Structure of Nucleus and its Components

overview

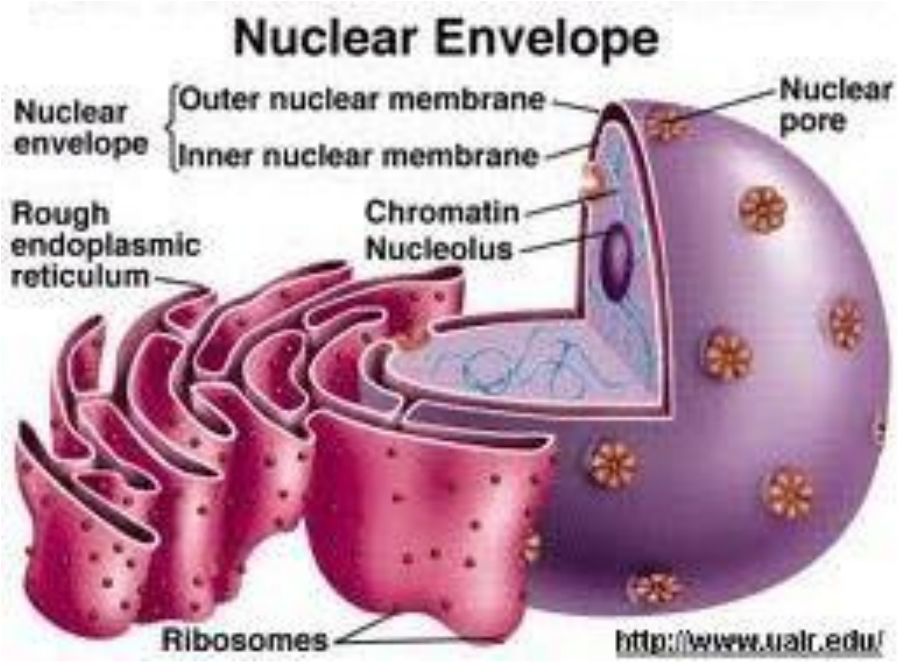
- Introduction
- Components of Nucleus
  - The Nuclear Envelope
  - The Nuclear Pore
  - The Nuclear pore complex
  - The Nucleolus
  - Chromatin/ Molecular structure of chromosome
- Nuclear DNA
- Mitochondrial DNA

Introduction

- Prominent & Characteristic features
- ‘Eukaryon’ means ‘true nucleus’
- Very essence of eukaryote – membrane bounded nucleus
- Imp functions;
  - Physically separates DNA from the cytoplasm’s complex metabolic machinery
  - Nuclear membrane serve as boundary

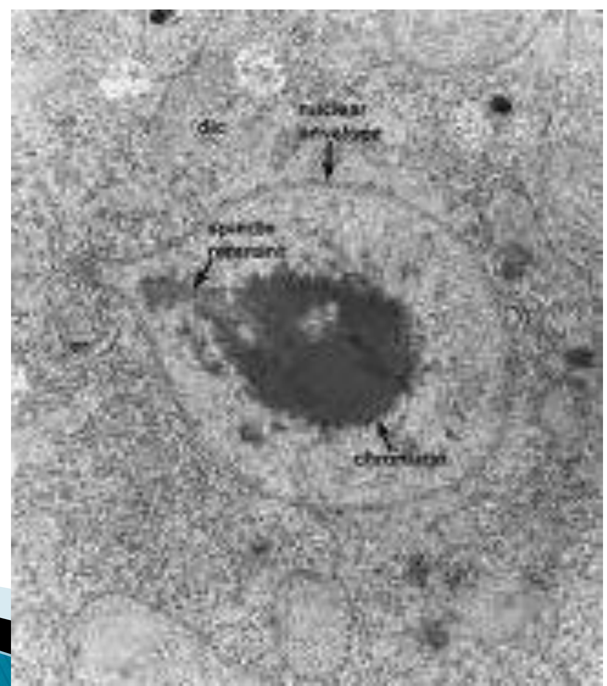
Components of Nucleus

1. Nuclear Envelope – pore riddled
2. Nucleoplasm – Fluid interior portion
3. Nucleolus – Dense cluster of RNA & Proteins – ribosomes
4. Chromatin – all DNA + Proteins



The Nuclear Envelope

- Existence of nuclear membrane – late 19th century
- Phase contrast microscopy – 20th century
- Further investigations - double membrane with space between 2 phospholipid bi layers
- Additional insight – advent of Electron microscopy.



- Inner and outer nuclear membrane with perinuclear space
- 7-8 nm thick
- Inner membrane lined with fiber network – Nuclear lamina- 10 to 40 nm
- Nuclear lamina – intermediate filament (protein) called as Lamins
- Nuclear lamina – support to NE & attachment sites for chromatin.

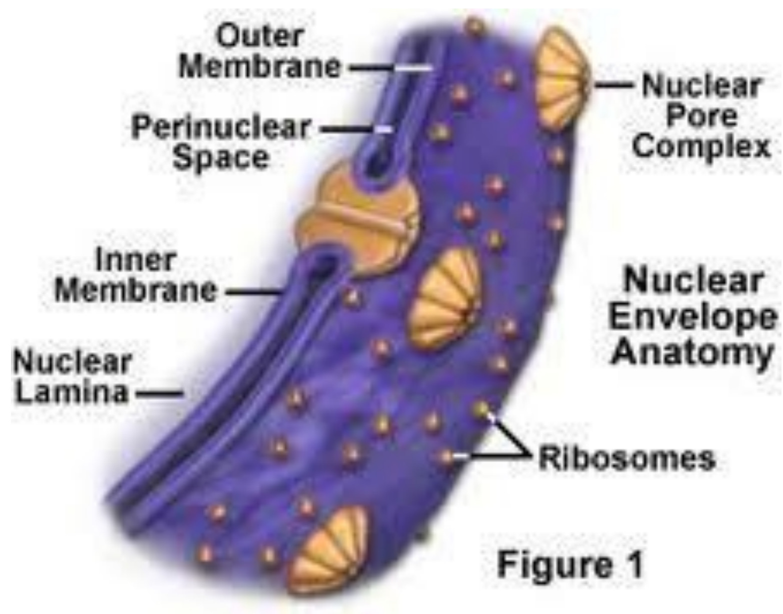
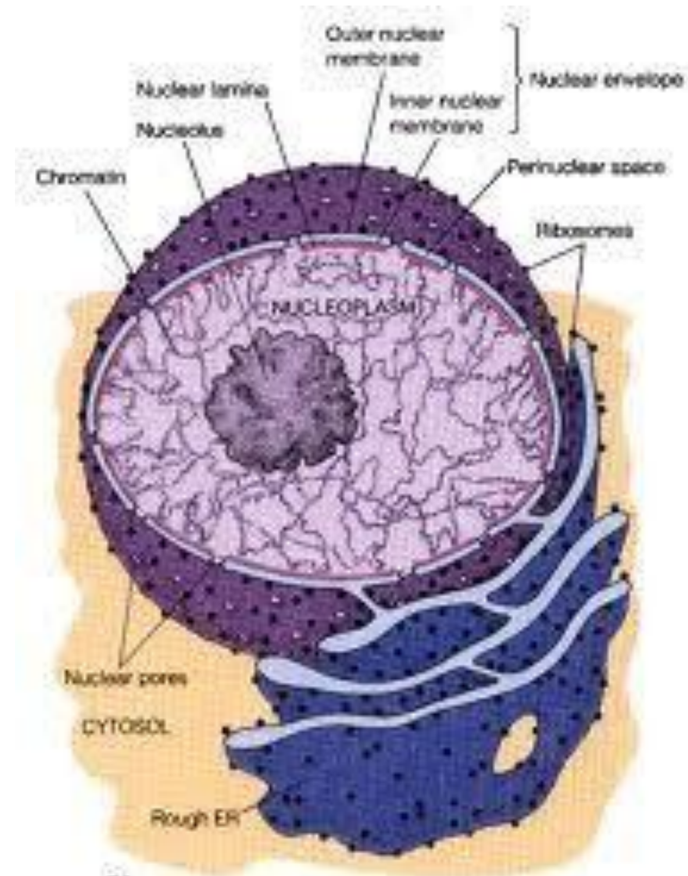


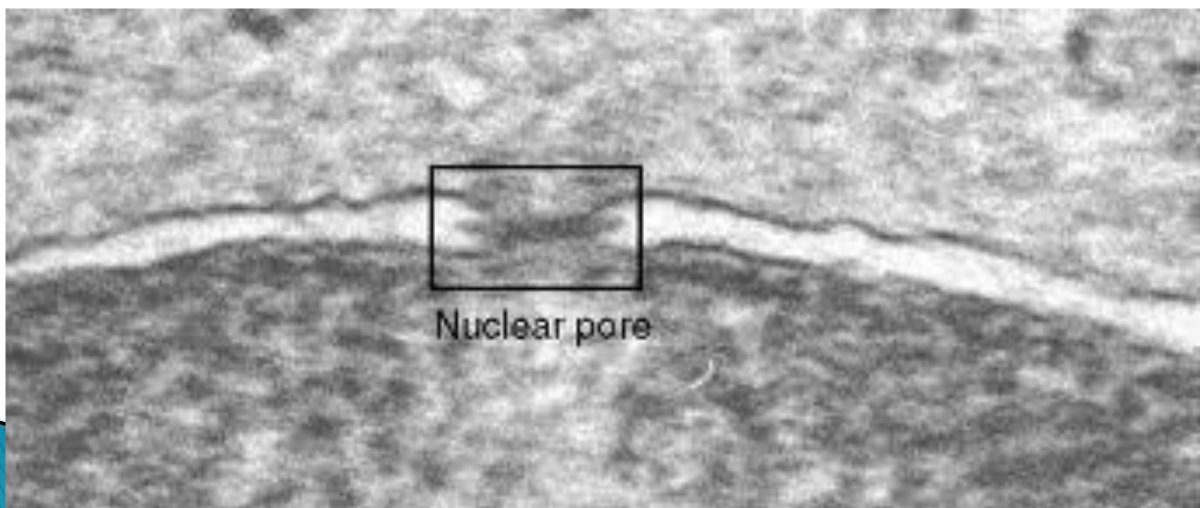
Figure 1

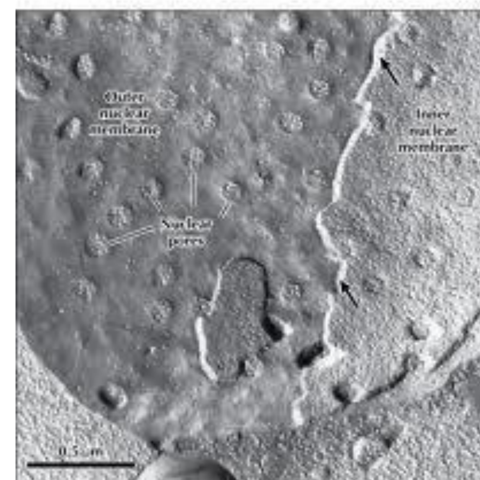
- Outer membrane - continuous with ER
- Outer membrane studded with ribosomes – protein synthesis.
- Perinuclear space – 20 to 40 nm continuous with cisternae of ER
- E/M - filaments of cytoskeleton extend outward cytoplasm – anchored to organells/ plasma membrane – known as Nuclear matrix
- Matrix - shape of nucleus



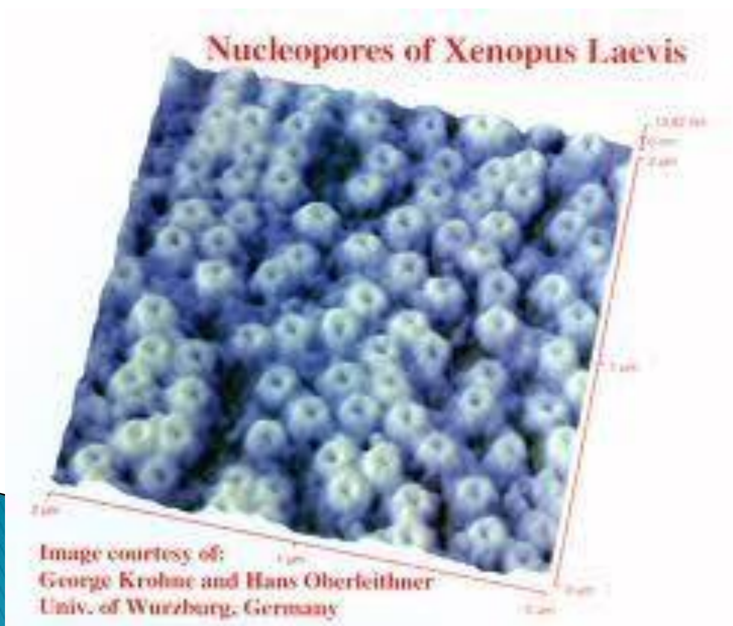
The Nuclear Pore

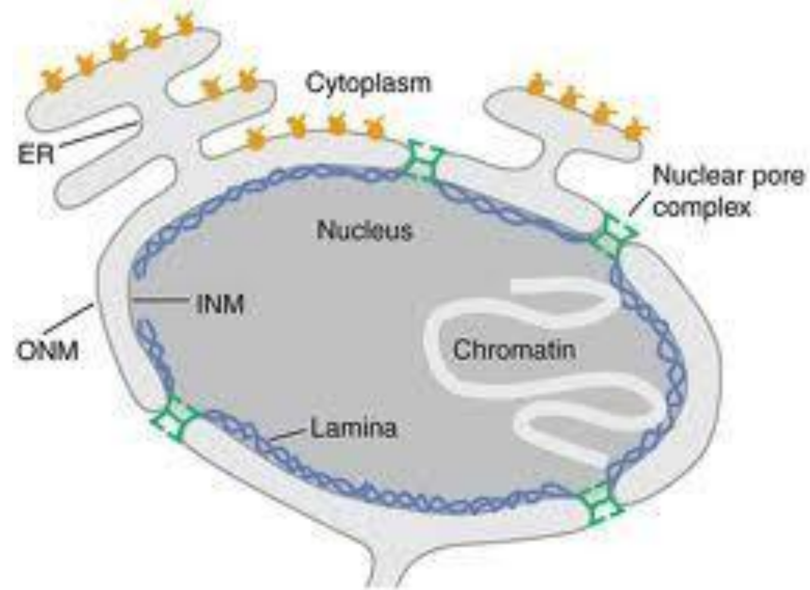
- Most distinctive feature of NE
- Small cylindrical channels –direct contact b/w cytosol & Nucleoplasm
- Readily visible – freeze fracture microscopy
- Density - cell type & activity





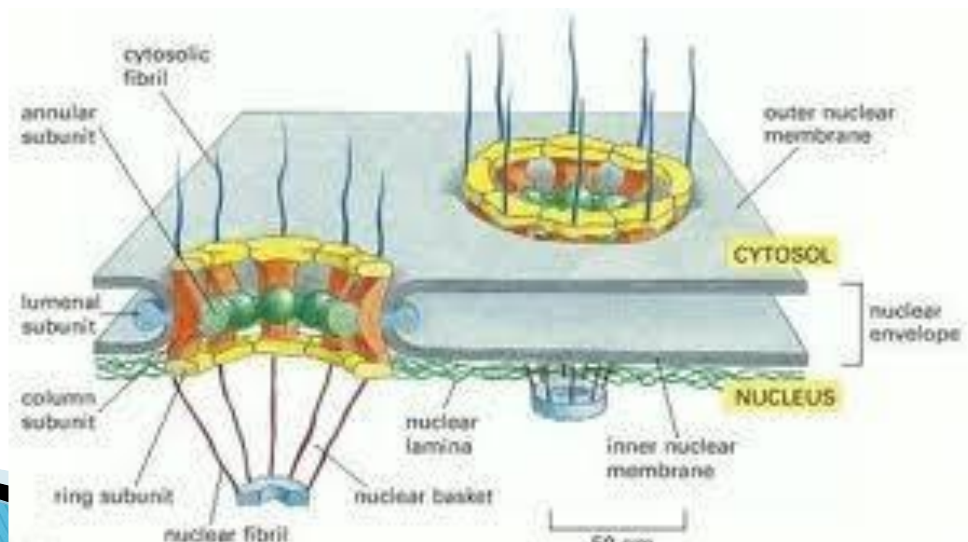
- Mammalian nucleus – 3000 to 4000 pores
- Density 10-20 pores/sq.micrometer
- Oocytes of *Xenopus laevis* (South African clawed toad) – Large nuclei, > 10 million
- Inner & outer membranes fused
- Structural complexity – control transport of key molecules



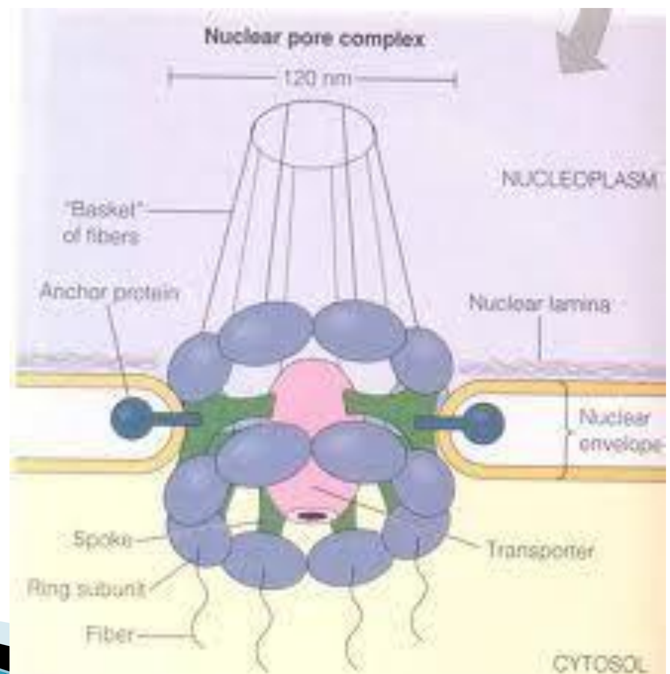


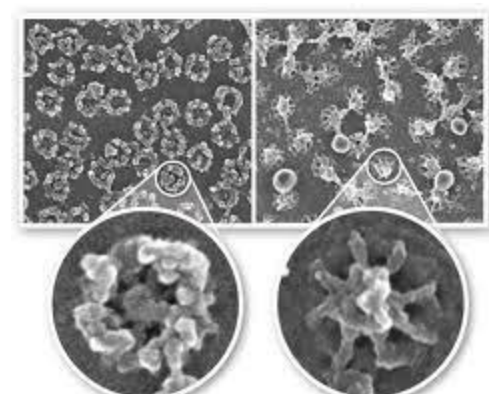
The Nuclear Pore Complex(NPC)

- Nuclear pore - lined with intricate protein structure
- Diameter 120 nm, overall mass 120 million da, 100 or more different polypeptide subunits
- E/M – octagonal arrangement of subunits
- Shape – wheel lying on its side within NE



- Two parallel rings – rim of wheel – 8 subunits
- 8 spokes extend from rings to wheel hub – Central granule transporter (move macromolecules across NE)
- Anchor protein – proteins extend from rim into perinuclear space.
- Fibers extend from rings to cytosol & Nucleoplasm (form a basket – cage/ fish trap)





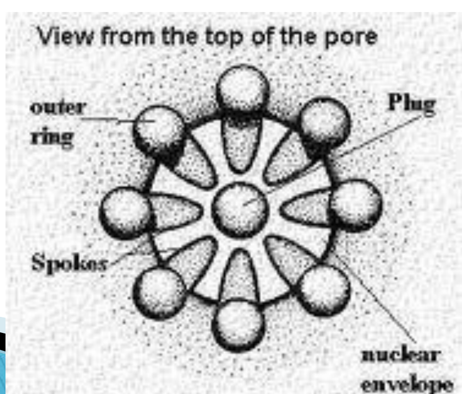
Transport across NE

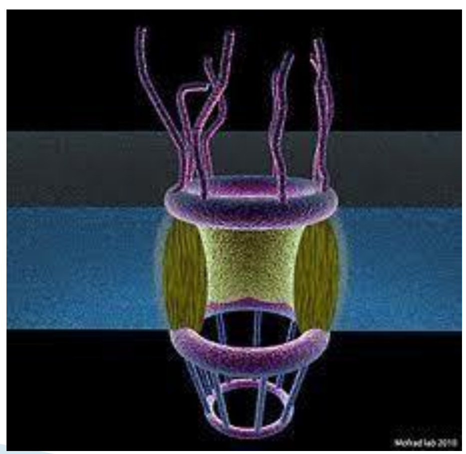
- Enzymes & proteins – replication & transcription must be imported from cytoplasm
- RNA & ribosomes for protein synthesis in cytoplasm must be obtained from nucleus
- Nature’s solution – evolution of eukaryotic NE with pores

- Ribosomes partially assembled in nucleus – subunits – RNA+Protein
- For protein synthesis – cytoplasm – subunits combined to form functional ribosomes
- Actively growing mammalian cell – 20,000 ribosomal units per minute
- 3000 to 4000 nuclear pores
- Transport rate of ribosomal subunits – 5 to 6units/minute/pore
- During replication histones needed @ 3,00,000 molecules/min
- Rate of inward movement – 100 histones/minute/pore

Passive transport of small molecules

- Apart from Macromolecules pores allow – small molecules and ions.
- Acqueous channels – direct contact cytosol and nucleoplasm
- Permeable to small molecules & ions
- Nucleoside triphosphates required for DNA & RNA synthesis diffuse freely through pores
- Small molecules – metabolic pathways
- Eight 9nm channels between the spokes + 9 nm channel at center of transporter





The Nucleolus

- Ribosome factory
- large, prominent structures
- Doesn't have membrane
- E/M it consists;

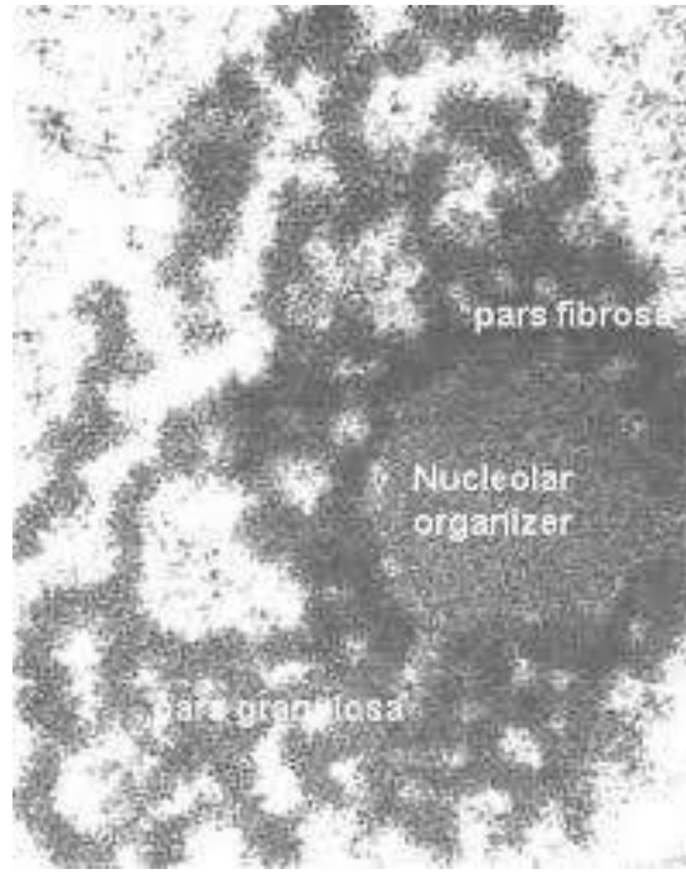
1. Fibrillar component

- DNA (unraveled chromatin loops) +
  RNA component of ribosome

- DNA carries genes for rRNA - NOR

- RNA is r RNA – synthesized & processed

- dense areas, transcription going on

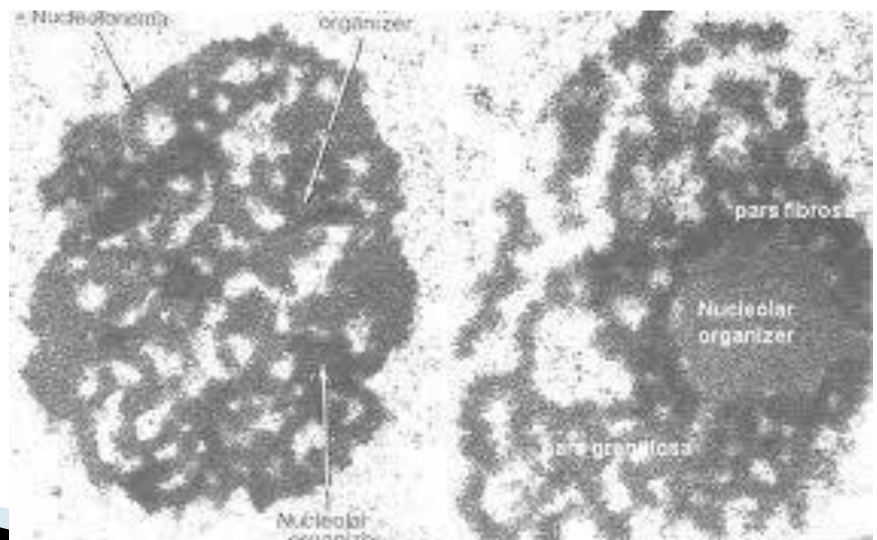


2. Granular component

- rRNA molecules + Proteins

- forms ribosomal subunits – exported to cytoplasm

- Size correlated with level of activity
- Cells having high rate of protein synthesis –many ribosomes –20 to 25% of nucleus
- Main difference – granular component present



- During cell division – condensation of chromatin into compact chromosomes
- Shrinkage and disappearance of nuclei
- rRNA & protein disperse/ degraded
- After mitosis – chromatin uncoils, NOR loop out, rRNA synthesis resumes
- Many tiny nucleoli visible – fuse & become large nucleolus

Chromatin/ Molecular structure of chromosomes

- Eukaryotic chromosomes – two broad components.

1. Nucleic acids:

   - DNA (primary nucleic acid)

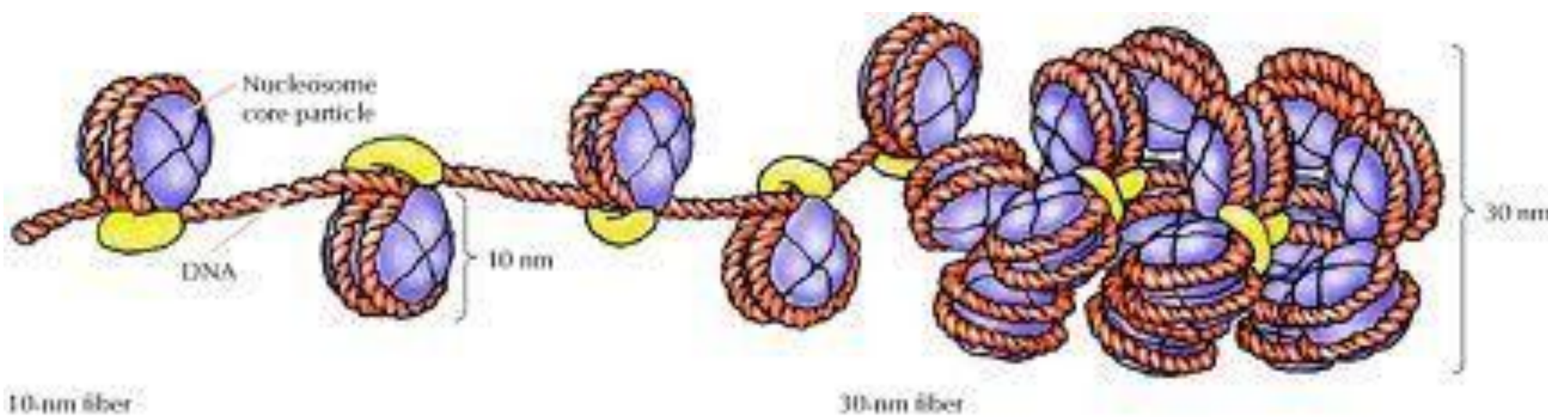
   + small amount of RNA (transit to the cytoplasm)

2. Proteins:

   i. Histones (basic pH) – core histones (H2A, H2B, H3 & H4), Linker histone (H1)

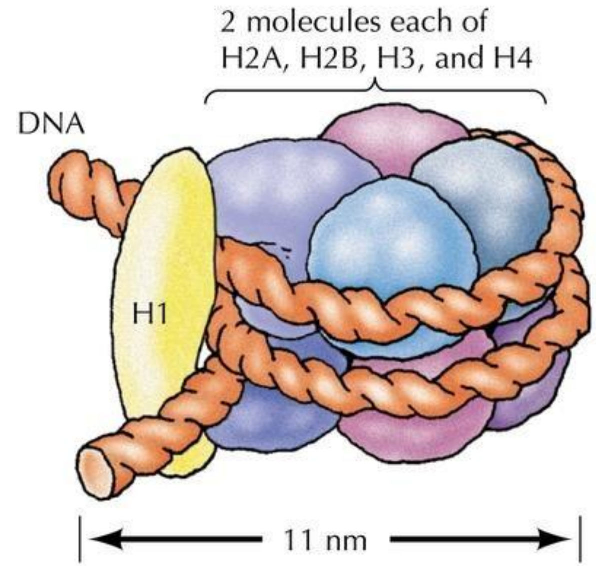
   ii. Non Histone proteins

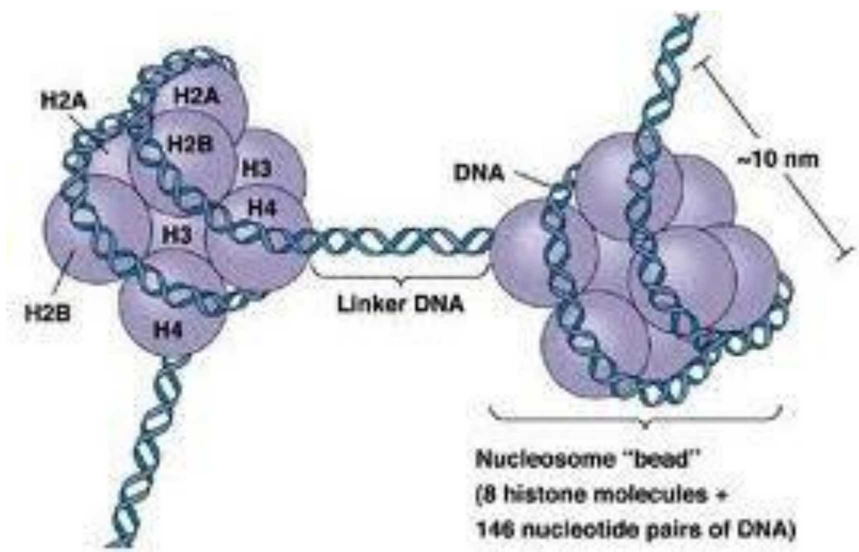
- Histones bind to –vely charged DNA – stability to the DNA
- Mixture of DNA & proteins – basic structural unit of chromosomes - chromatin fiber
- E/M examination of intephase chromatin – ellipsoidal beads joined by linker DNA known as Nucleosomes.



Nucleosome

- Simplest packing strand of DNA
- 146 bp DNA wrapped around histone octamer
- Octamer = 2 copies of 4 core histones
- DNA length varies b/w species
- Core DNA – DNA associated with histone octamer
- Linker DNA – DNA b/w histone octamer – 8 to 114 bp





Model of packing of chromatin and the chromosome scaffold in metaphase chromosome

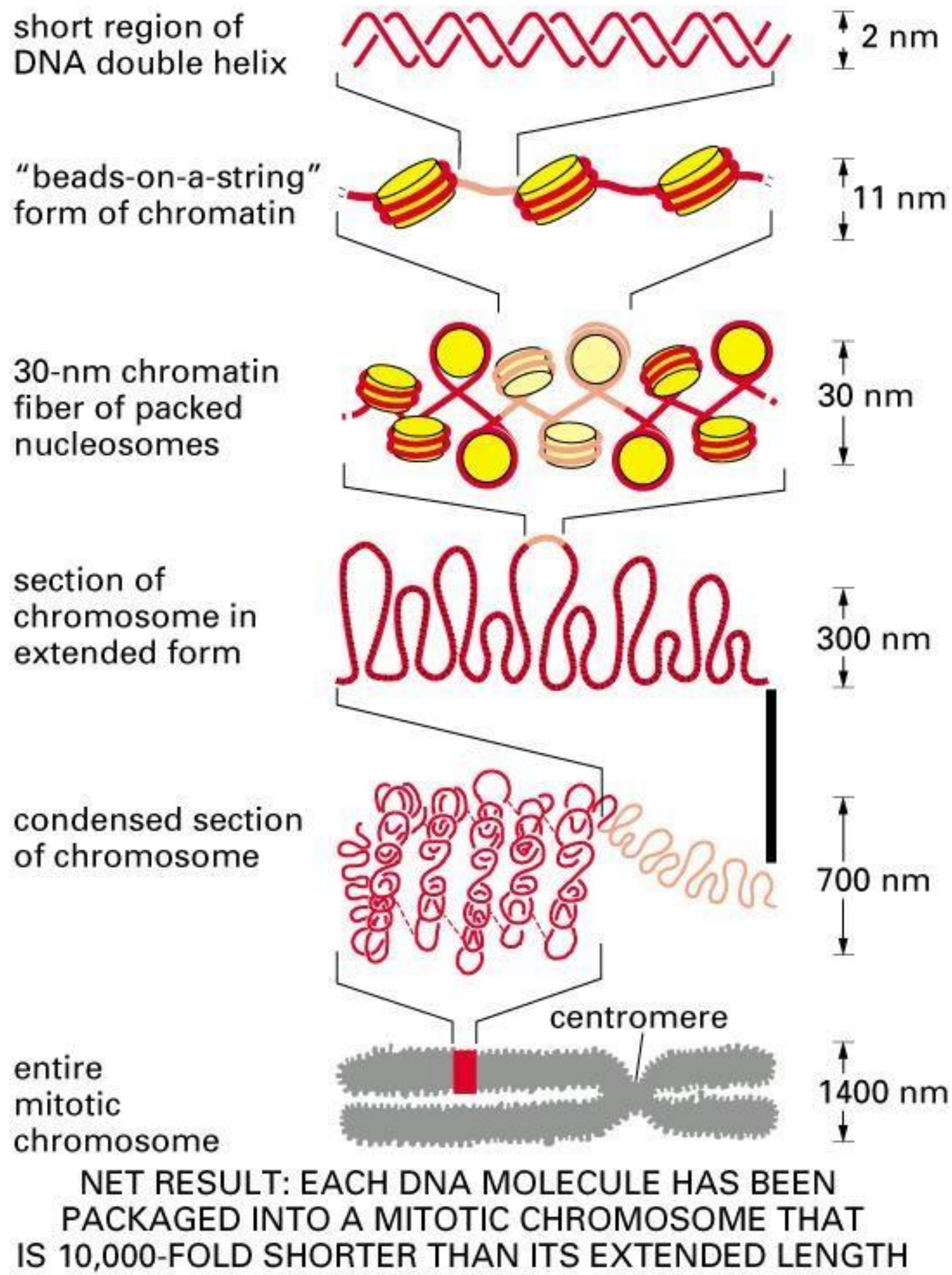
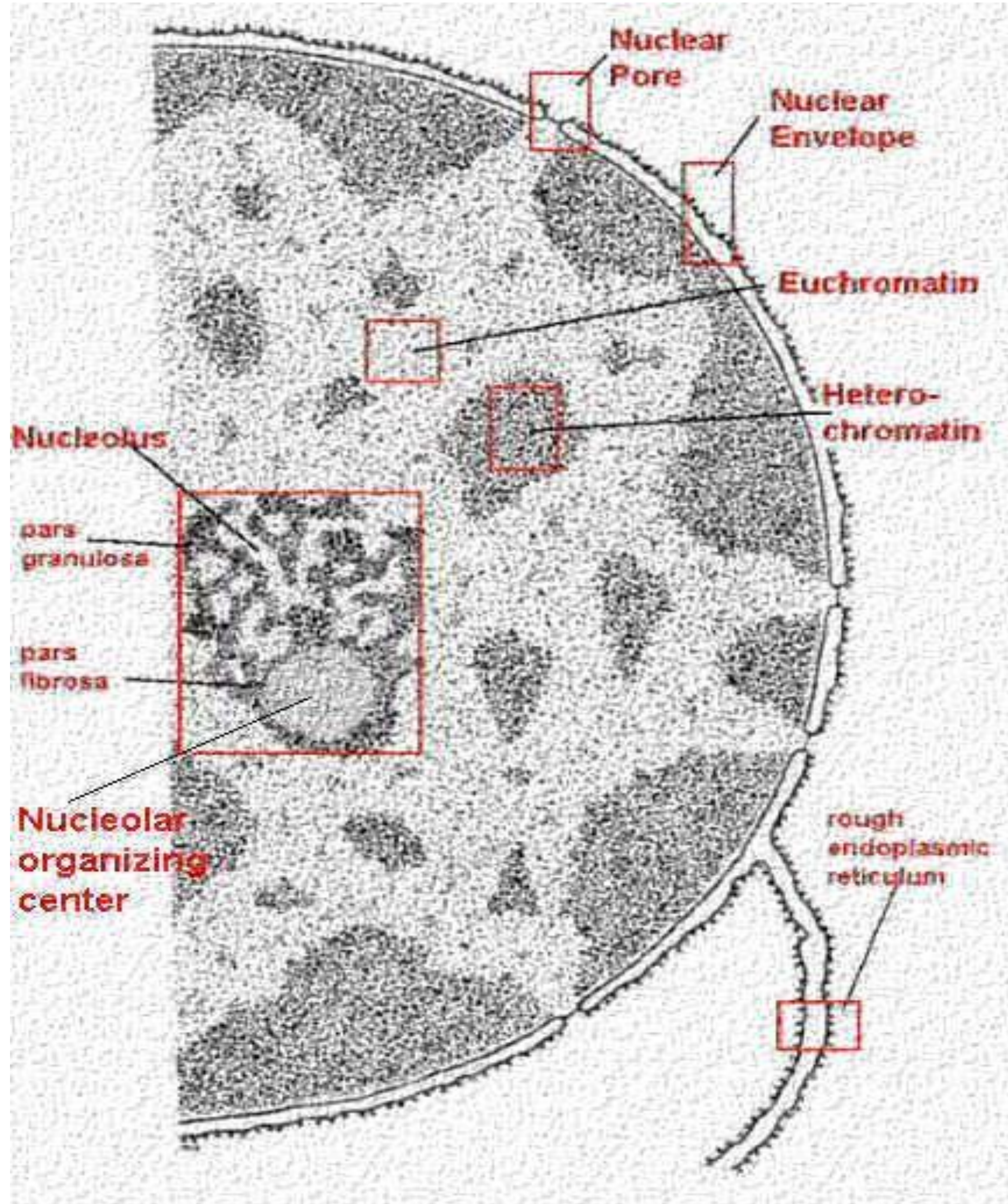


Figure 4–55. Molecular Biology of the Cell, 4th Edition.

- Chromatin can be differentiated into two regions (during interphase & early prophase)

1. Euchromatin – lightly staining
2. Heterochromatin – densely staining



Euchromatin	Heterochromatin
Lightly staining regions	Darkly staining
Less tightly packed chromatin fibers therefore non condensed	Tightly packed chromatin fibers therefore condensed
Not visible – light microscope, undergo regular changes in morphology with cell division	Visible, remain highly condensed in all stages
Genetically active regions	Genetically inactive regions – either they lack genes/ contain genes that are not expressed
Replicates earlier during S phase	Replicates later during S phase
GC rich	AT rich

- Heterochromatin – some parts of chromosome don’t always encode protein
- Heterochromatin regions – centromere and tip of the chromosome
- Pattern of Heterochromatin & Euchromatin – good markers for chromosome characterization

Heterochromatin – Two forms

1. Constitutive heterochromatin

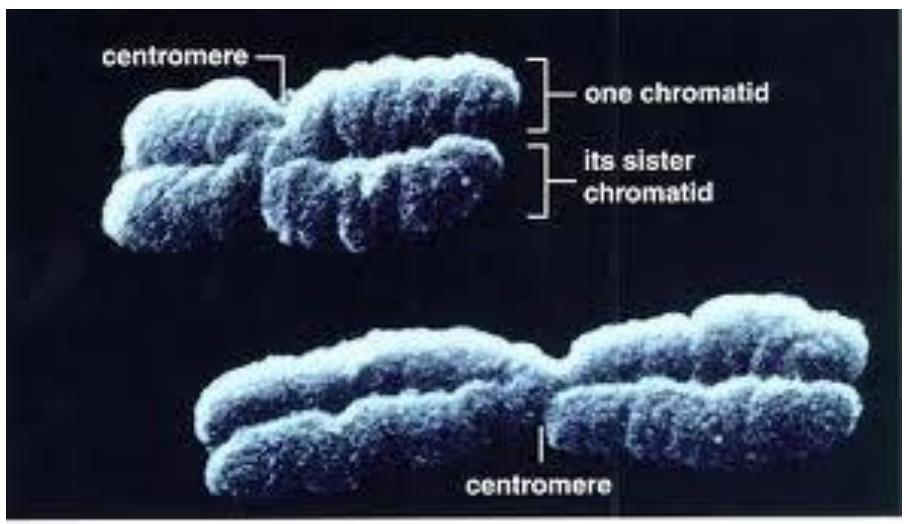
- Remains compacted
- Permanently heterochromatic
- Around centromere
- Highly repeated sequences

2. Facultative heterochromatin

- Either heterochromatic/ euchromatic
- Females have two X
    - » Only one is transcriptionally active
    - » Other condensed – *Barr body*

Chromosome Number

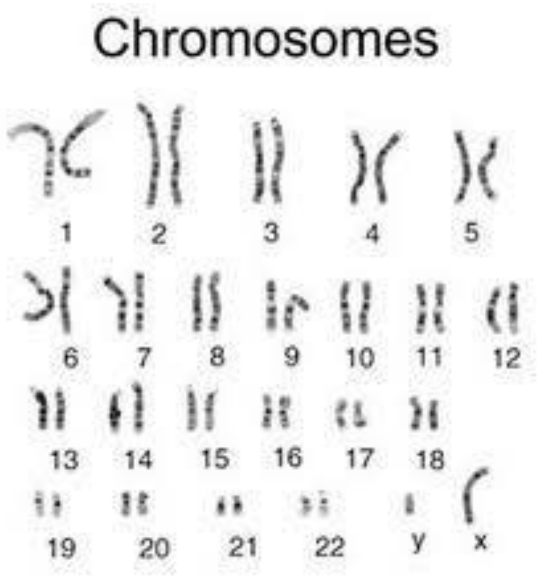
- Somatic cells – chromosomes occur in pairs
- Two members – similar shape & size – homologous
- Diploid Number – chromosomes found in nucleus of somatic cell
- Haploid Number –chromosomes found in nucleus of sex cells



Chromosome number (2n) of some species of animals

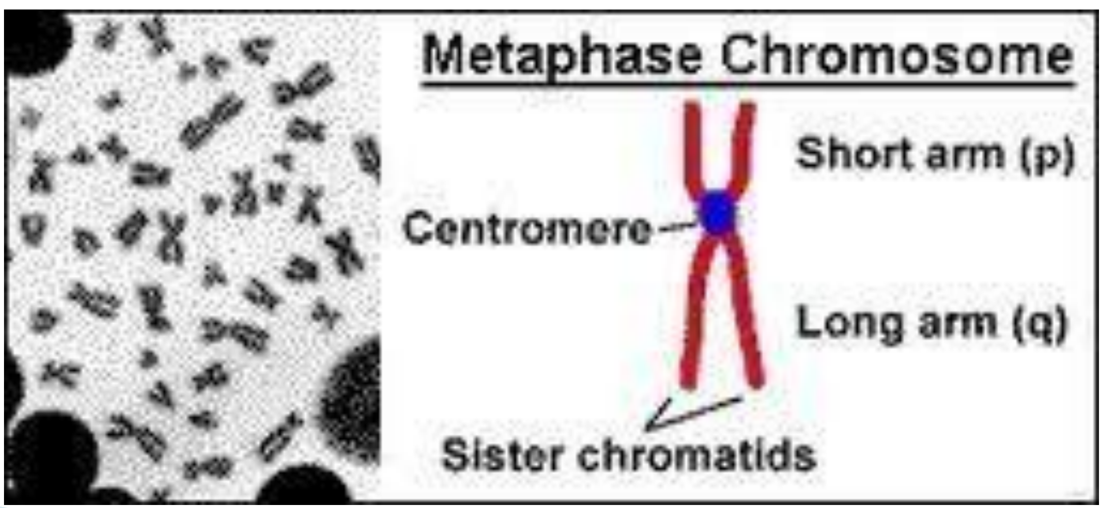
Buffaloe	48	Cat	38
Cattle	60	Camel	74
Goat	60	Elephant	56
Sheep	54	Human	46
Pig	38	Fruit fly	8
Horse	64	Mouse	40
Donkey	62	Guinea pig	64
Poultry	78	Monkey	42
Dog	78	Frog	26

- In most diploid species of animals – two types of chromosomes;
- – Autosomes : chromosomal pairs identical in size & shape
  – Sex Chromosomes: chromosomal pairs differ in size & shape
-



Chromosome morphology

- Morphological features visible – metaphase
- Chromosome appears as double structure
- Two parallel strands (sister chromatids) connected by a common centromere

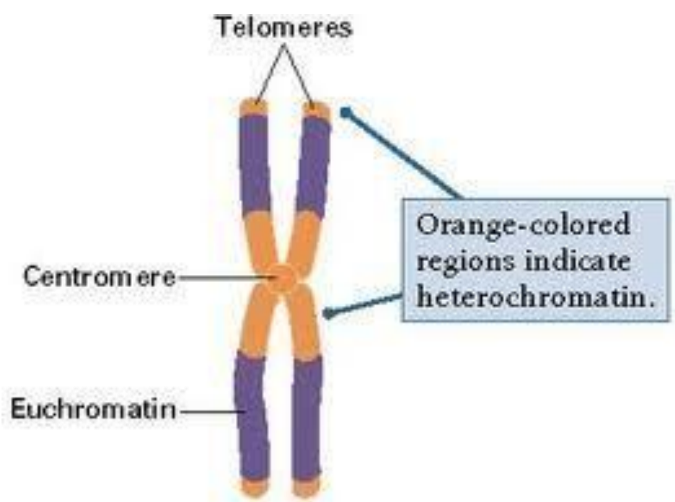


Chromosome Size

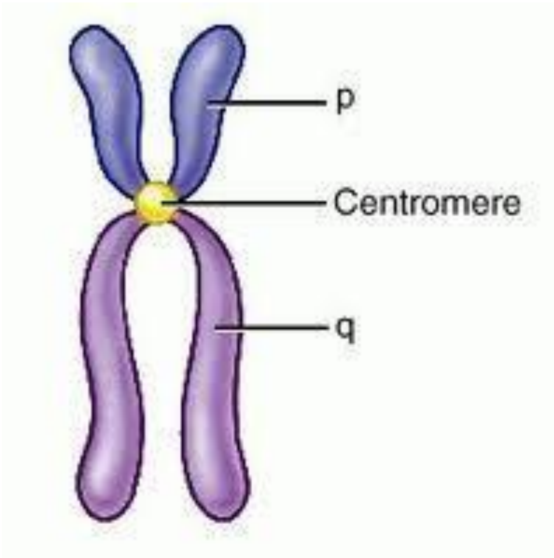
- Best measured at metaphase – condensed and thickest
- Length 1-30 micron, diameter 0.2-2 micron
- Varying degree of contraction – absolute lengths vary at different stages of cell cycle.

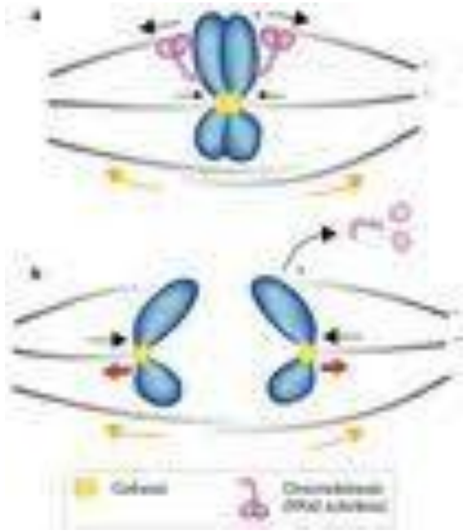
Centromere

- Chromosome shape determined by position of centromere
- Single differentiated, permanent, well defined organelle
- Nucleoproteins – recognized by its less intense staining.
- Heterochromatic – structural not informational
- Transverse constriction on chromosome – primary constriction

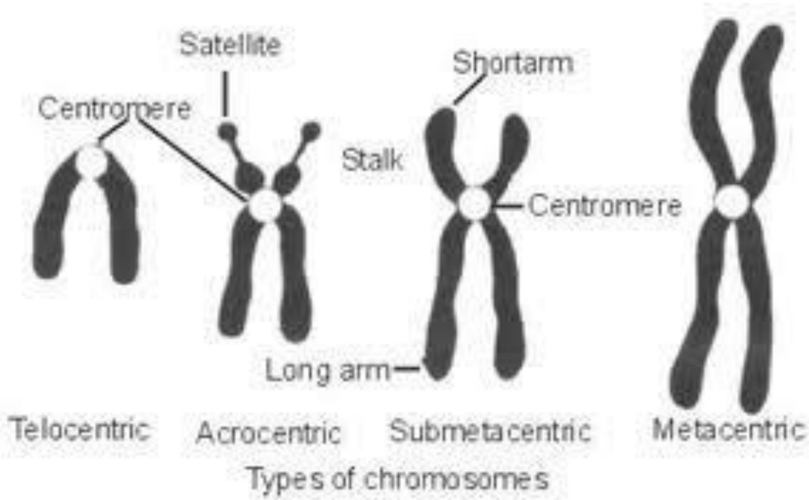


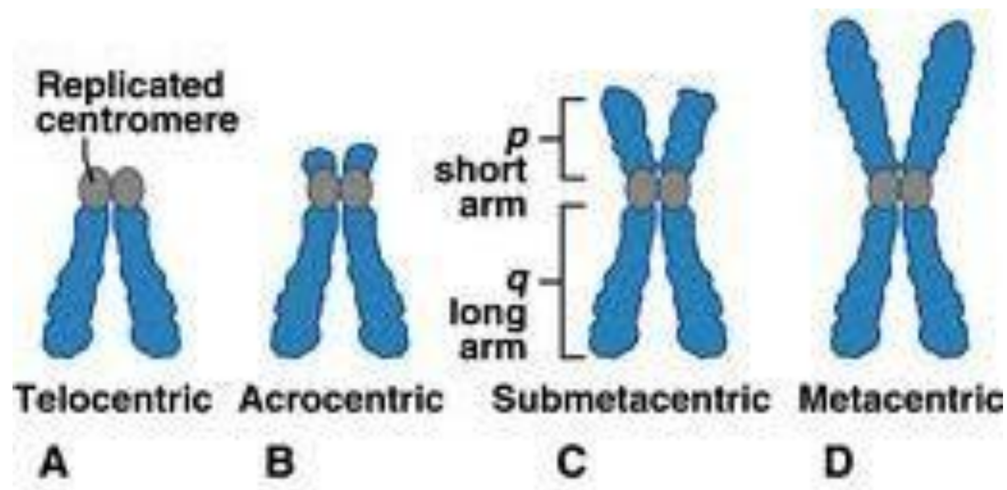
- Shorter arm (p) and Longer arm (q)
- Two imp functions;
  - Holding together of siser chromatids
  - Chromosome movement



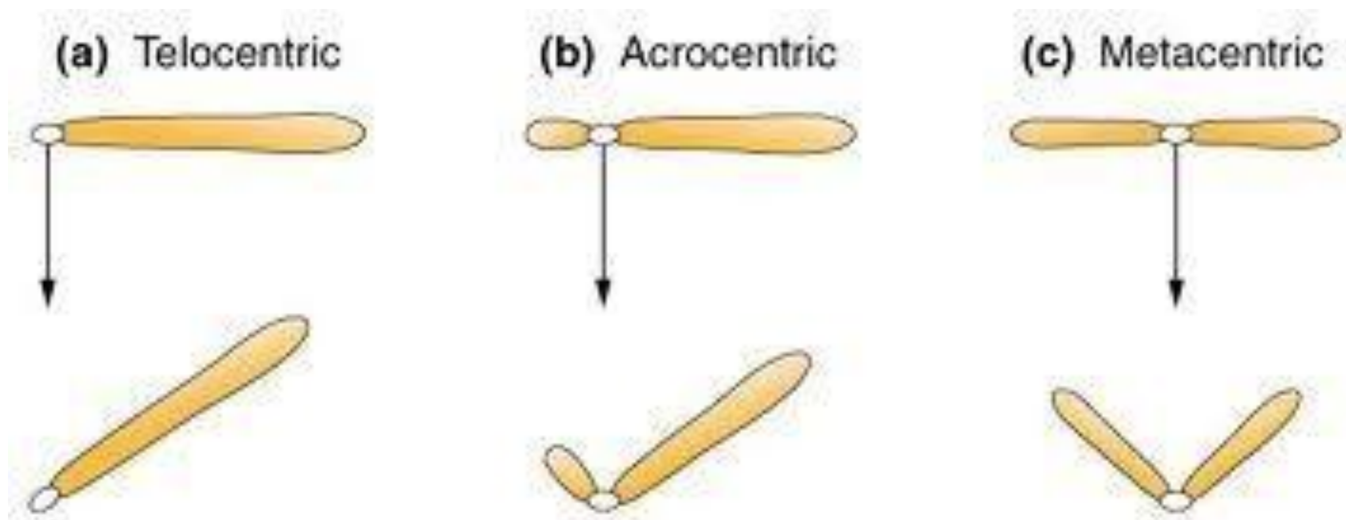


- Based on position of centromere 4 major morphological types of chromosomes are recognised





- Chromosome show different shape as they move towards poles at anaphase
  - A metacentric/ submetacentric appears V or U shape
  - A acrocentric/ telocentric appears I or J shape



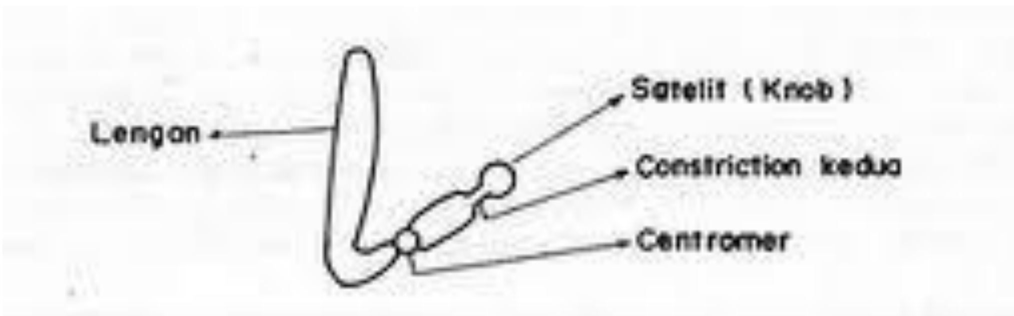
Other features of chromosome

1. Telomeres:

- Distal regions at both ends
- Specific structural importance
- Prevent fusion b/w ends of different chromosomes – hair pin
- Length is maintained by telomerase enzymes
- Age dependent decline in telomere length
- Non sticking & Maintaining chromosome length

2. Satellite:

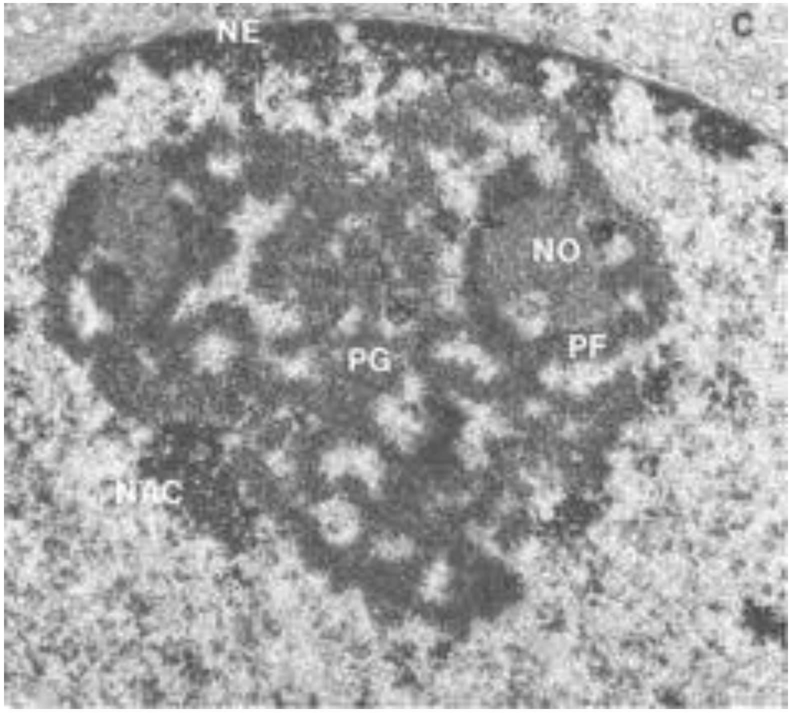
- Tiny terminal extension
- Cytologically not visible
- Satellite produces narrow constriction – secondary constriction
- Secondary can be distinguished from primary
- Angular deviation (bend) at primary constriction



- No. of chromosomes having satellite varies from species to species
- In humans 5 of 23 pairs
- Not found in cattle, sheep, goats, pigs and horses
- Chromosome having satellite – SAT chromosome

3. Nucleolar organizer Region(NOR):

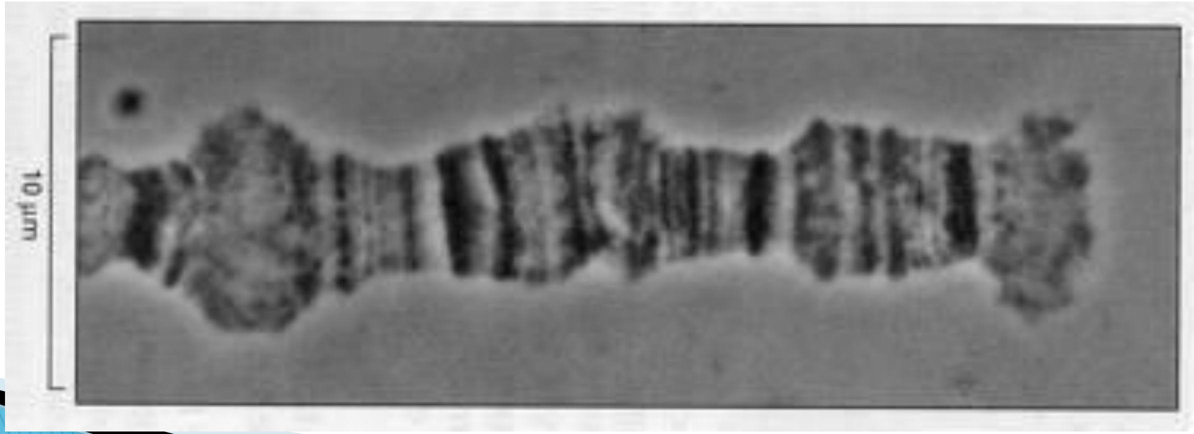
- Specific region of chromosome contains
- genes for synthesis of rRNA
- Nucleolus can be seen attached to NOR
- NOR's usually found at telomeric ends



Special Chromosomes

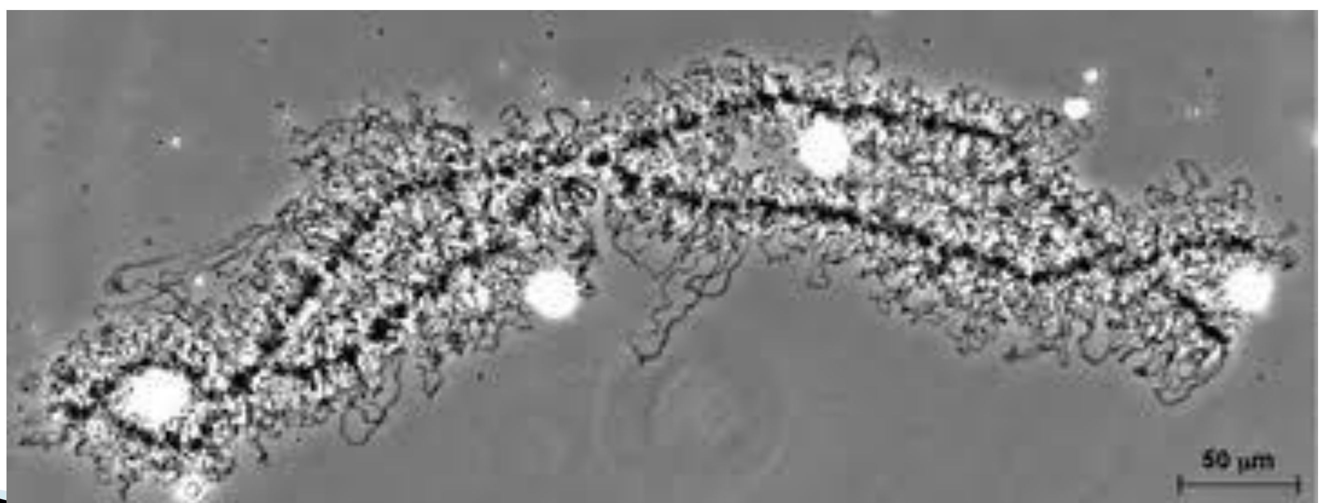
1. Polytene chromosome:

- Giant chromosome
- Repeated division of chromosome without nuclear division
- Chromosome don’t separate
- 200 times larger
- Found in insects of order diptera – flies, mosquitoes
- Also found in salivary glands & gut epithelium of larva of Drosophila melanogaster



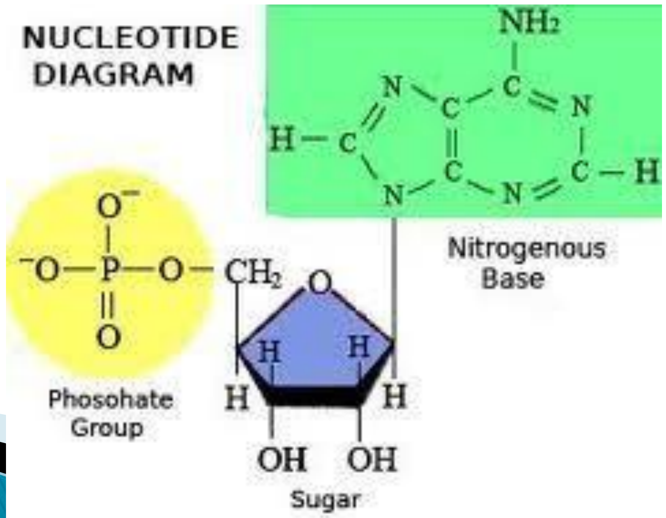
2. Lamp brush chromosome:

- Seen in maize & amphibians
- Growing oocytes (immature eggs) of most animals, except mammals
- During the diplotene stage of meiotic prophase I due to an active transcription of many genes
- 800 to 1000 micron long

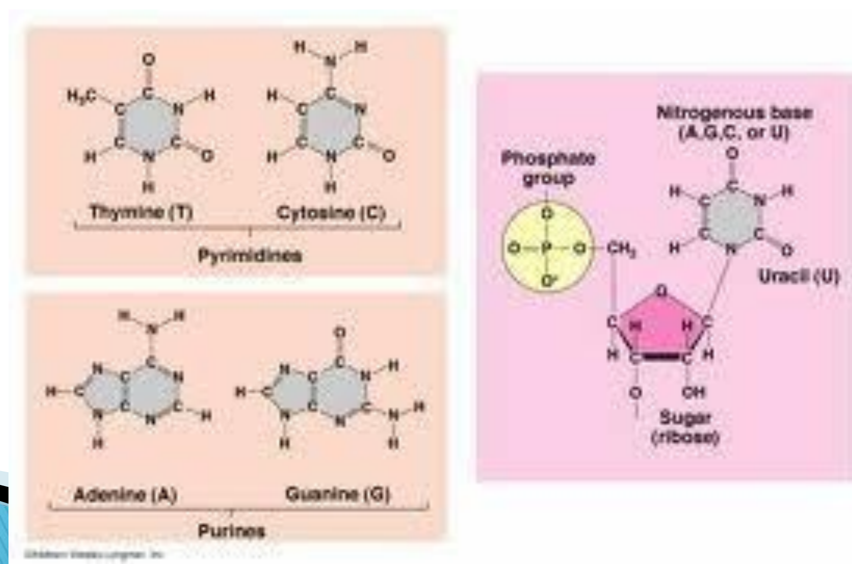


Nuclear DNA

- Macromolecules – mol wt. few thousand daltons
- Polymeric molecules made of 4 different monomeric units called Nucleotides.
- Nucleotide:
  - A pentose (5-carbon) sugar
  - A Nitrogenous base
  - A phosphate group
- Isolated from nuclei & acidic in nature – Nucleic acids



- For RNA – pentose sugar is ribose
- For DNA – pentose sugar is deoxyribose
- Nitrogenous bases ; two classes
  - Purines – adenine(A) & guanine (G)
  - Pyrimidines – thymine (T) & cytosine (C)
- In RNA thymine (T) replaced by Uracil (U)
- Nucleoside = base + sugars
- Base, PO4 gp & OH grp – 1st, 5th & 3rd position



- In 1953 James D Watson and Francis H.C. Crick published paper – model for physical & chemical structure of DNA
- molecule

It was based on earlier findings by different scientist

1. Erwin Chargoff – hydrolyzed DNA of no. of organisms & quantified the purines & pyrimidines released

   - mol. Concentration [A]=[T] & [G] = [C]
   - Total concentration of purines = pyrimidines [A+G] = [C+T]
   - the ratio [A+G] &[C+T] varied b/w organisms
   - these equivalencies – Chargoff rules

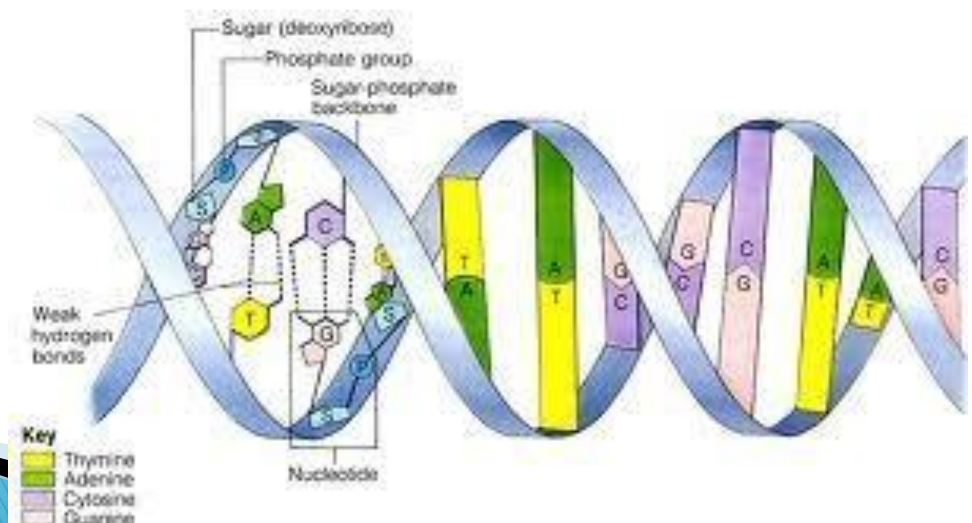
2. Rosalind Franklin & Maurice H. F. Wilkins studied isolated fibers of DNA by X-ray diffraction technique

- Distance b/w 2 turns 3.4 nm
- distance b/w 2 nucleotides 0.34 nm
- bp per turn 10
- diameter 2 nm

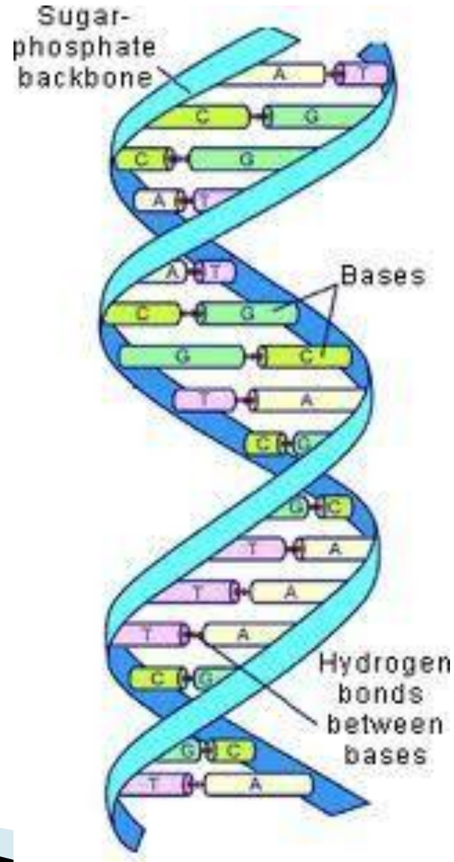
- Watson & Crick considered all evidence & began to build three – dimensional models for structure of DNA

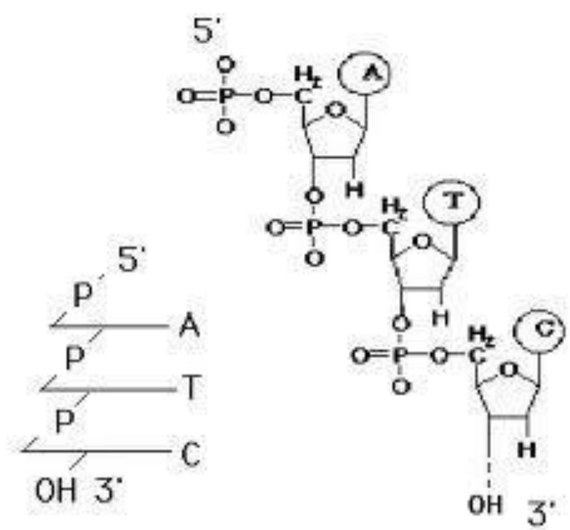
<u>Main features</u>:

- Double helical structure, two chains coiled
  Chains run in opposite direction – antiparallel
- Two strands are complimentary
- Bases stacked inside, sugar on axis
- Diameter of helix – 2 nm, bases separated by 0.34 nm along axis & rotated at 360^o



- Two chains held together by hydrogen bonds
- b/w A&T double bonds, b/w G&C triple bonds
- Phosphate gp on 5th position of sugar – OH gp on 3rd Position forming phophodiester bonds

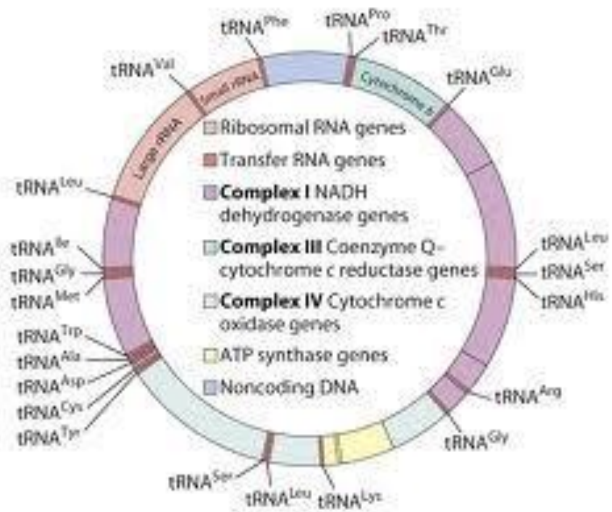




Mitochondrial Genome/ DNA

- Circular, double stranded, super coiled
- Linear mt DNA - protozoa & fungi
- GC content of mt DNA differs from nDNA
- mt DNA devoid of histones & similar to genomes of prokaryotes – endosymbiotic origin
- nDNA - 3 billion nucleotides, 1% of which representative of 20 -25 thousand active genes
- mt DNA have only 17000 nucleotides – 37 distinct genes

- mt DNA codes for 13 polypeptides (oxidative phosphorylation), 22 tRNA & 2 rRNA
- Other components (eg DNA polymerase, RNA polymerase, ribosomal proteins etc) are encoded by nDNA & must be imported
- mt DNA – heavy strand (purine rich) & light stand (pyrimidine rich)
- mt genome is strictly maternally inherited
- Sperm contributes no mt DNA – fertilizing egg



- Biological maternal relatives all share their mt DNA – nDNA is unique
- Each cell has one nucleus but hundreds to thousands of mt
- But many mt represent only many copies of same DNA
- Higher rate of mutation seen in mtDNA compared to nDNA
- mt DNA not subjected to recombination during sexual transmission