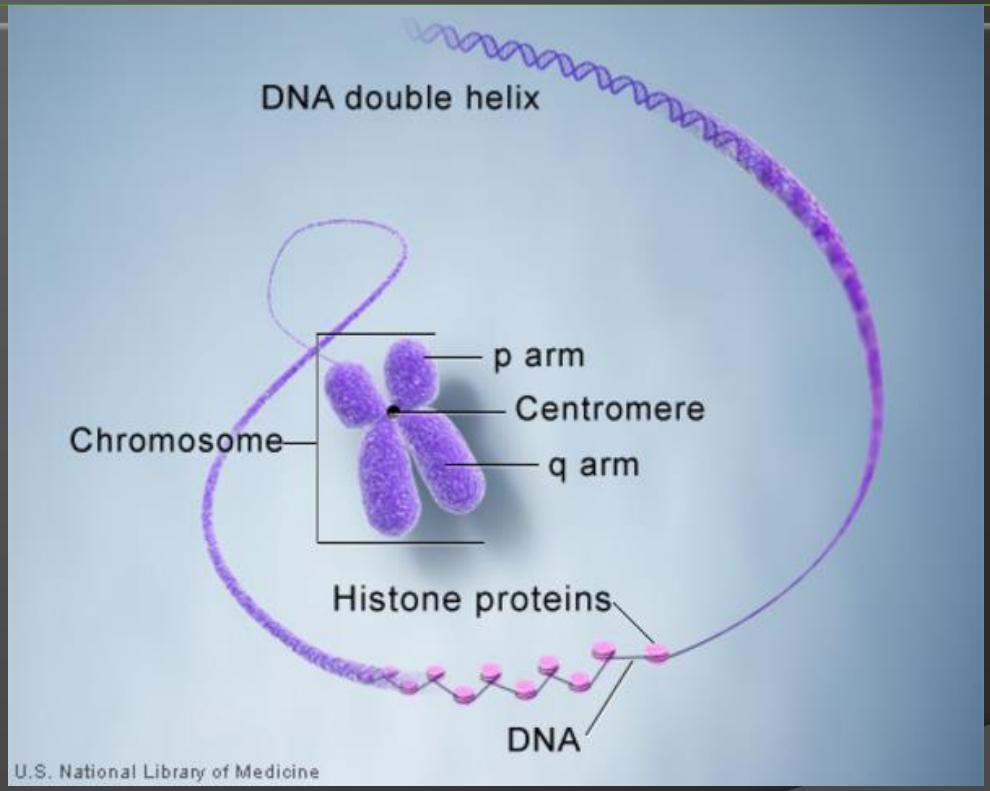
CHROMOSOME



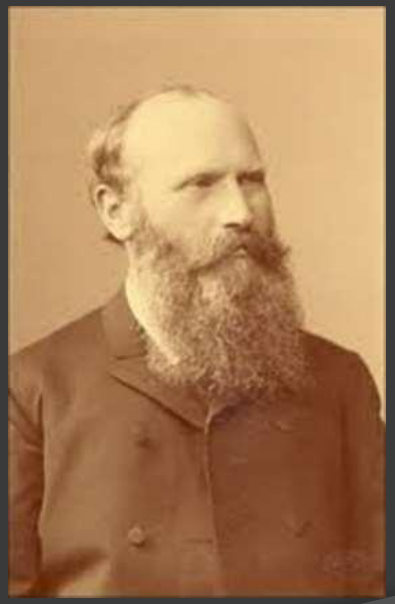
Munmun Chatterjee

Introduction

- Chromosome : Greek word
  - Chroma: color
  - Soma: body
- Contain most of DNA of living organisms.
- Duplicated chromosomes contain two identical copies known as chromatids or sister chromatids

Discovery

- Schleiden, virchow, butschli – first recognize the structure
- The term coined by Von Waldeyer-hartz



Length of chromosome

- The length of chromosomes varies
  - 1 micron (eg:fungi) to 30micron (eg:Trillium).
- This excludes the salivary gland chromosomes of Diptera(flys).
  - 2mm long.
- All the chromosomes of a species may be similar size (symmetrical karyotype) or different chromosomes may vary in size (asymmetrical karyotype).

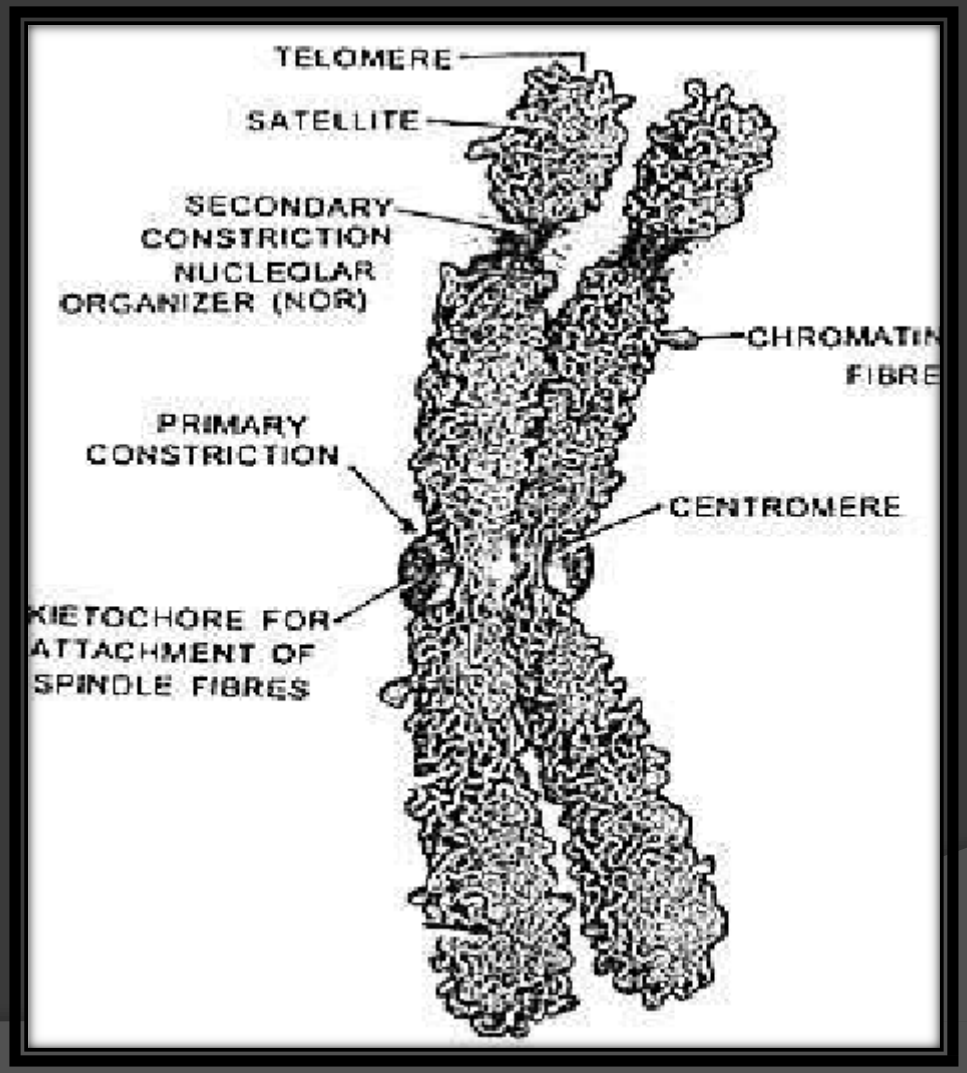
3 billions base pairs are Organized into 46 chromosome

General structure of chromosome

- Chromatids: two chromatids or arms which are cylindrical rod shaped, parallel to each other held at point called centromere
- Short arm “p” & long arm “q”
- Chromonema: thread like structure even called as chromatin fiber
  - Heterochromatin: tightly coiled
  - Euchromatin: loosely coiled
- Centromere : layers of protein complex which help in attachment of spindle fibers during cell division

- Secondary constriction : the narrow region in both arms of chromosomes
- Present a SAT chromosome above the secondary constriction (SAT=Sino Acido Thymidine) or satellite chromosome
  - In human somatic cells there are 10 homologous pairs of SAT chromosomes i.e 13,14,15,21,22 pairs.
- Nucleolus always associated with secondary constriction , So secondary constriction also called as nucleus organiser region(NOR)
- SAT chromosome referred to as nucleolus organiser chromosome (NOC)

- Telomer :blunt tips of chromosome
  - Doesn't unite with other telomere, and prevent attachment



Types of chromosome based on the position of centromere

- Metacentric chromosome : centromere is exactly at center
- Sub-metacentric chromosome: centromere is slightly away from the center
- Acrocentric chromosome: centromere is sub terminal with very long and short arm
- Telocentric chromosome: centromere is at terminal position with only one arm

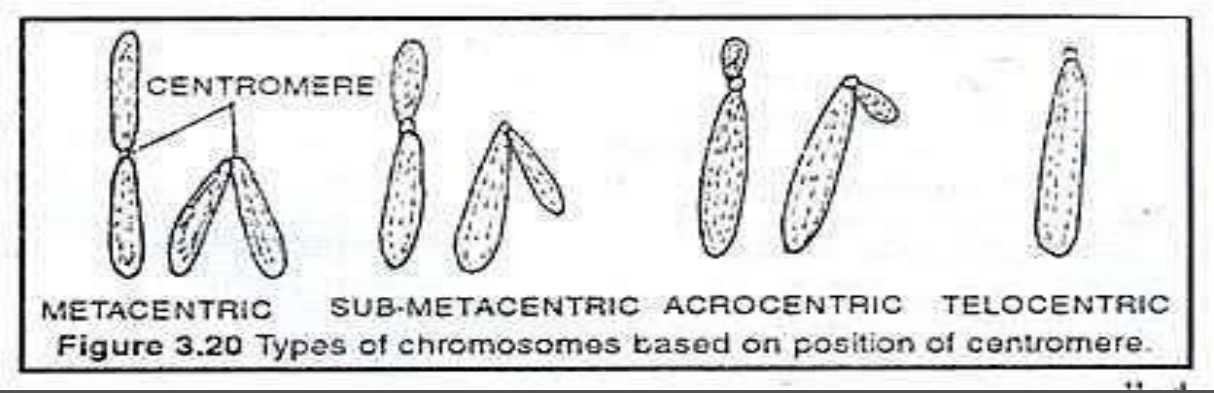
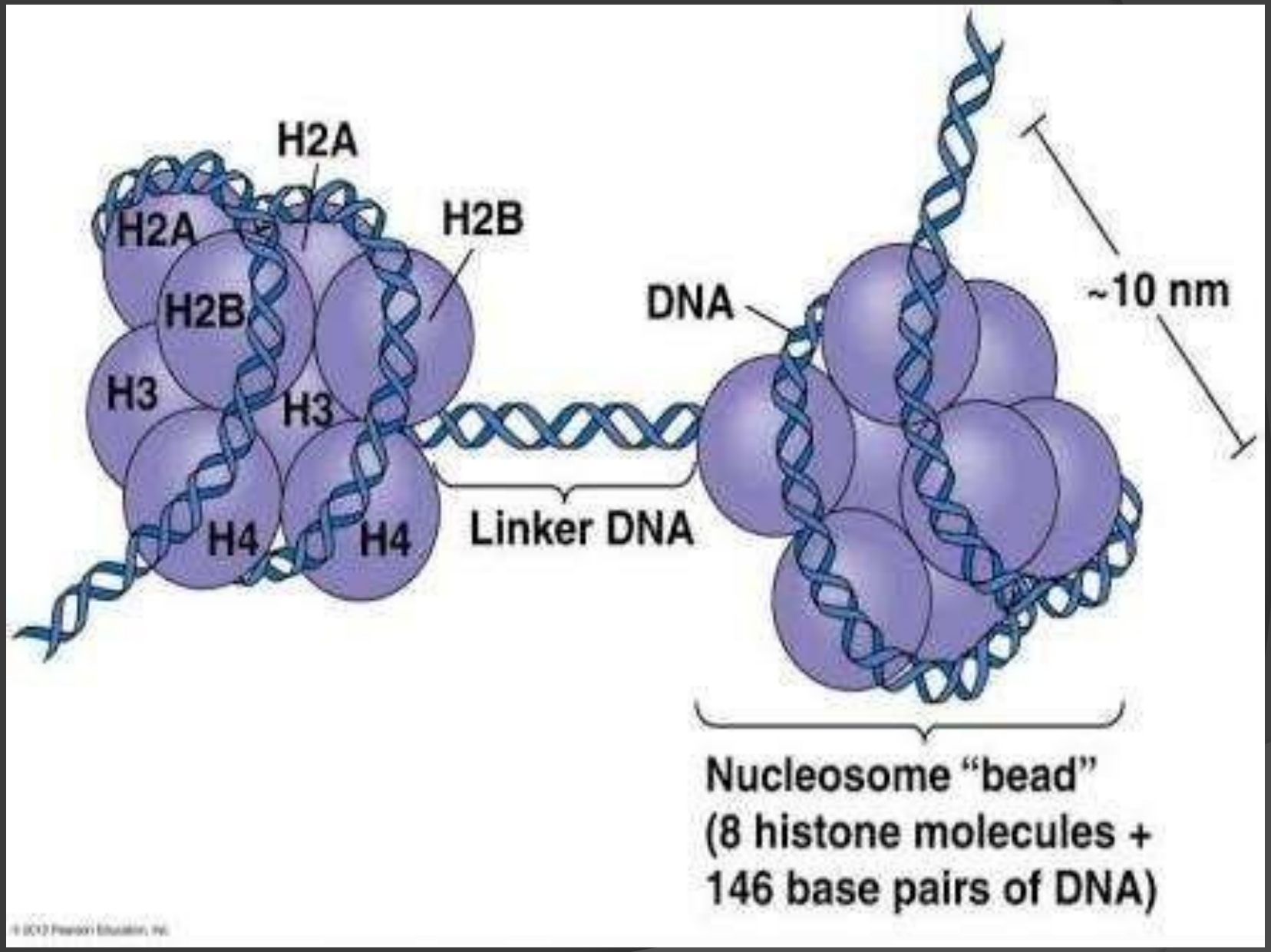


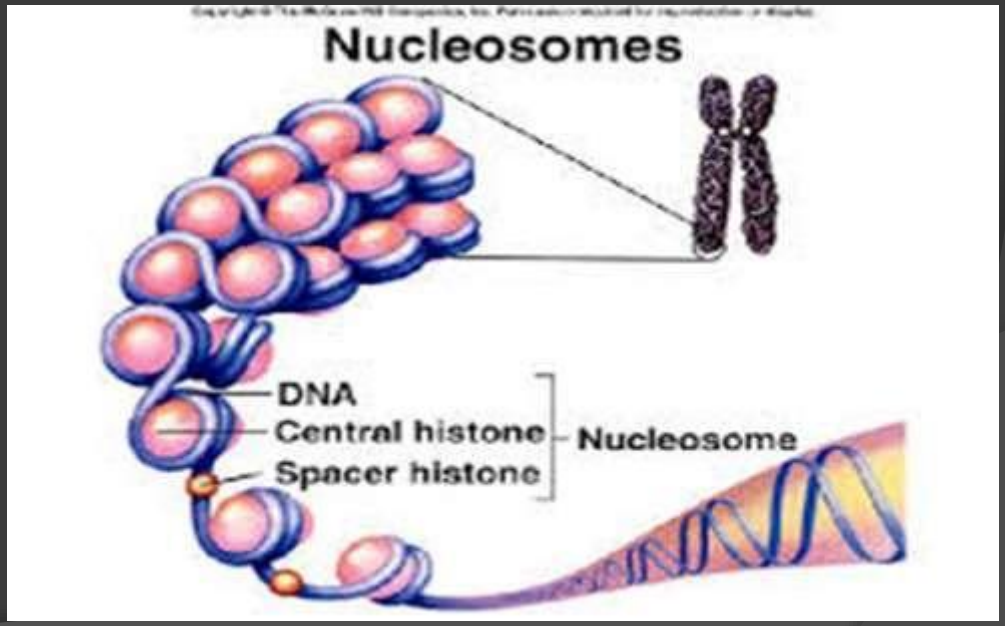
Figure 3.20 Types of chromosomes based on position of centromere.

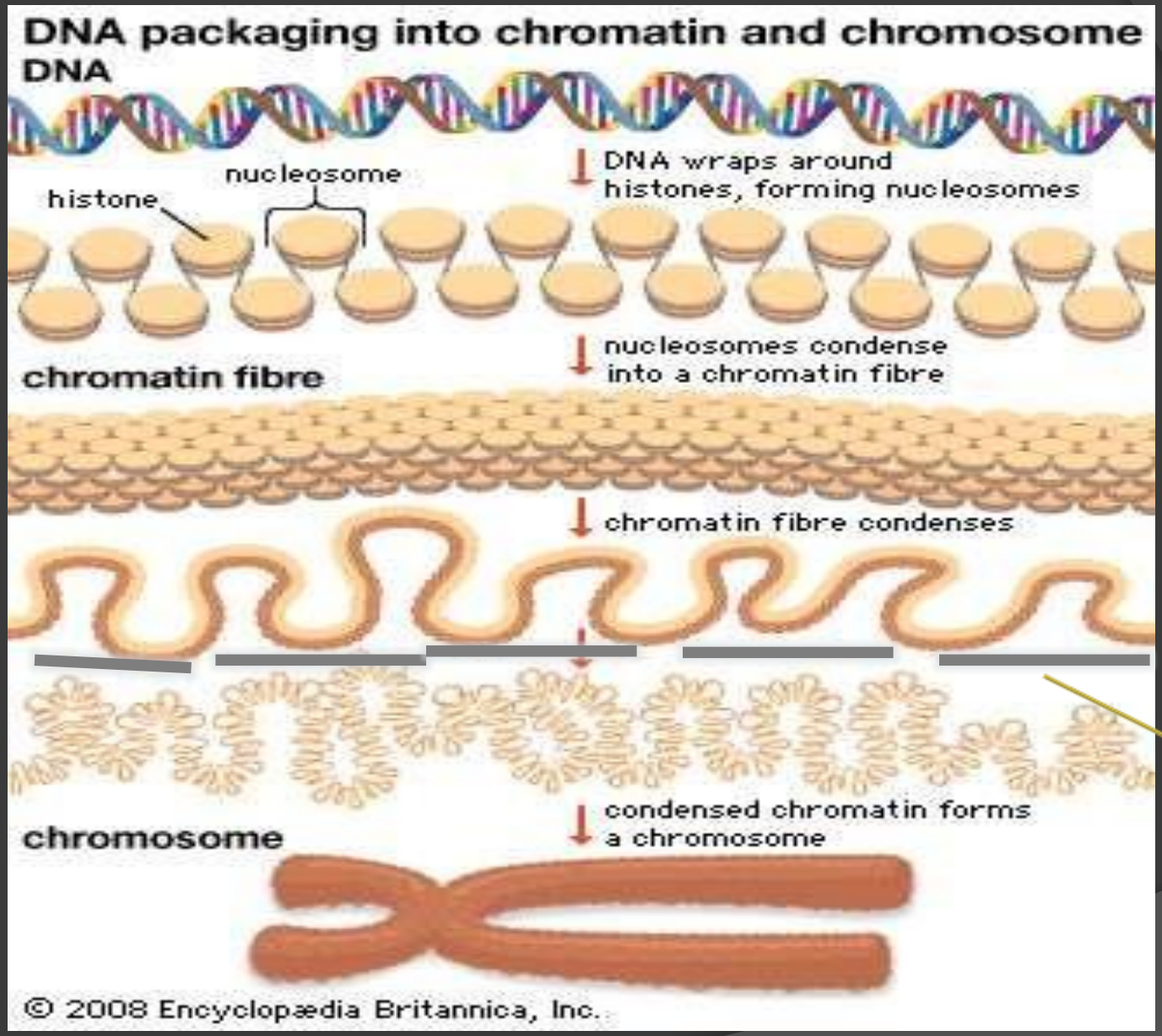
Ultra structure of chromosome

- single molecule of DNA bind with many molecules of histones.
- Histones
  - H1/H5,H2A,H2B,H3,H4,H5
- A small number of non-histone proteins are also present.
- During most of the cell's life cycle, chromosomes are elongated and cannot be observed under the microscope.
- During the S phase of the mitotic cell cycle the chromosomes are duplicated.
- At the beginning of mitosis the chromosomes are replicated.


H2A
H2A
H2B
H2B
H3
H3
H4
H4
Linker DNA
DNA
~10 nm
Nucleosome "bead"
(8 histone molecules +
146 base pairs of DNA)

- These duplicated condensed chromosomes are known as dyads.
- The duplicated chromosomes are held together at the region of centromeres.
- The centromeres are made up of protein called kinetochore.




DNA packaging into chromatin and chromosome
DNA
DNA wraps around histones, forming nucleosomes
nucleosome
histone
nucleosomes condense into a chromatin fibre
chromatin fibre
chromatin fibre condenses
condensed chromatin forms a chromosome
chromosome
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scaffold

Heterochromatin

- Heterochromatin is condensed
  - This is in fact what defines heterochromatin, and it is applicable to both constitutive HC and facultative HC. This high condensation renders it strongly chromophilic and inaccessible to DNAse 1 and to other restriction enzymes in general
- Heterochromatin DNA is late replicating
  - The incorporation of various nucleotide analogues shows that the DNA from both constitutive and facultative HC, is late replicating.
- Heterochromatin DNA is methylated
  - The DNA of HC is highly methylated on the cytosines. An anti-5-methyl cytosine antibody therefore strongly labels all the regions of HC
- In heterochromatin, histones are hypo-acetylated
  - Histones may undergo post-translational modifications of their N-terminal ends which may affect the genetic activity of the chromatin.

- Histones from heterochromatin are methylated on lysine 9
  - Methylation of the histone H3 lysine 9 (H3-K9) has only very recently been found to be involved in the process of heterochromatinisation of the genome, both in Heterochromatin is transcriptionally inactive
  - relatively poor in genes, and its genes are not usually transcribed in a heterochromatic context.
- Heterochromatin does not participate in genetic recombination
  - It is generally accepted that HC does not participate in genetic recombination
- Heterochromatin has a gregarious instinct
  - This tendency of the heterochromatin to aggregate appears to be strongly linked to the presence of satellite DNA sequences, but it may also involve other additional sequences.

Euchromatin

- It is a lightly packed chromatin that is enriched in genes and often under active transcription
- Human genome is made up of 92% is euchromatin
- The structure of euchromatin is reminiscent of an unfolded set of beads along a string, wherein those beads represent nucleosomes
- Nucleosomes consist of eight proteins known as histones , with approximately 147 base pairs of DNA wound around them
- Euchromatin appears as light-colored bands when stained in G banding and observed under an optical chromosome

Difference between the heterochromatin and euchromatin

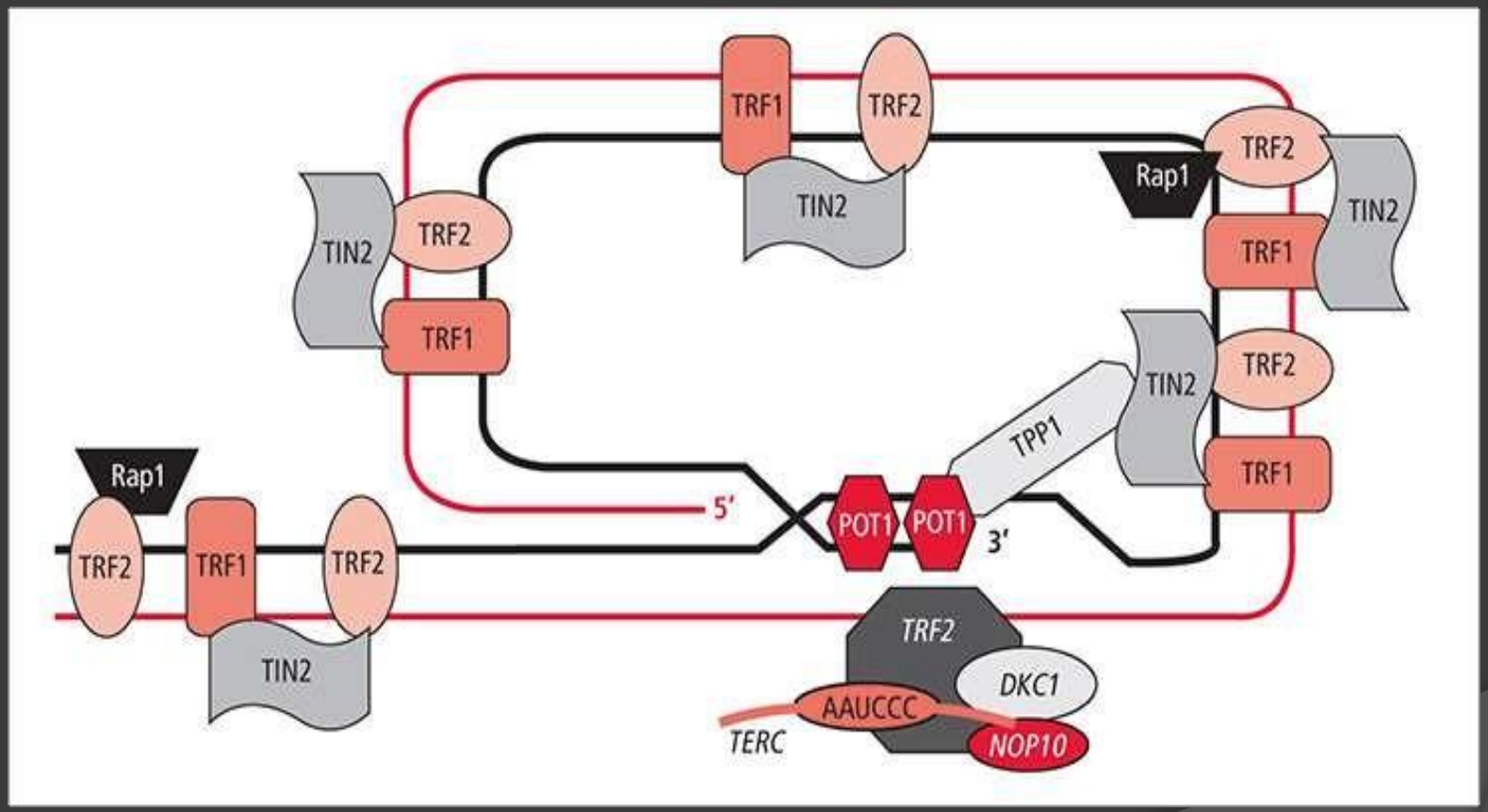
Euchromatin	Heterochromatin
The chromatin fibres in this region are loosely coiled as compared with hetero chromatic regions	The chromatin fibres in this region are more tightly folded than euchromatic regions
Euchromatin is deeply stained in divisional cycle but less stained in interphase.	Heterochromatin is deeply stained in interphase but less stained in divisional cycle
Euchromatic regions are able to synthesize mRNA in vitro.	Heterochromatic regions are unable to synthesize mRNA in vitro
Due to addition or loss of this region phenotype is affected.	Addition or loss of this region does not affect phenotype

The crossover frequency is more in euchromatin.	Crossover frequency is less than euchromatin.
Euchromatin does not show heteropycnosis.	Heterochromatin shows heteropycnosis.
Euchromatin is less affected than hetero chromatin by temperature, sex, age, etc.	Heterochromatin is more affected than euchromatin by temperature, sex, age of parents, proximity to the centromere

Telomere

- Region at the end of chromosome
- In 1970 Russian theorist Alexei Olovnikov
- Sequence : TTAGGG with complementary DNA strand being AATCCC
- Average telomere length decline from about 11kb at birth to less than 4kb in old
- Telomeres are protected by shelterin protein
- The telomere end become shorter due to each cell division
- they are replenished by an enzyme telomerase reverse transcriptase

- At the very distal end of the telomere is a 300 bp single-stranded portion, which forms the T-Loop.
- This loop is analogous to a *knot*, which stabilizes the telomere, preventing the telomere ends from being recognized as break points by the DNA repair machinery.
- non-homologous end joining occur at the telomeric ends, chromosomal fusion will result. The T-loop is held together by several proteins, the most notable ones being TRF1, TRF2, POT1, TIN1, and TIN2, collectively referred to as the shelterin complex
- In humans, the shelterin complex consists of six proteins identified as TRF1, TRF2, TIN2, POT1, TPP1, and RAP1


TRF1
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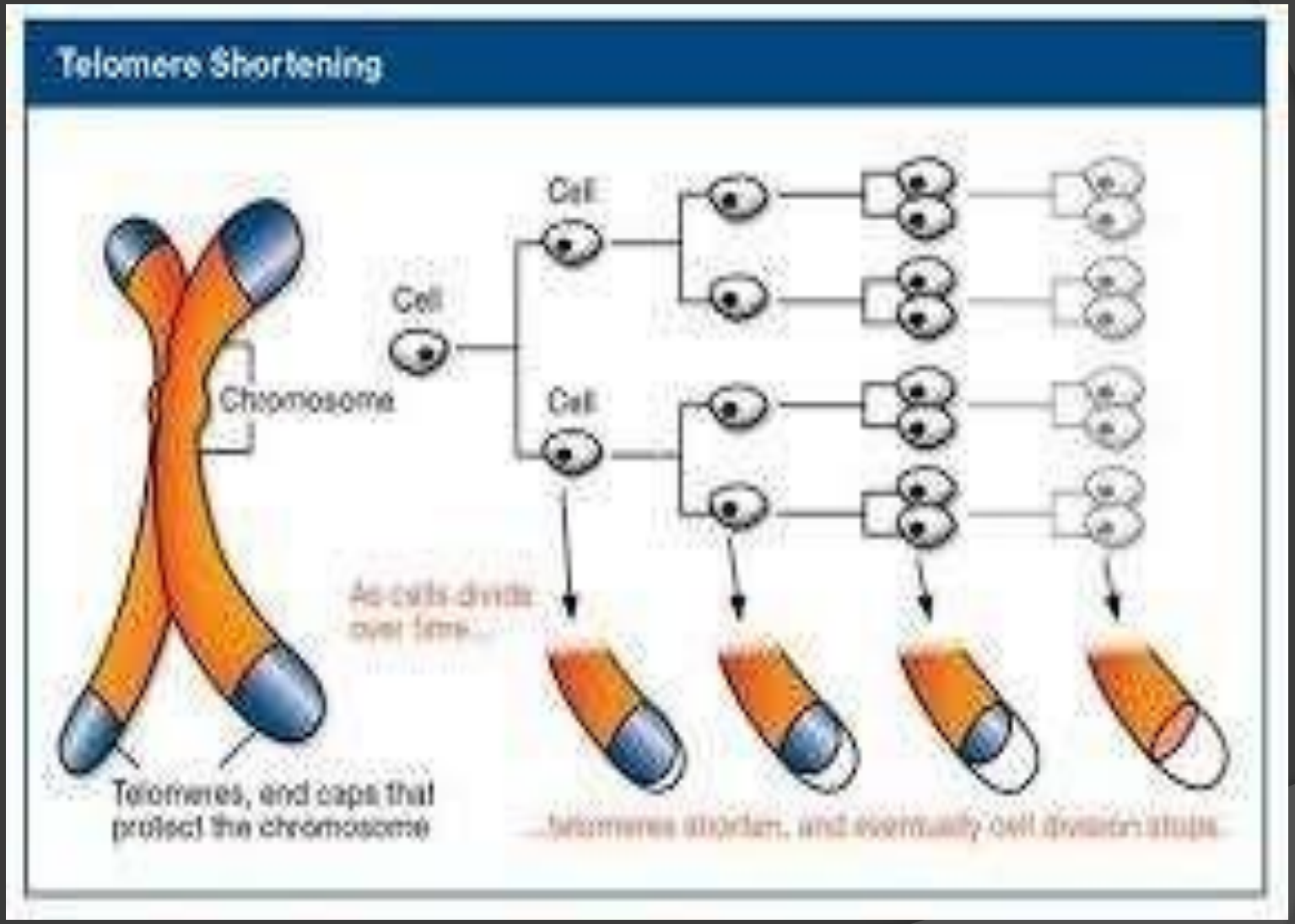
TIN2
TRF2
TIN2
TRF1
Rap1
TRF2
TIN2
TRF1
TIN2
TRF2
TPP1
TRF1
Rap1
5'
POT1
POT1
3'
TRF2
TRF1
TRF2
TIN2
TRF2
DKC1
AAUCCC
TERC
NOP10

- Telomeres are critical for maintaining genomic integrity and study shows that telomere shortening is commonly acquired during tumor formation
- short telomere can lead to genomic instability chromosome loss and the formation of non reciprocal translocations
- The precursor lesions are significantly shorter than surrounding normal tissue
- It can be found is most of the cancers

- Shorter telomeres have a negative effect on our health.
- Telomere shortening is the main cause of age-related break down of our cells.
- When telomeres get too short, our cells can no longer reproduce, which causes our tissues to degenerate and eventually die.
- Some cells, like those found in the skin, hair and immune system, are most affected by telomere shortening because they reproduce more often.

- An enzyme called telomerase can slow, stop or perhaps even reverse the telomere shortening that happens as we age. The amount of telomerase in our bodies declines as we age.
- Telomerase maintains and may even lengthen telomeres. Exposing human cells to telomerase slows cell aging and allows cells to begin copying again and longer telomeres cause gene expression to change to a younger phenotype which makes cells function as though they were younger.
- There are other things we can do that might help restore telomere length or at least slow the loss of telomere length: reduce stress, stop smoking, lose weight, exercise more and eat a healthier diet.


Telomere Shortening
Cell
Cell
Cell
Chromosome
Telomeres, and caps that protect the chromosome

Centromere

- The centromere is unique region on the chromosome that enquired for attachment to the mitotic spindle and chromosome segregation
- The centromeres are made up of protein called kinetochore
- This kinetochore protein ensures the attachment of microtubules that is spindle fibers
- When cells enter mitosis, the sister chromatids are linked along their length by the action of the cohesin complex.
- **Cohesin** is a protein complex that regulates the separation of sister chromatids during cell division, either mitosis or meiosis.

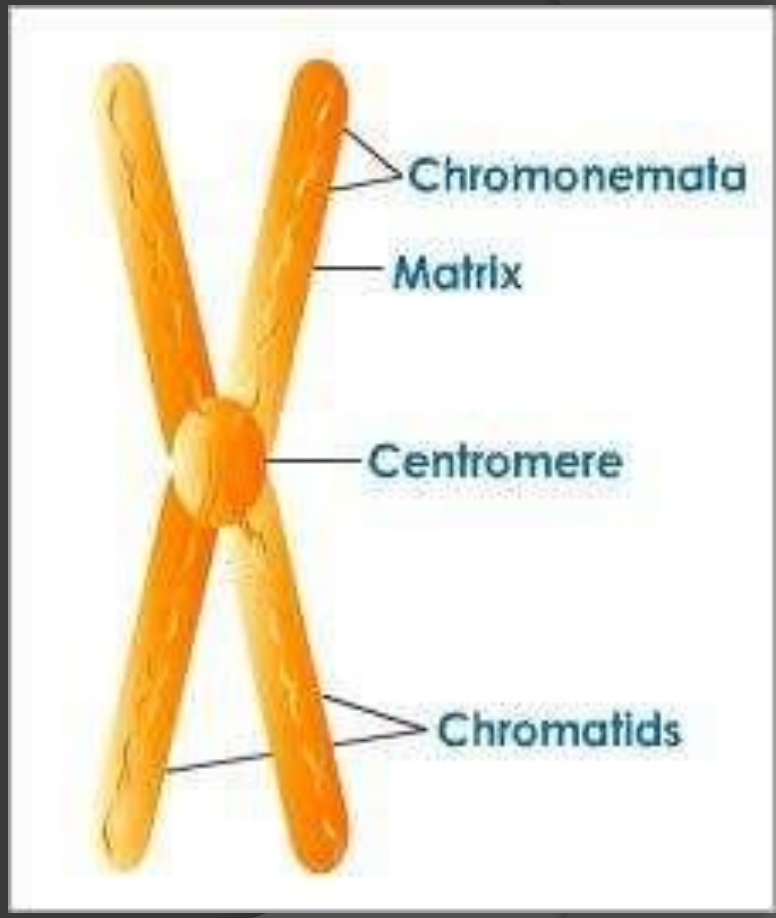
- Cohesin hold sister chromatids together after DNA replication until anaphase when removal of cohesin leads to separation of sister chromatids.
- It is now believed that this complex is mostly released from chromosome arms during prophase, so that by the time the chromosomes line up at the mid-plane of the mitotic spindle, the last place where they are linked with one another is in the chromatin in and around the centromere
- It even acts as motor protein

Prokaryotic chromosome

- Prokaryotes like the bacteria and archaea typically have a single circular chromosome
- 160,000 base pairs to 12,200,00 base pairs
- The DNA of the archaea are more organized, they are packaged within structures similar to eukaryotic nucleosomes
- The DNA are released into the relaxed state for the process of transcription, replication and regulation.

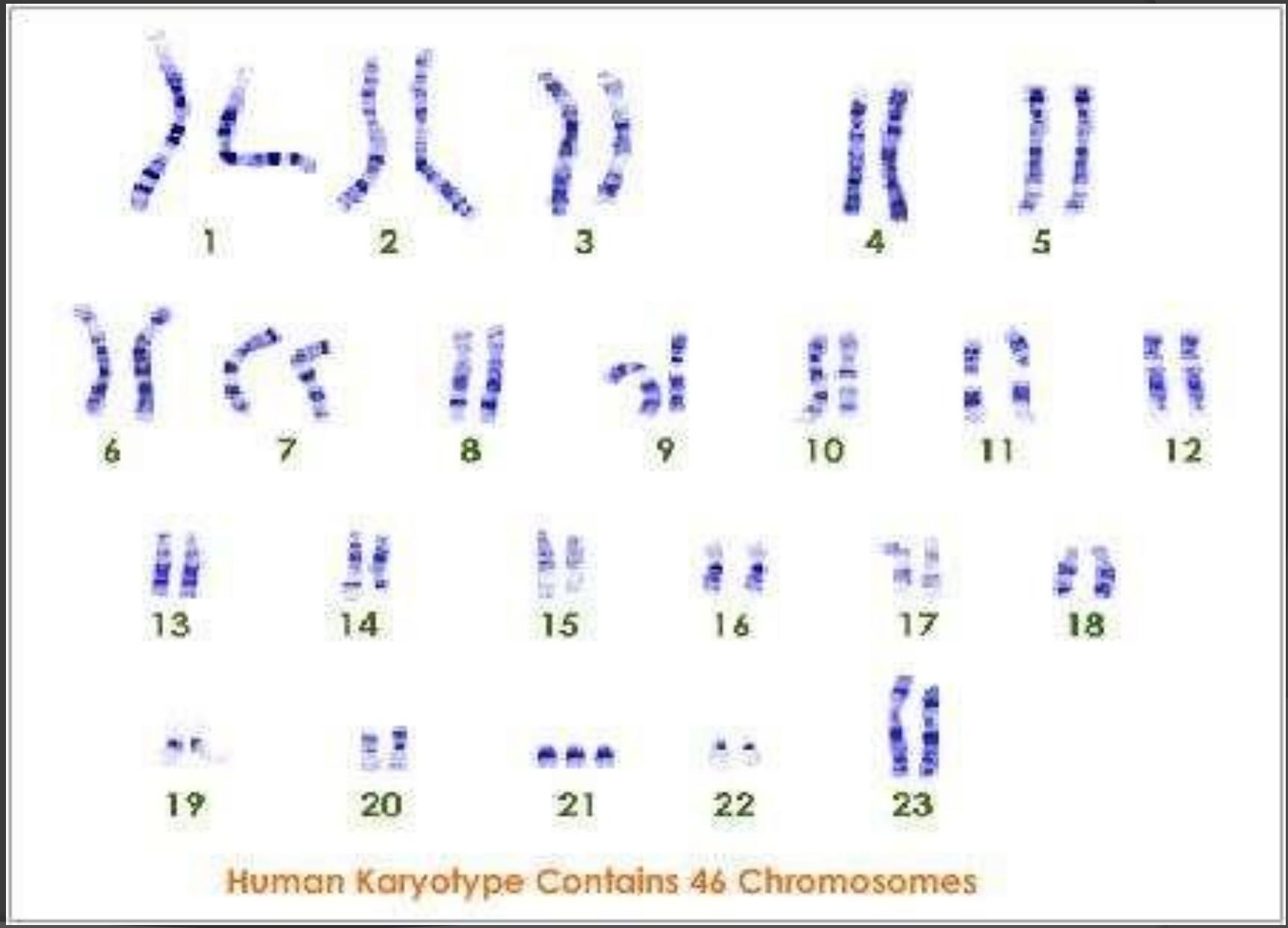
Eukaryotic chromosome

- In eukaryotes the chromosomes are multiple large, linear and are present in the nucleus of the cell
- chromosome typically has one centromere
- the chromosomes of eukaryotic organisms consists of complexes made of DNA and protein
- Eukaryotic genomes contain levels of complexity that are not encountered in prokaryotes.
  - One from each parent. Some flowering plants are polyploidy that is they carry several copies of the genome.



Human chromosome

- Humans chromosomes are of two types autosomes and sex chromosomes
- Genetic traits that are linked to the sex of the person are passed on through the sex chromosomes
- The rest of the genetic information is present in the autosomes
  - 23 pairs of chromosomes in their cells, of which 22 pairs are autosomes and one pair of sex chromosomes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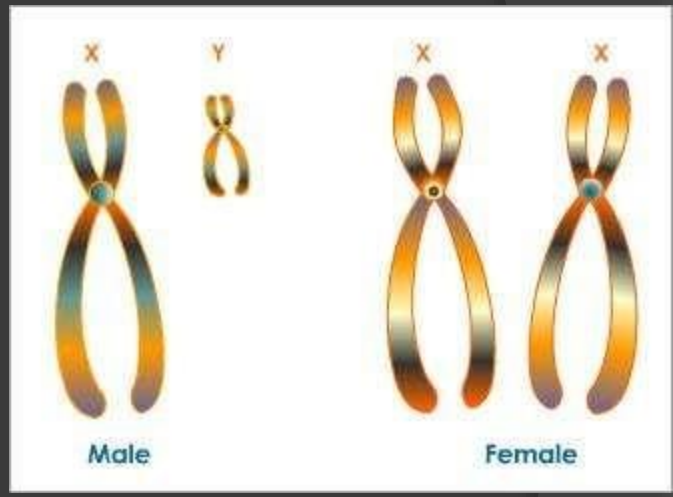
Human Karyotype Contains 46 Chromosomes

Sex Chromosomes

- Sex chromosomes differ in form of size, behavior from the ordinary chromosome.
  - determine the sex of an individual during reproduction
- These sex chromosomes differ between the male and the females.
  - Females have two copies of X chromosome, males have one X chromosome and one Y chromosome



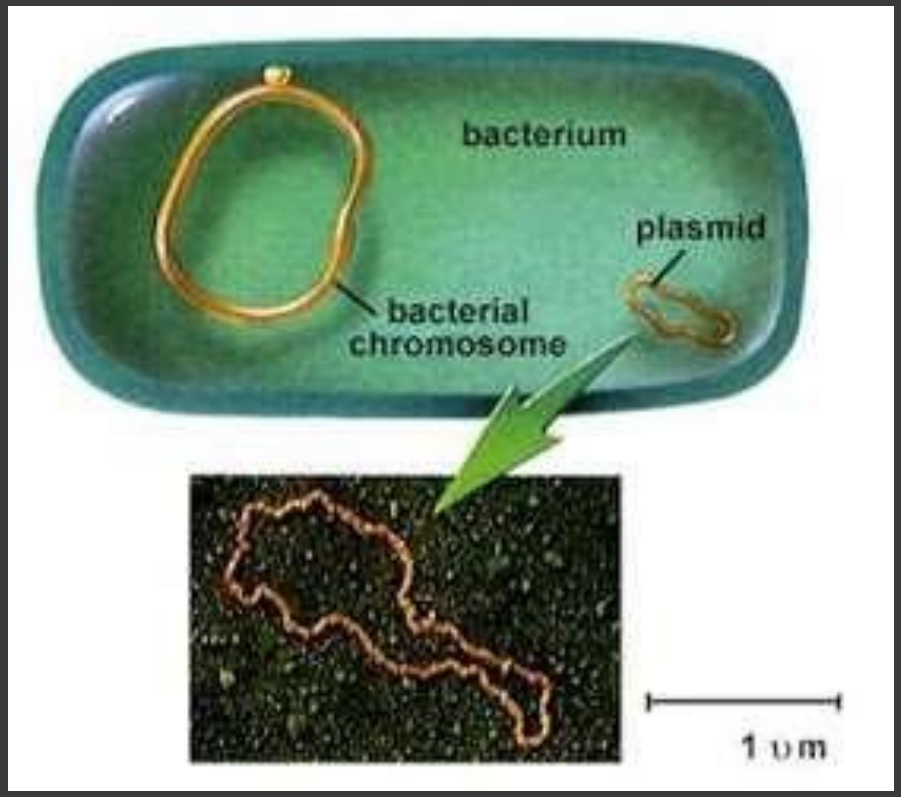
Homologous chromosomes

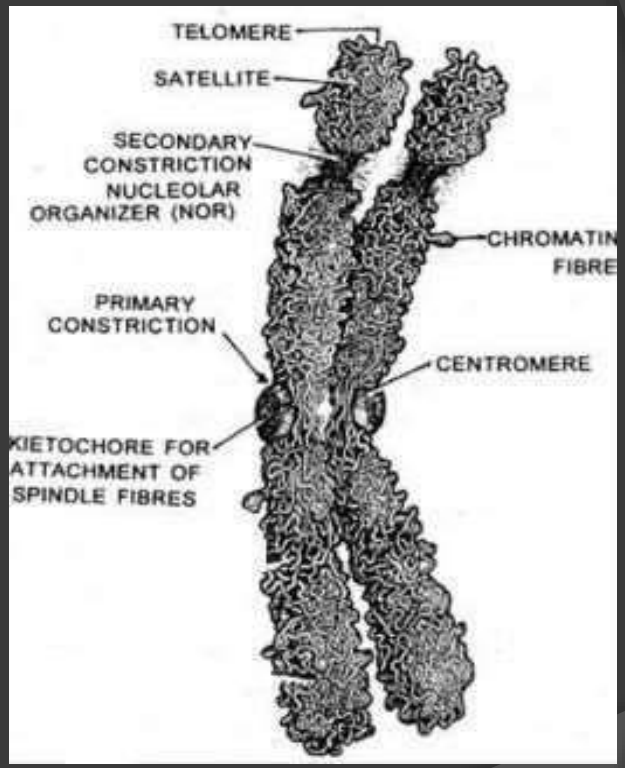
- Homologous chromosomes are also known as homologs or homologues.
- same length, position of centromere, and pattern of staining, genes for the same characteristic are at a corresponding loci.
- These chromosomes are usually not identical, but they carry the same type of genes
- During the process of mitosis the daughter chromosomes carry the same sequence of nucleotide
- The genes are carried in the same order, but the alleles for the trait may not be similar.

Difference between prokaryotic and eukaryotic chromosome

Prokaryotic chromosome	***Eukaryotic chromosome***
Found in cytoplasm ,nucleoid	Found in nucleus
Circular chromosome attached to the inside of cell membrane	Linear chromosome
Single chromosome plus plastid	Many chromosome
Made only of DNA	Made of chromatin, nucleoproteins
Copies its chromosome and divides immediately afterwards	Copies chromosome, then cell grow, then goes to mitosis to divide chromosomes equally
Histone proteins are absent	Histones proteins are present

Prokaryotic and eukaryotic chromosome



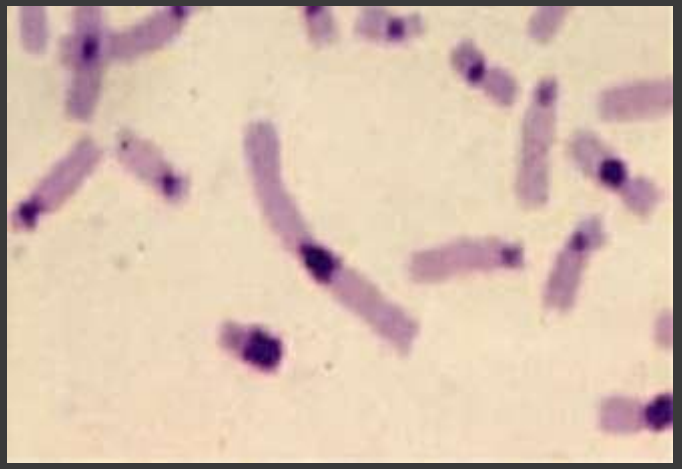


Functions

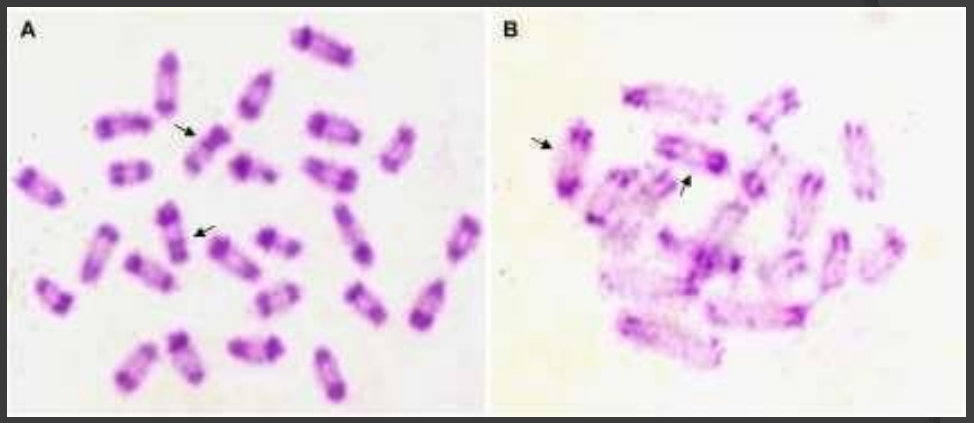
- Genetic Code Storage
  - code for specific proteins
- Sex Determination
  - Females have two X chromosomes and males have one X and one Y chromosome.
- Control of Cell Division
  - Chromosomes check successful division of cells during the process of mitosis.
- Formation of Proteins and Storage
  - chromosomes direct the sequences of proteins formed in our body

Chromosome banding

- C-Banding
- C-banding stains areas of heterochromatin, which is tightly packed and repetitive DNA. C-banding is specifically useful in humans to stain the centromeric chromosome regions and other regions containing constitutive heterochromatin - secondary constrictions of human chromosomes 1, 9, 16, and the distal segment of the Y chromosome long arm.



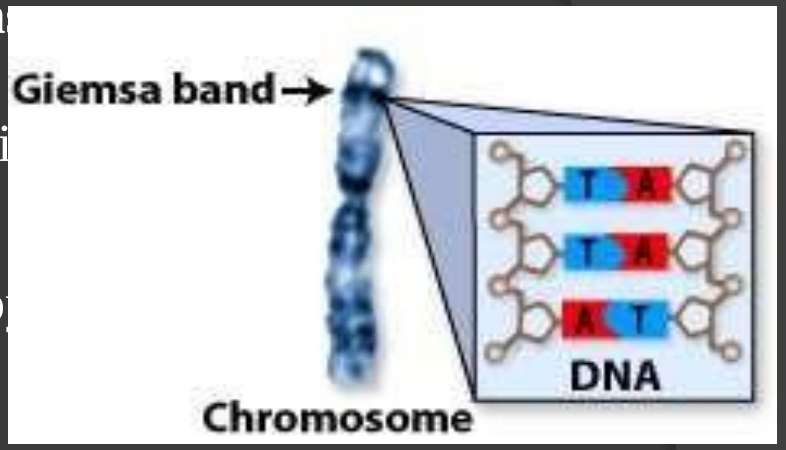
Representative partial metaphase illustrating C banding



C-banding of chromosomes from male Latrodectus.(A) L. hesperus with arrows pointing to sex chromosomes X1 and X2, and (B) L. geometricus with arrows pointing to LG-4

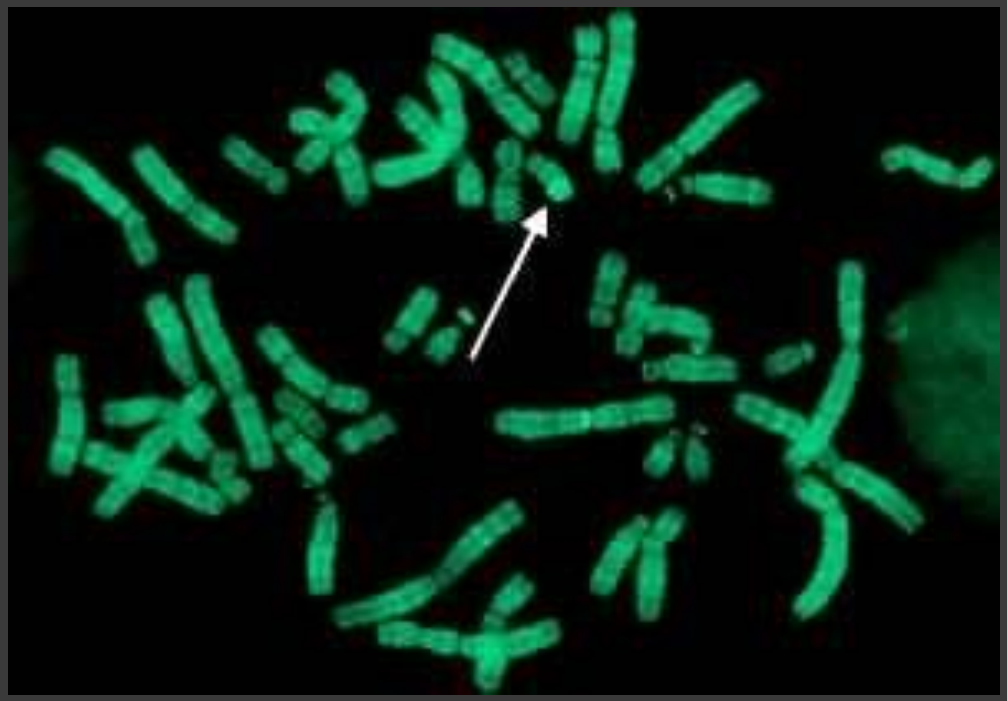
G-Banding

- The technique of G-banding involves **Giem** staining following pretreatment with weak **trypsin solution, urea or protease**. It provi greater detail than C-banding.
- It was first used for human chromosomes b Summer et al. in 1971.
- G-bands may reflect a stronger chromatin condensation. However, this technique is not suitable for plant chromosomes.
- Dye gives chromosomes a striped appearance because it stains the regions of DNA that are rich in adenine (A) and thymine (T) base pairs.



Q-Banding

- **Quinacrine mustard**, an alkylating agent, was the first chemical to band chromosomes viewed under a fluorescence microscope.
- **Quinacrine dihydrochloride** has subsequently been substituted by **quinacrine mustard**. The alternating bands of bright and dull fluorescence are called Q bands.
- The bright bands are primary composed of DNA rich in adenine and thymine, while the dull bands are rich in guanine and cytosine.
- Q bands are especially useful for distinguishing the human Y chromosome and various chromosome polymorphisms involving satellites and centromeres of specific chromosomes.

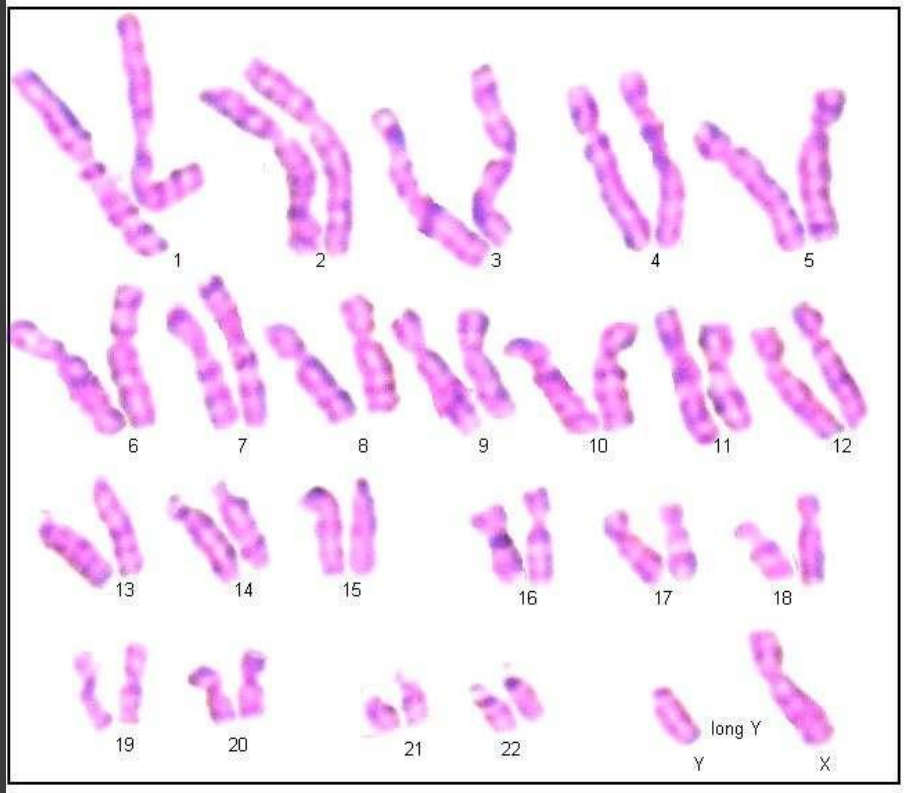


Q-banded metaphase from a healthy male with the Y chromosome (arrow)

R-Banding

- Reverse banding (R-banding) involves the incubation of slides containing metaphase chromosomes in hot phosphate buffer and stained with **Giemsa.**
- The banding pattern that results is essentially the reverse of G bands. R bands are G-C-rich.
- The A-T-rich regions are selectively denatured by heat leaving the G-C-rich regions intact.
- **Fluorochromes** that are GC specific also produce a reverse chromosome banding pattern. R-banding is helpful for analyzing the structure of chromosome ends, since these areas usually stain light with G-banding.

R-Banding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long Y
Y
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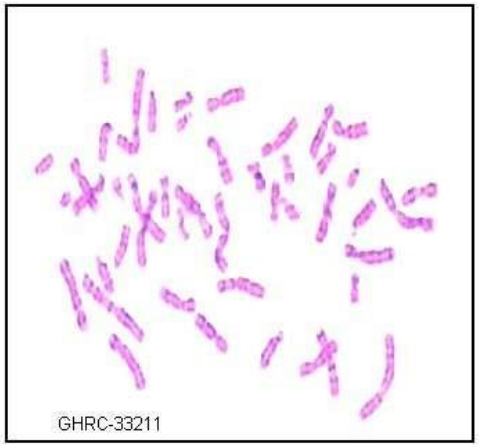
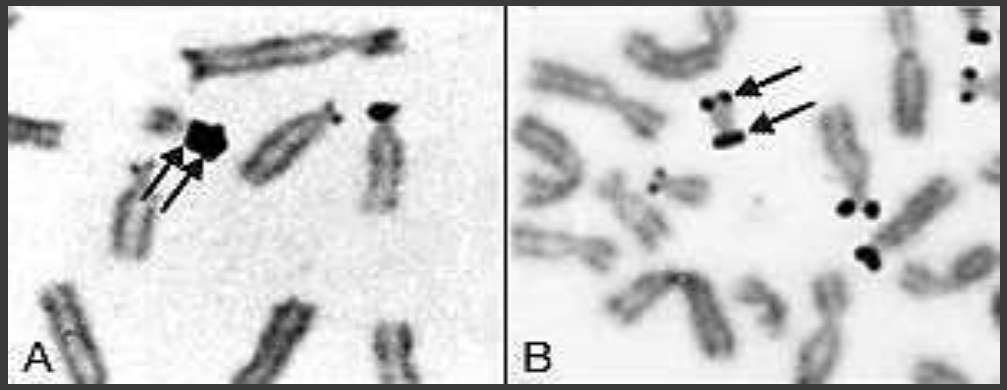

GHRC-33211

NOR-banding

- NOR-banding involves silver staining (silver nitrate solution) of the "nucleolar organizing region", which contains rRNA genes.

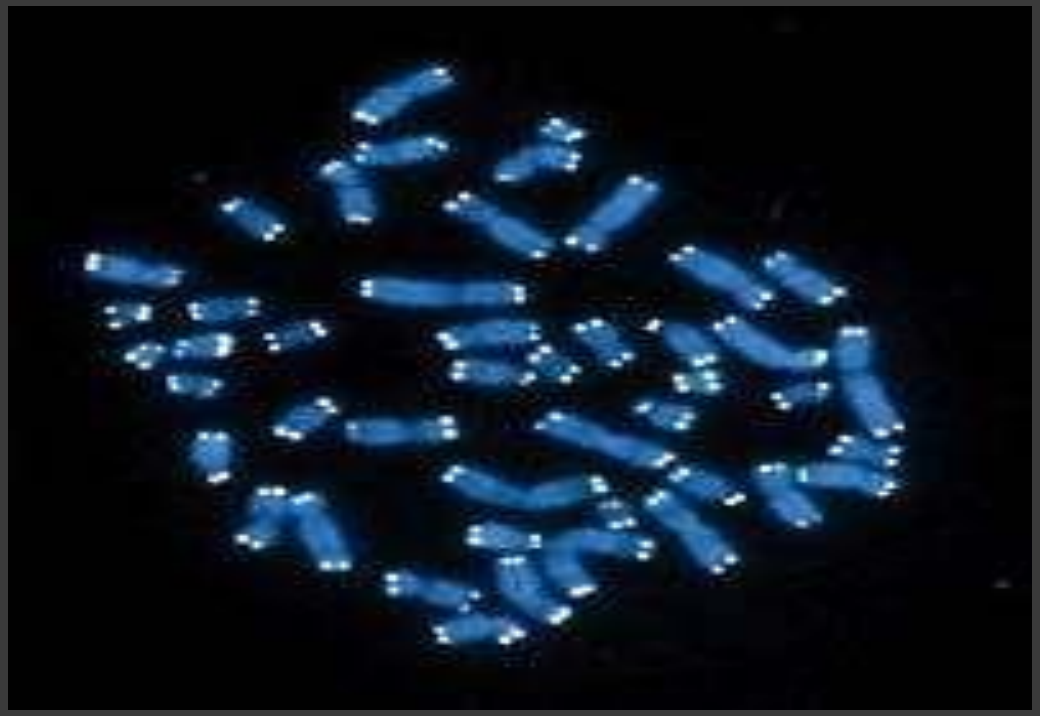


- Representative nucleolar organizing region-banded partial metaphases illustrating double satellites (A) and an isodicentric chromosome with satellites at both ends (B)

T-Banding

- T-banding involves the staining of telomeric regions of chromosomes using either **Giemsa** or **acridine** orange after controlled thermal denaturation.
- T bands apparently represent a subset of the R bands because they are smaller that the corresponding R bands and are more strictly telomeric.

T-banding

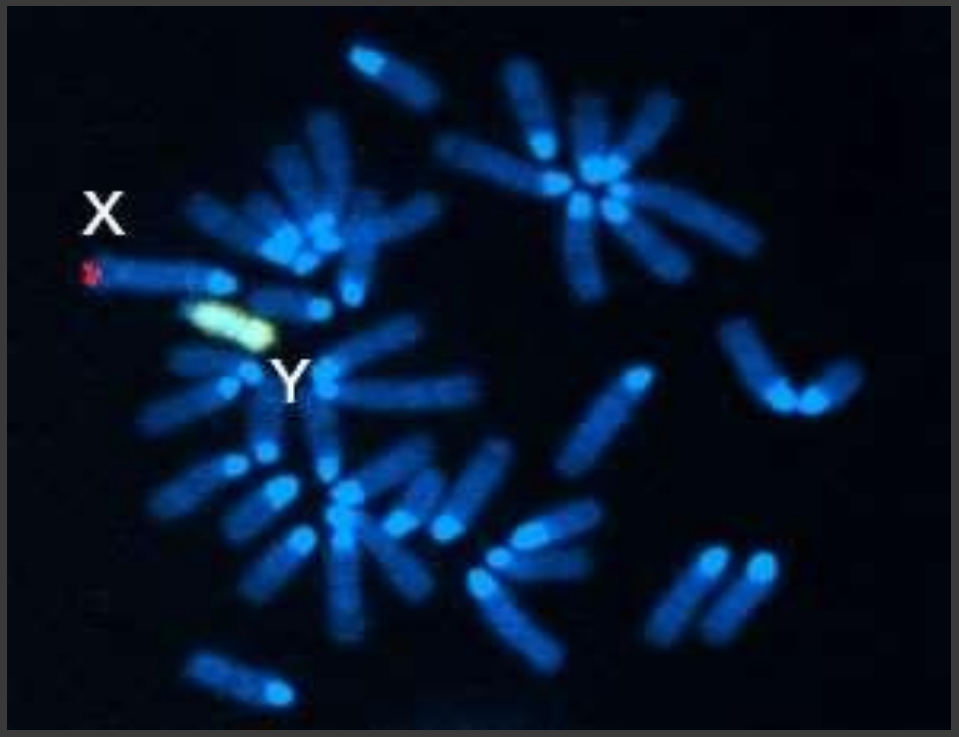


- N-Bands:The technique of N-banding was originally described by Matsui and Sasaki in 1973. Briefly, air-dried chromosomes slides are stained for 90 minutes with Giemsa following extraction with 5% trichloroacetic acid at 95°C for 30 minutes and then 0.1 NH_4Cl at 60″C for 30 minutes.
- The N-bands are generally located at the secondary constriction, satellites, centromeres, telomeres and heterochromatic segments. It is suggested that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar organizer region of the eukaryotic chromosomes.

- The N- banding patterns have been used for the location of nucleolar regions in the different organisms, such as, mammals, birds, amphibians, fishes, insects and plants. N-banding patterns differ in the chromosomes of different species

FISH

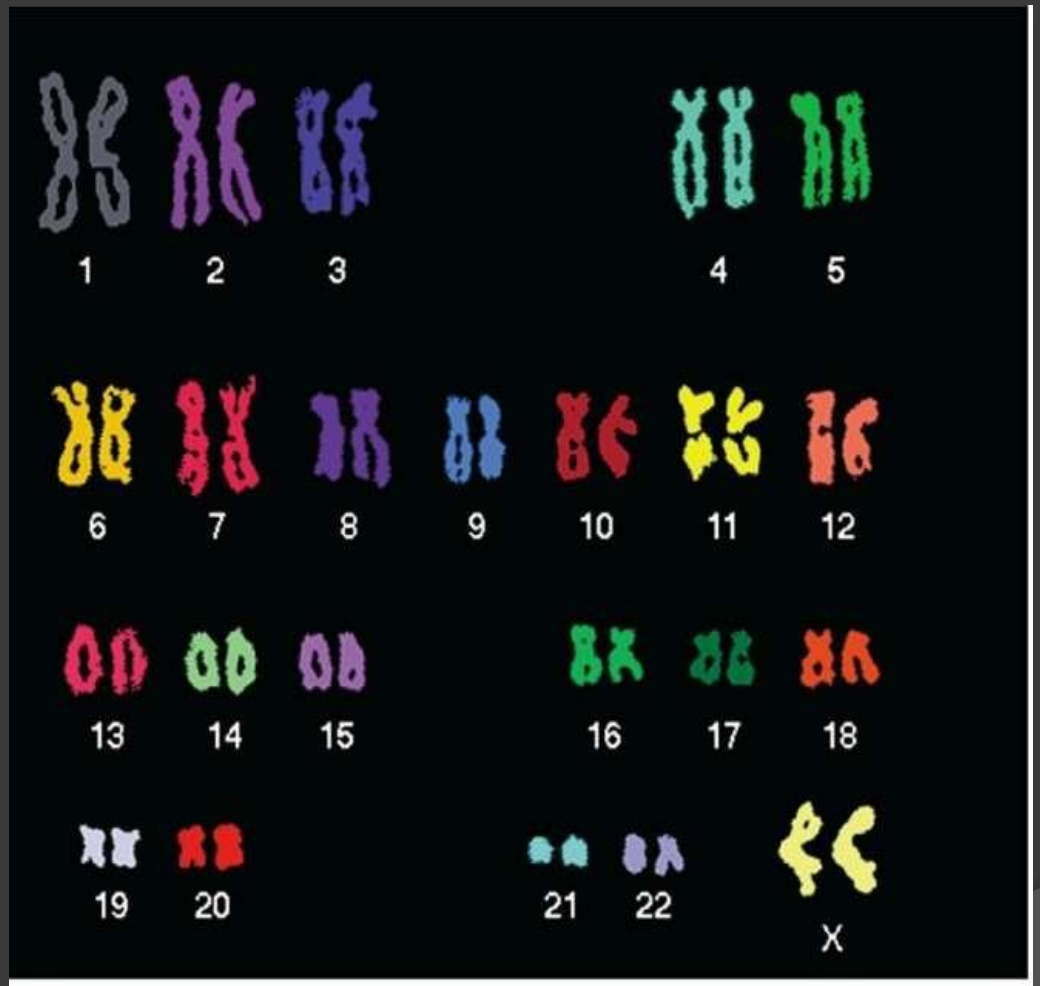
- **Fluorescence *in situ* hybridization** (**FISH**) is a cytogenitics technique that uses fluorescence probes that bind to only those parts of the chromosome with a high degree of sequence complementarity.
- It was developed by biomedical researchers in the early 1980s[1] and is used to detect and localize the presence or absence of specific DNA sequencing on chromosomes .
- Fluroscence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification.
- FISH can also be used to detect and localize specific RNA targets in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expressions within cells and tissues



FISH signals on mouse metaphase spreads
Yellow:Y, Red: X

Chromosome painting

- Chromosome painting involves the use of fluorescent-tagged chromosome specific DNA sequences to visualize specific chromosomes or chromosome segments by in situ DNA hybridization and fluorescence microscopy
- Chromosome painting refers to the hybridization of fluorescently labelled chromosome-specific, composite probes to cytological preparations. Chromosome painting allows the visualization of individual chromosomes in metaphase or interphase stages and the identification of both numerical and structural chromosomal aberrations with high sensitivity and specificity
- which is also called colour karyotyping.



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thank
you