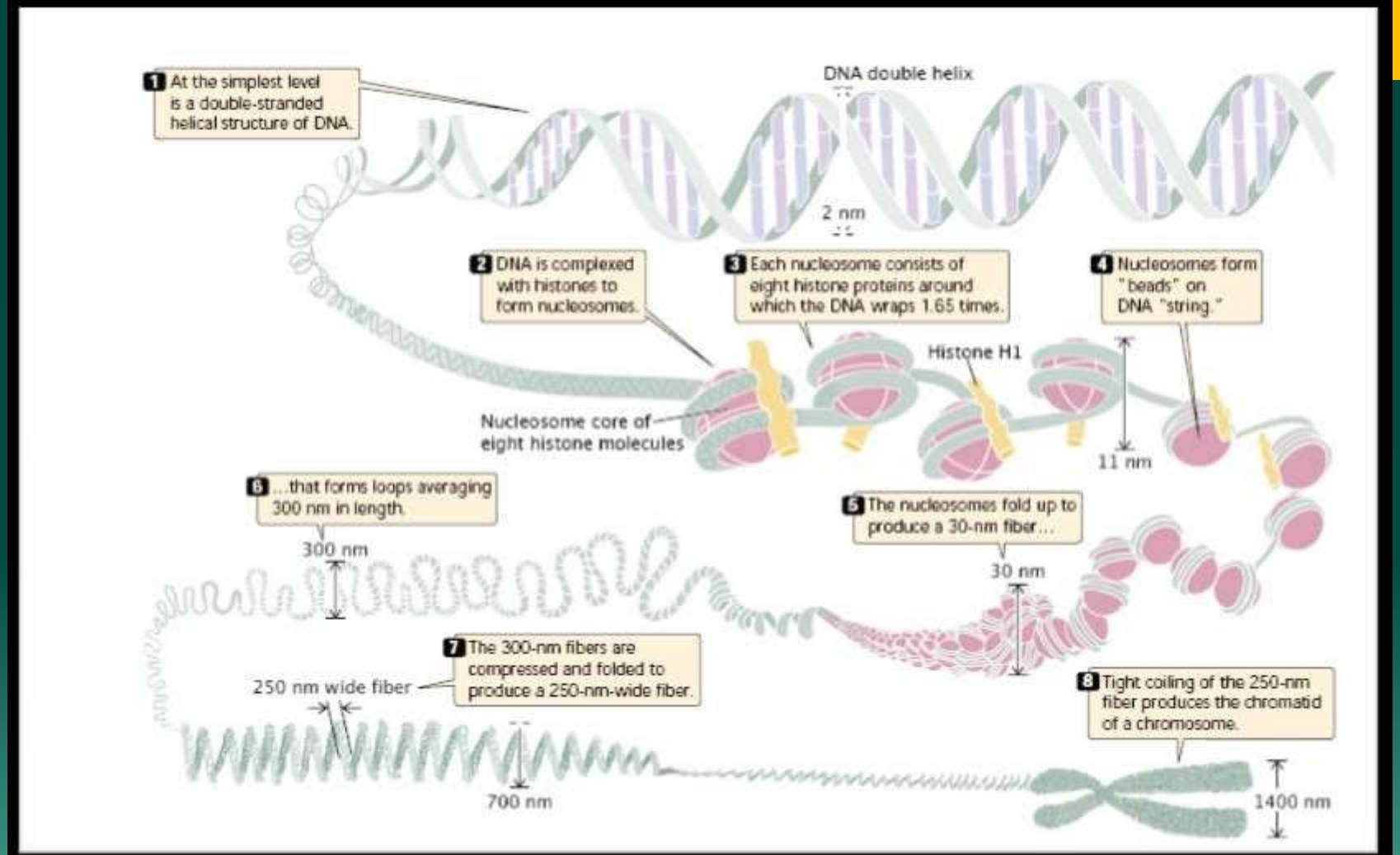
Radial loop scaffold model: 3rd stage of DNA compaction

- The next model that depicts the maximum condensation of chromatin fiber is the radial loop scaffold model.
- This model propose that certain non-histone proteins including topoisomerase II bind to chromatin every 60-100 kb and tether the supercoiled nucleosome-studded 30 nm fiber into structural loops (due to formation of knots on a straight chromatin fiber, its length can be reduced).
- Other non-histone proteins may, in turn, gather the loops into daisy-like rosettes and additional non-histone proteins may then compress the rosette centers into a compact bundle.
- A range of non-histones thus form the condensation scaffold. This proposal of looping and gathering is known as the ‘radial loop scaffold model’ of compaction.



Radial loop scaffold model: 3rd stage of DNA compaction

- Recent analysis of DNA indicates that special, irregularly spaced, repetitive base sequences associate with non-histone proteins to define the chromatin loops. These stretches of DNA are known as scaffold associated regions or SARs. SARs are most likely the sites at which the DNA is anchored to the condensation scaffold.
- Other forms of SARs are called matrix attachment regions or MARs.
- The non-histone proteins exists as matrix proteins in the interphase and give rise to scaffolds. The sequence of DNA that binds to SAR at metaphase, binds with MAR during interphase. Hence, both SAR and MAR have same DNA sequence bound to them.
- Now it achieves a 250-fold compaction of roughly 40-fold compacted 30nm fiber.
- This radial loop scaffold model is a hypothetical structure.


1 At the simplest level is a double-stranded helical structure of DNA.
DNA double helix
2 nm
2 DNA is complexed with histones to form nucleosomes.
3 Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
4 Nucleosomes form "beads" on DNA "string."
Histone H1
Nucleosome core of eight histone molecules
11 nm
6 ...that forms loops averaging 300 nm in length.
300 nm
5 The nucleosomes fold up to produce a 30-nm fiber...
30 nm
7 The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
250 nm wide fiber
8 Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.
700 nm
1400 nm

Thank you