Ivana FELLNEROVÁ

Introduction to CYTOGENETICS

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What is

CYTOGENETICS ?

Study of the CHROMOSOMES

- **Structure**
- **Function**
- **Behavior during mitosis and meiosis**
- **Evolution**

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LECTURE OVERVIEW

- **Eukaryotic Chromosome**
  [structure, function]
- **Human karyotype**
  [autosomes, sex chromosomes]
- **Cytogenetic techniques**
  chromosome banding
  FISH, M-FISH, SKY, CGH

chroma = "colour"

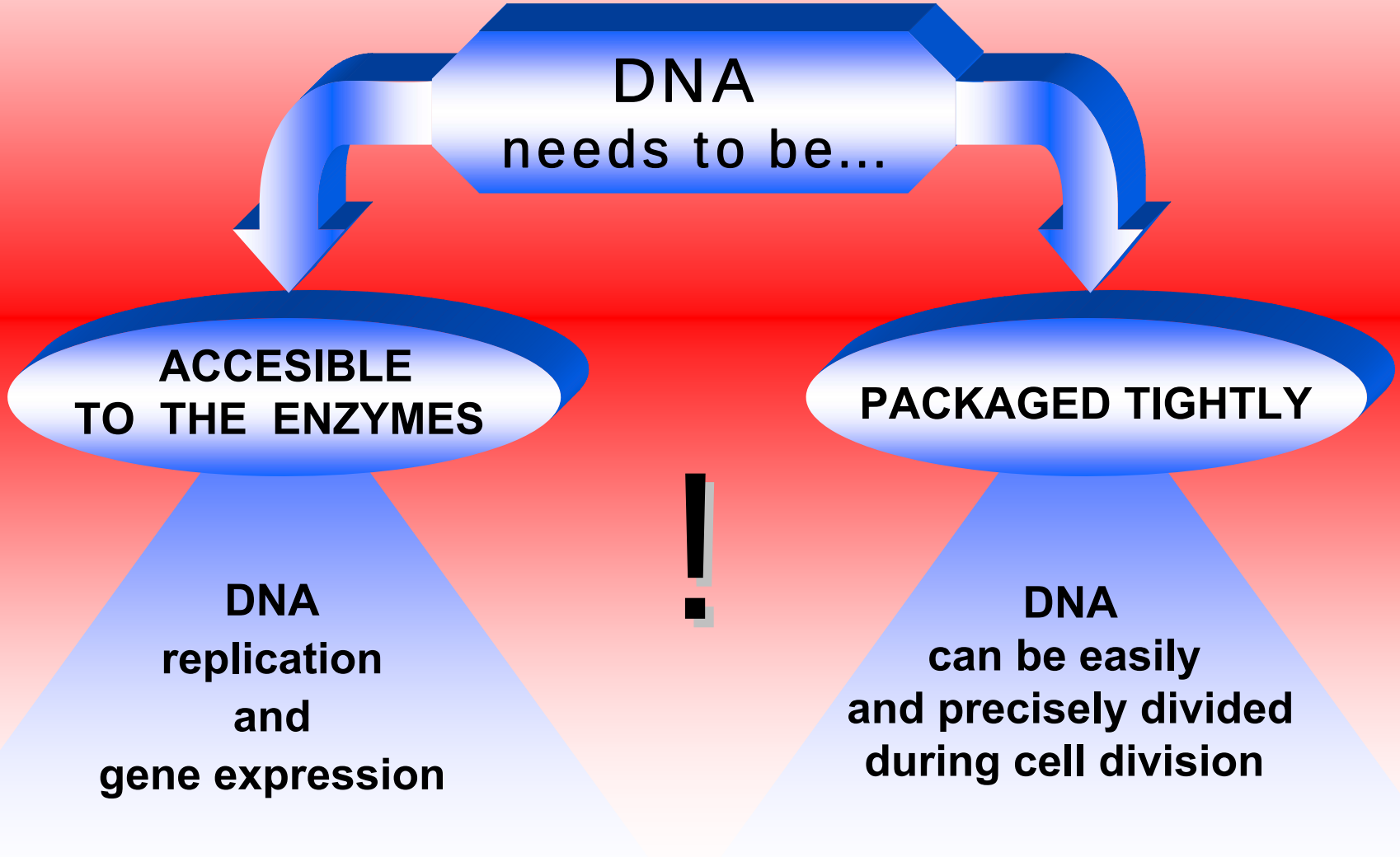
soma = "body"

What is eukaryotic

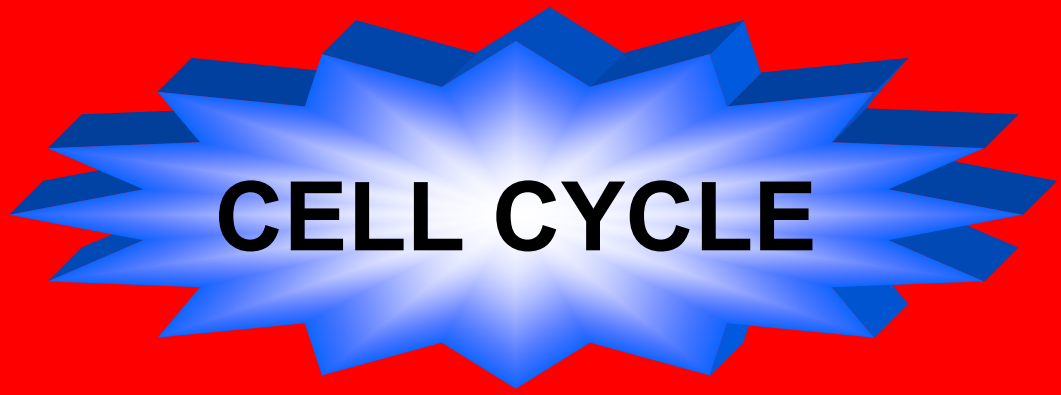
CHROMOSOME ?

- "Packages" that carry out genes
- Threadlike structures located in the <u>cell nucleus</u>
- Composed of substance called <u>chromatin</u>: DNA
  proteins

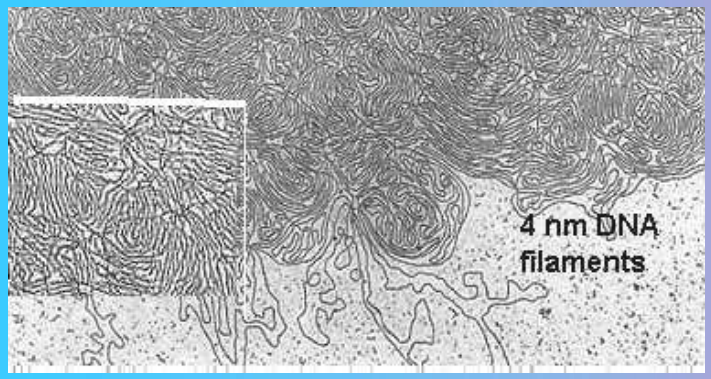
TWO conflicting requirement of the cell:

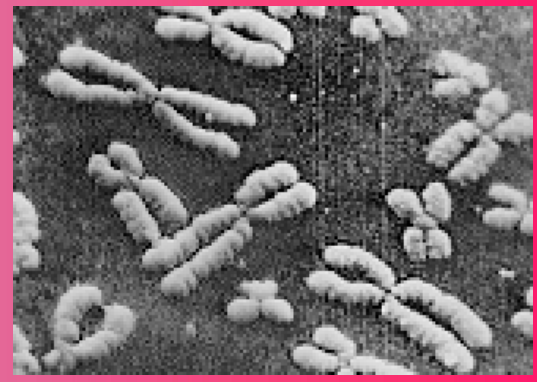


Chromosomes are dynamic structures
Gross morphology dynamically changes during the cell cycle



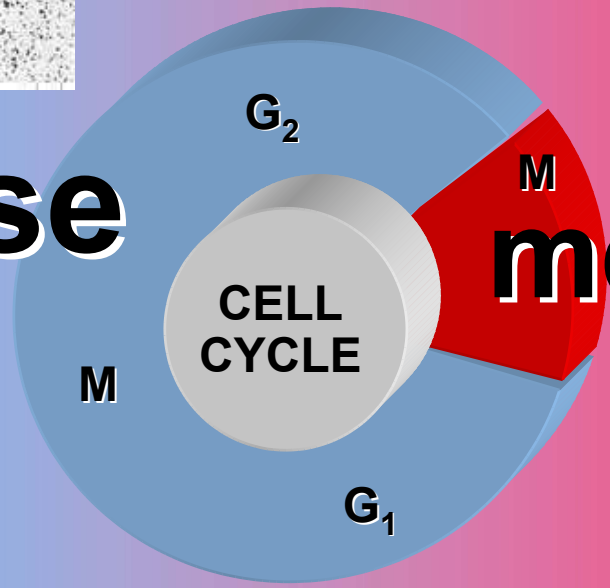
It is important to distinct between interphase and mitotic chromosomes





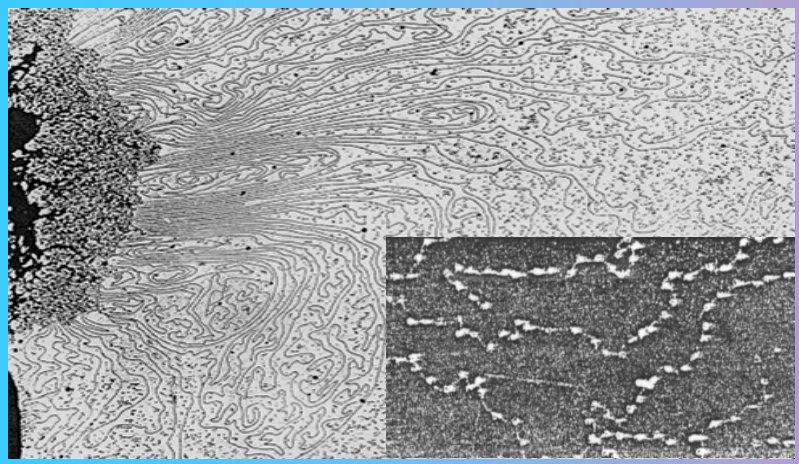
interphase

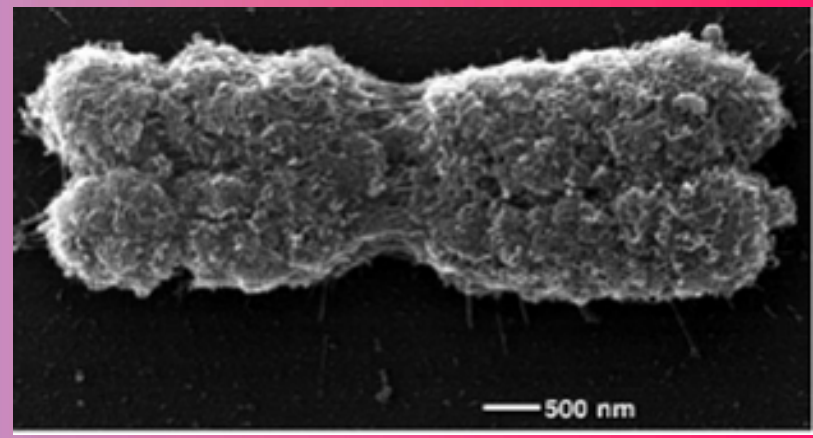
Chromosomes exist as ultrafine **threads of chromatin** dispersed throughout the nucleus



metaphase

Chromatin condense into short **cylindrical thick chromosomes**





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CHROMATIN STRUCTURE

Interphase chromosome

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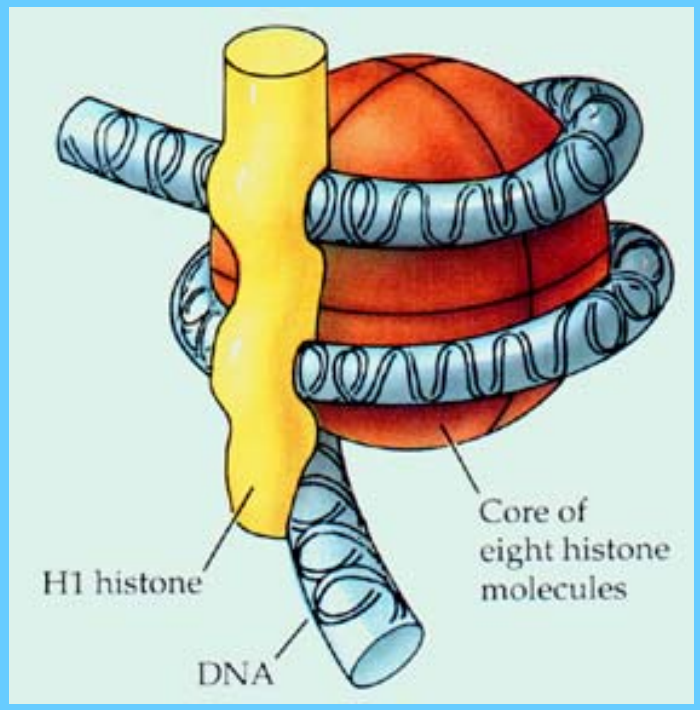
Molecular structure of

chromatin:

- ❑ **DNA**
- ❑ **histone proteins**
- ❑ **non-histone proteins**

  [RNA polymerase, DNA-binding proteins, gene regulators]

nucleosome...



11 nm

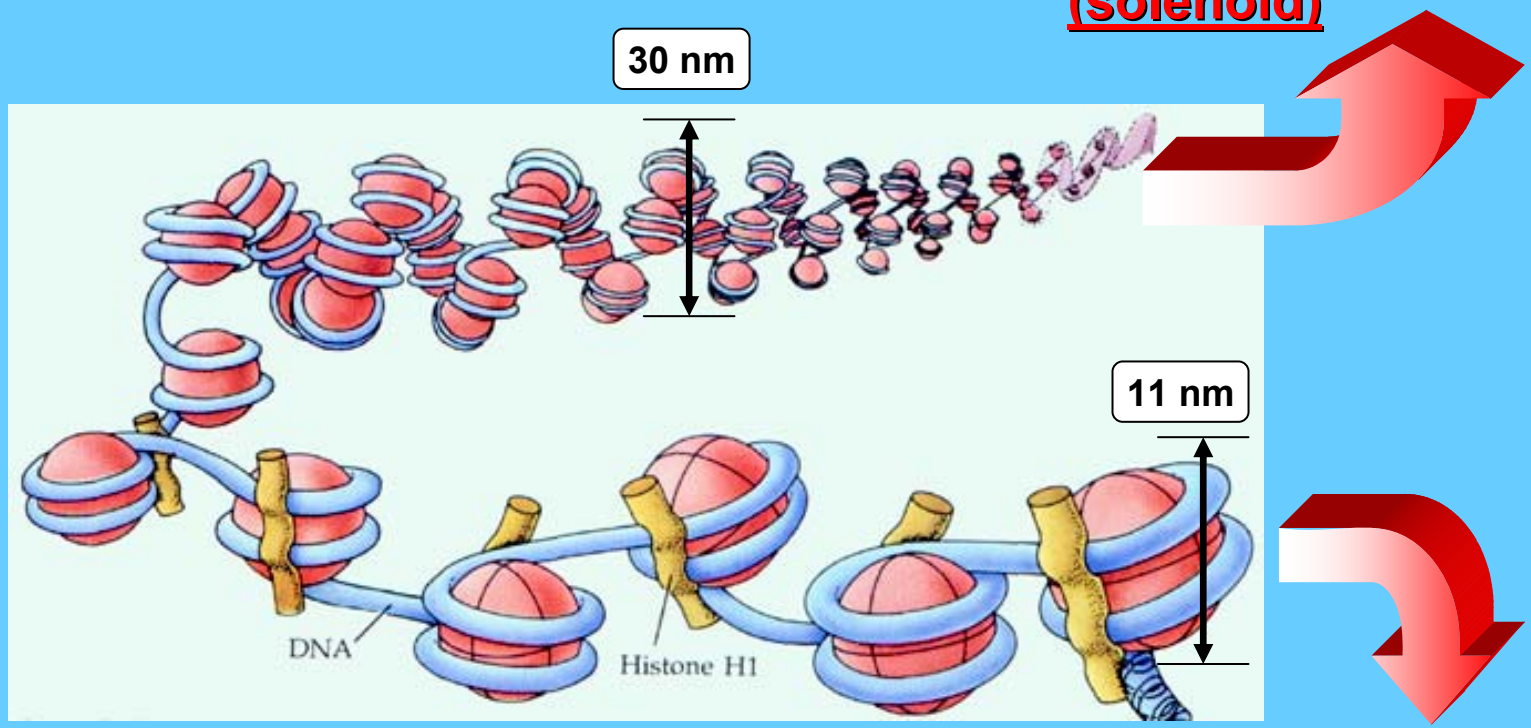
DNA double helix coils around a central core of **eight histone molecules** to make **nucleosome**

Another **histone (H1)** clamps DNA to the core

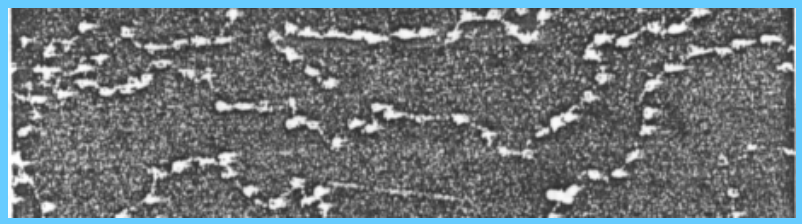
DNA per each nucleosome contain about 200 base pairs

...elementary structural unit of chromatin

Six nucleosomes create one helical turn of the **30 nm chromatin fiber (solenoid)**



Short region of naked DNA link each nucleosome: **11nm chromatin fiber**



The **“beads”** = nucleosomes
The **“threads”** = DNA

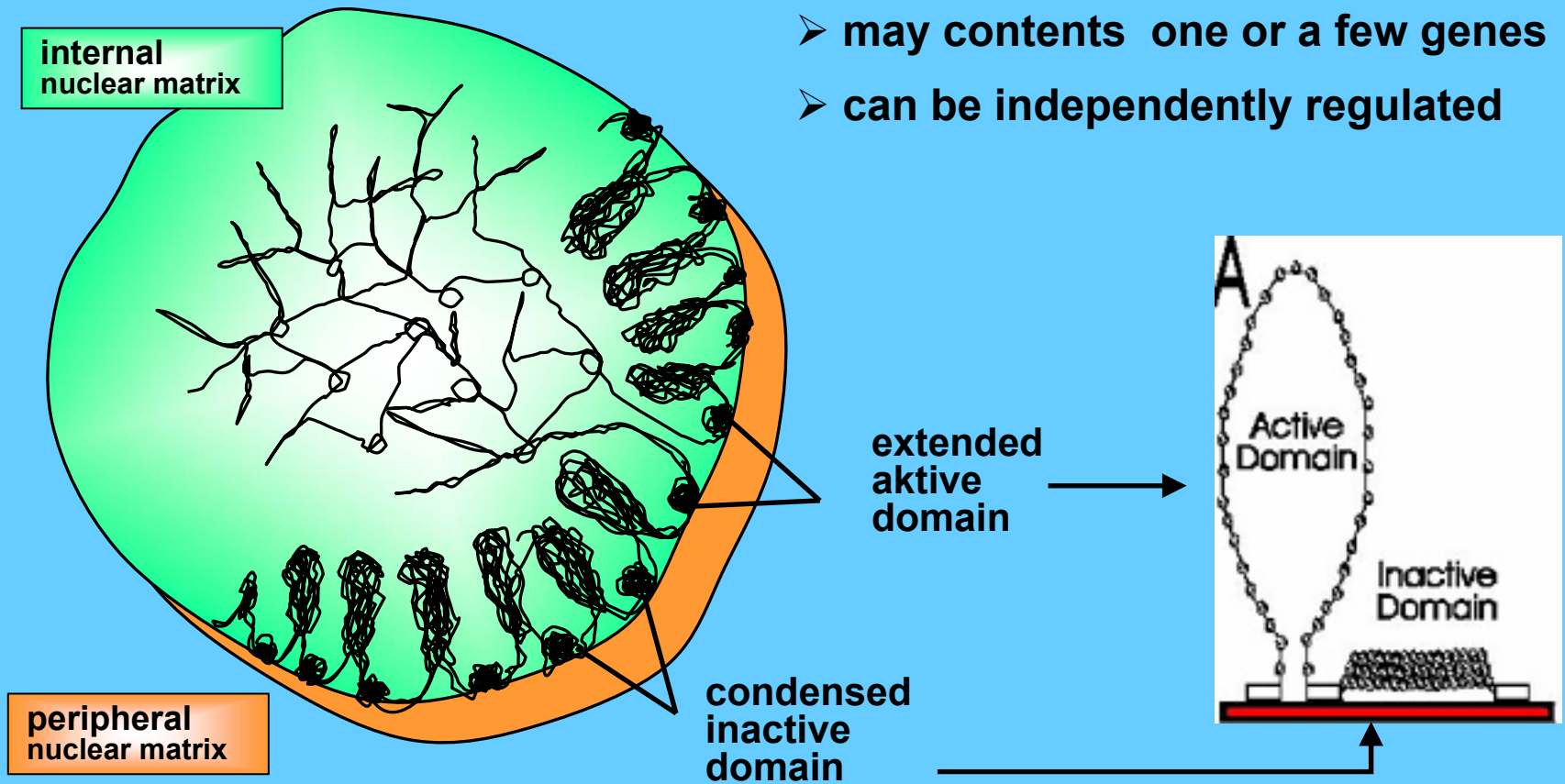
CHROMATIN in INTERPHASE NUCLEUS

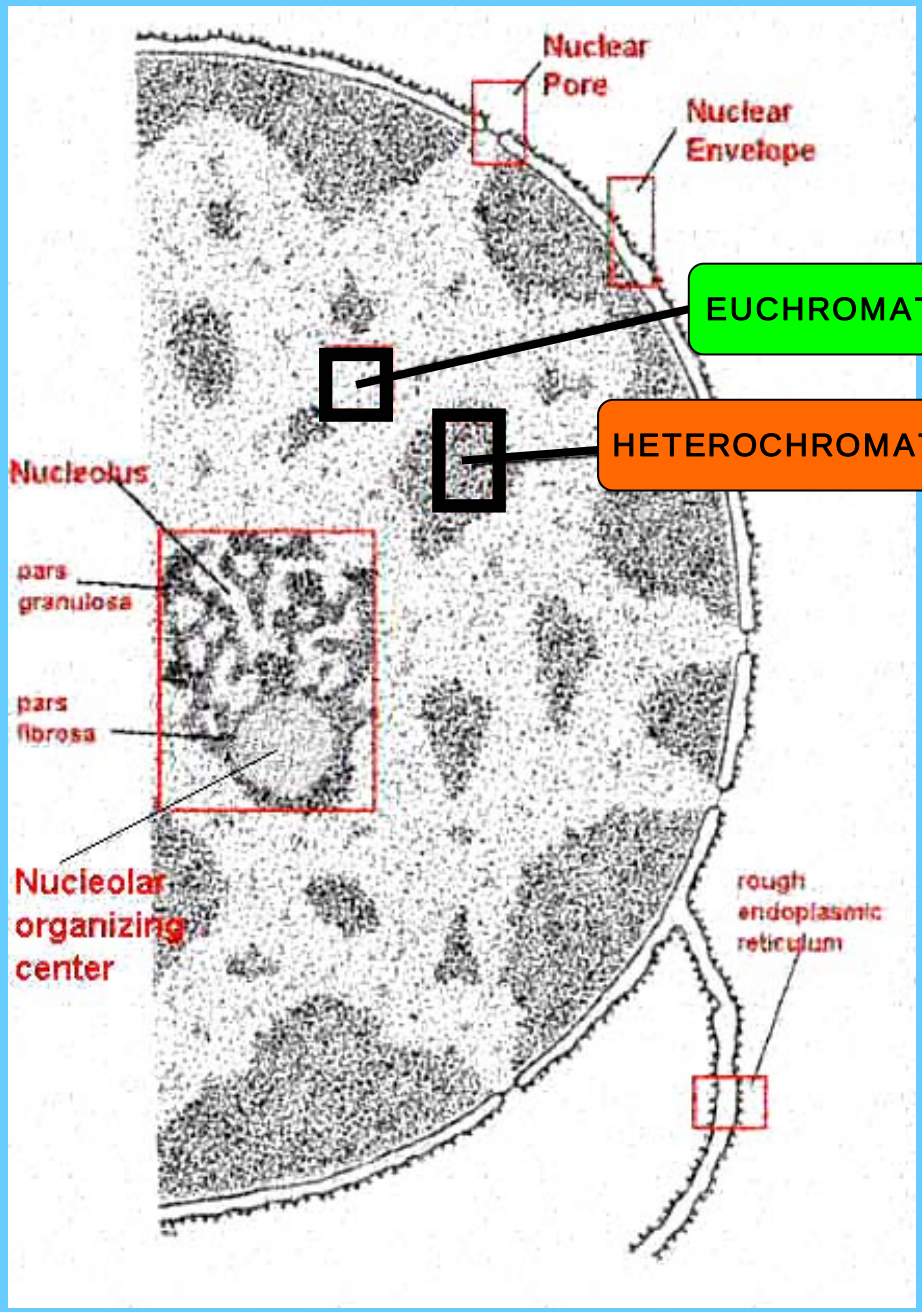
Most interphase chromatin is condensed into 30nm coil

Chromatin fiber in the nucleus is organized into discrete loop domains

EACH LOOP:

- **is attached to nuclear protein matrix**
- **may contents one or a few genes**
- **can be independently regulated**





Lightly-staining transriptional active

Darkly-staining transriptional inactive

Constitutive heterochromatin:

- contains few genes
- always condensed
- formed of sequences located in regions coincident with centromeres and telomers

Facultative heterochromatin

- composed of transcriptional active regions
- may be euchromatin in some developmental or physiological states and heterochromatin in others

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HETEROCHROMATIN	★	EUCHROMATIN
darkly - staining	⟷	lightly - staining
condensed	⟷	extended
transcriptional inactive	⟷	transcriptional active
peripheral nuclear matrix	⟷	fibrilar internal nuclear matrix
highly-repetitive DNA	⟷	single copy or middle repetitive
nucleosomes with H1	⟷	nucleosomes with HMG's

CHROMATIN PACKING

FORMING
METAPHASE CHROMOSOME

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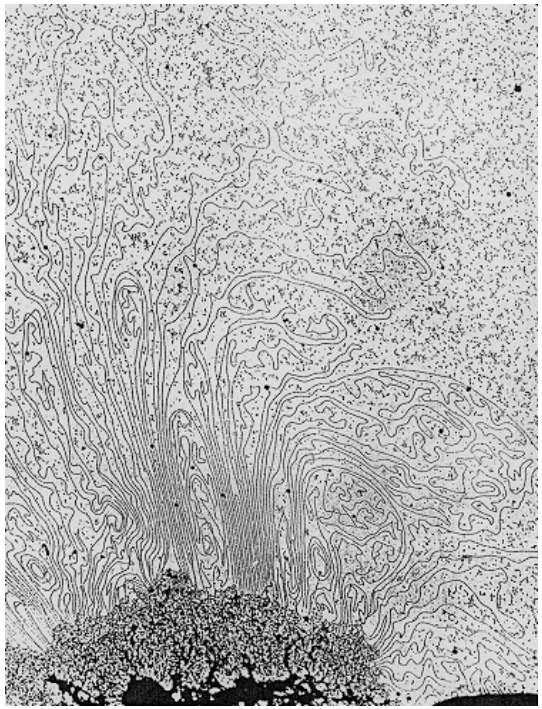
The way from the...

chromatin string

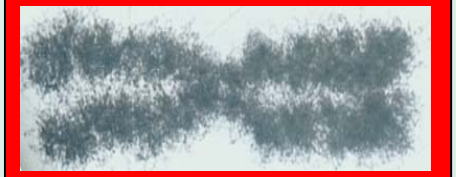


to the

condensed mitotic chromosomes…

…is a highly organized process

metaphase chromosome contains two sister chromatids



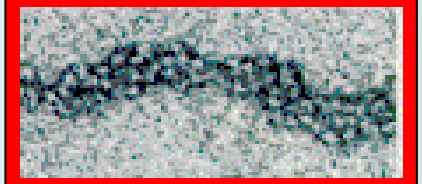
18 chromatin loops around nuclear matrix

supercoiled condense chromatin forms CHROMATID

Chromatin

Loosely condensed chromosome

„SOLENOID“ = left handed chromatin superhelix (30 nm)



Loops by 30nm chromatin fiber

Uncondensed chromatin fiber

DNA

Nucleosome

Histone

„beads on a string“

chromatin fiber 11 nm

DNA double helix (2 nm)

DNA

DNA wrapped around histones create nucleosom
nucleosomes are linked together by DNA

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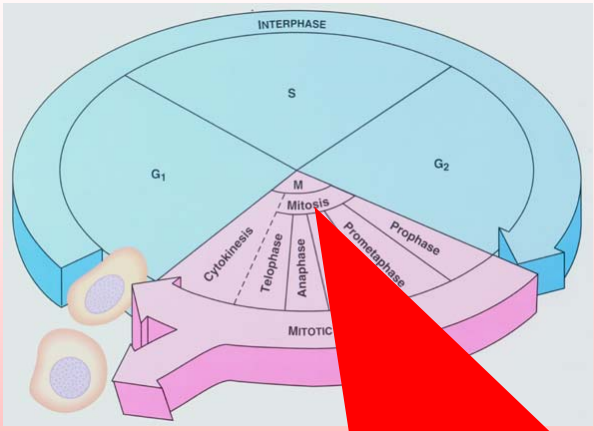
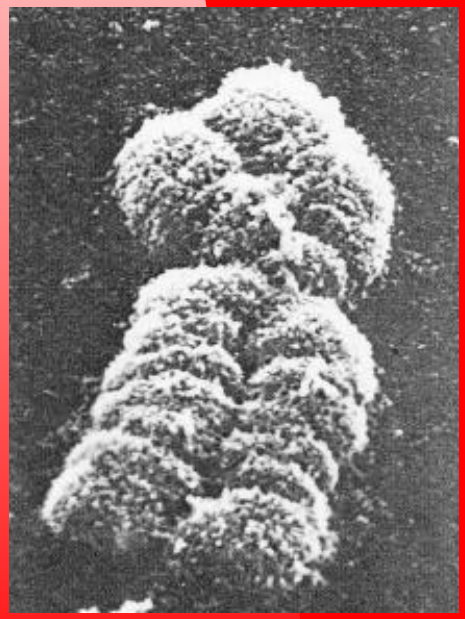
The highest level of chromosome organization appears during...



...METAPHASE

High-order helically coiled chromatin forms cylindrical chromosome



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Mitotic metaphase is the best stage for studying chromosome morphology

METAPHASE CHROMOSOME

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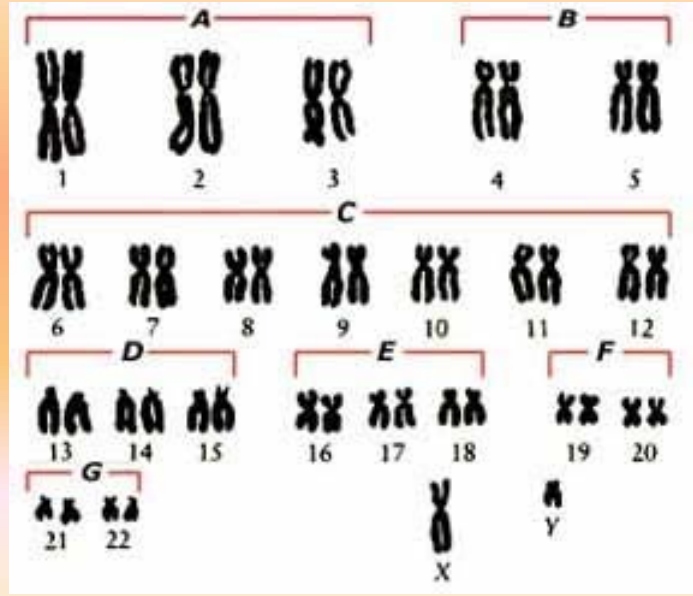
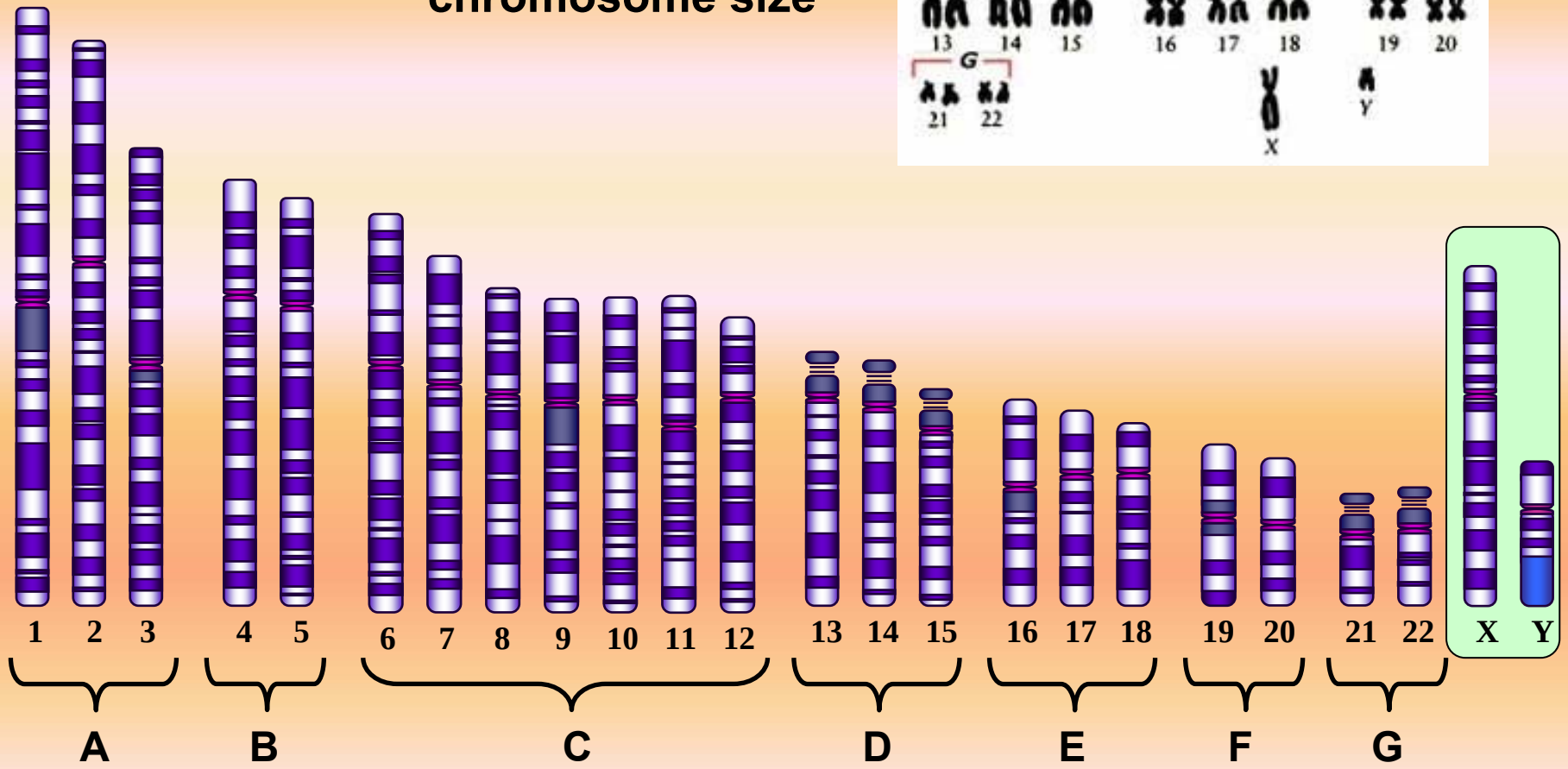
Morphological characteristic of CHROMOSOMES

- SIZE
- CENTROMERE **position [determines the ratio of arm length]**
- **Presence of** NUCLEOLAR ORGANISER **regions [NORs] and** FRAGILE SITE

Chromosome SIZE

Fourfold differences in human chromosome size





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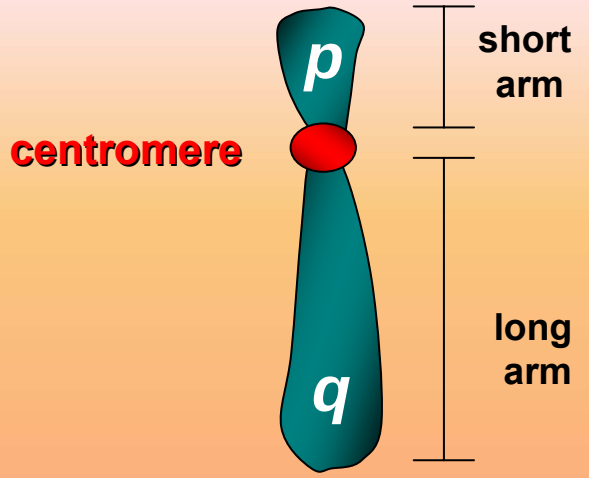
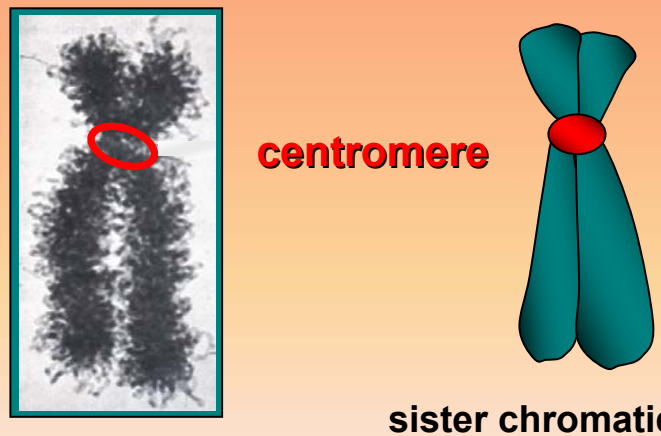
CENTROMERE

- **centromere** = **primary constriction**
  region of a mitotic chromosome
- divides chromosome into two arms "*p*" and "*q*"

Prophase chromosome



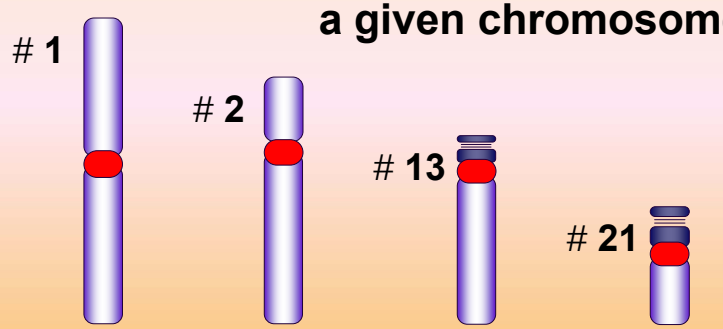
Metaphase chromosome

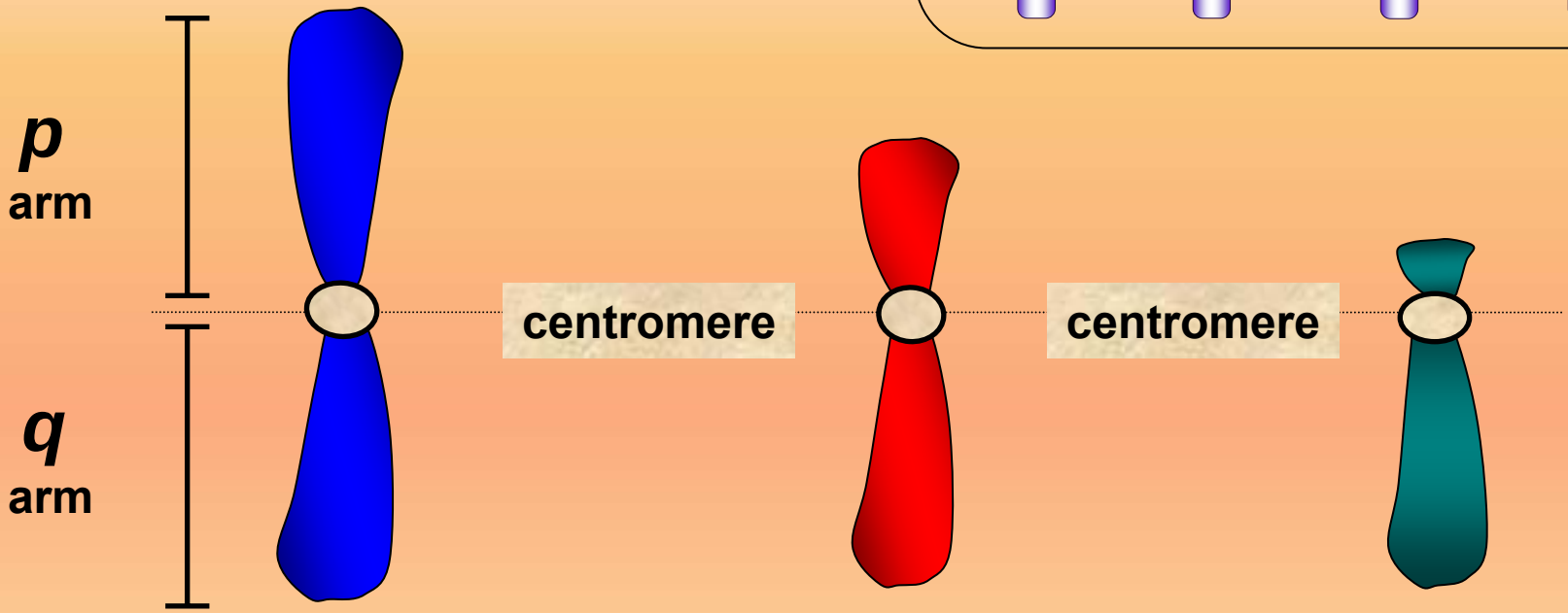


- holds **sister chromatids** together during mitosis

CENTROMERE position:

Centromere position is constant for a given chromosome





METACENTRIC
Centromere near the midle
$p = q$

SUBMETACENTRIC
Centromere near one end
$p < q$

ACROCENTRIC
Centromere near one end
$p <<< q$

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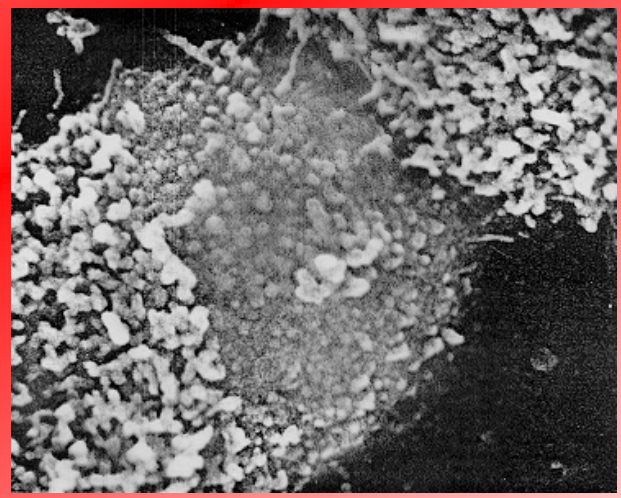
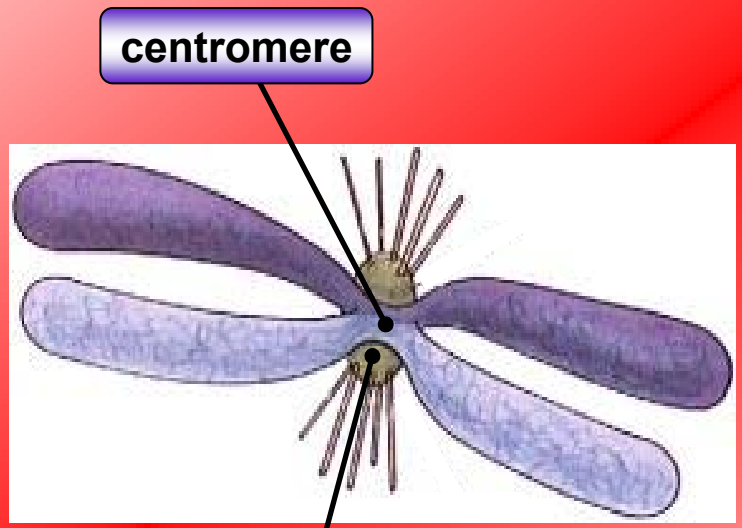
Closer look at CENTROMERE

- contents a special kind of DNA sequence
- represents constitutive heterochromatin





- centromere is the region where **spindle fiber** is attached spindle fiber separates sister chromatids during cell division

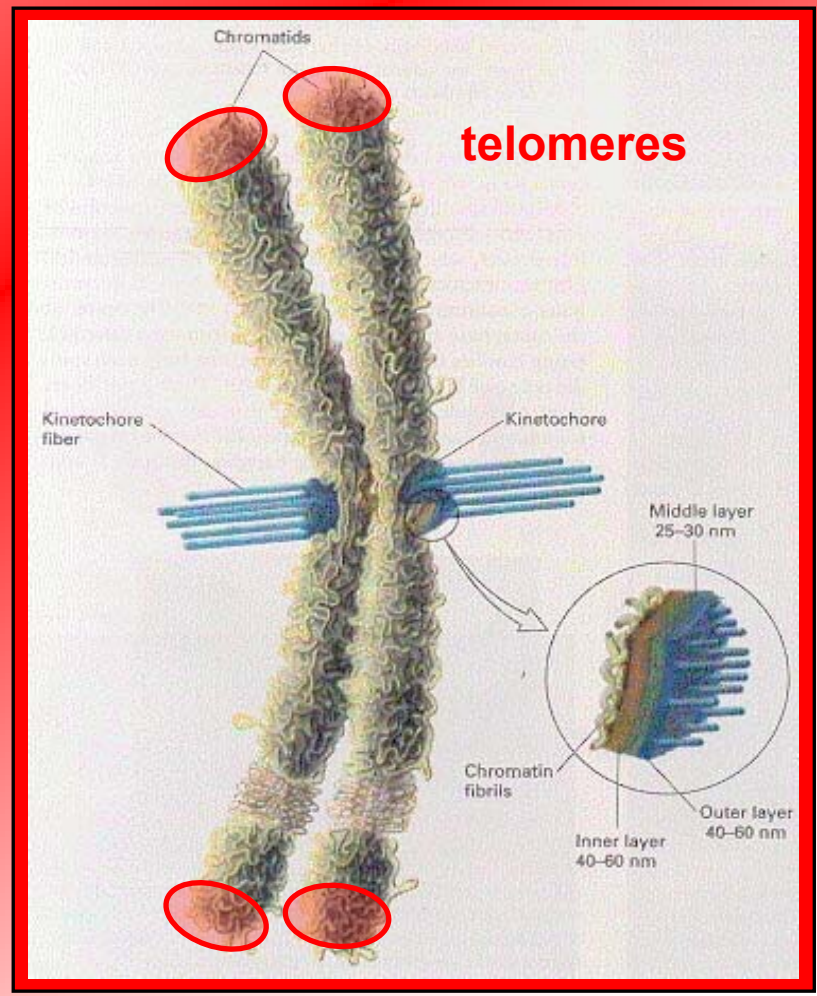
Other specific regions of chromosomes

KINETOCHORE

- A protein structure that forms at each centromere on mitotic chromosome
- Serves as the attachment point for the spindle fiber

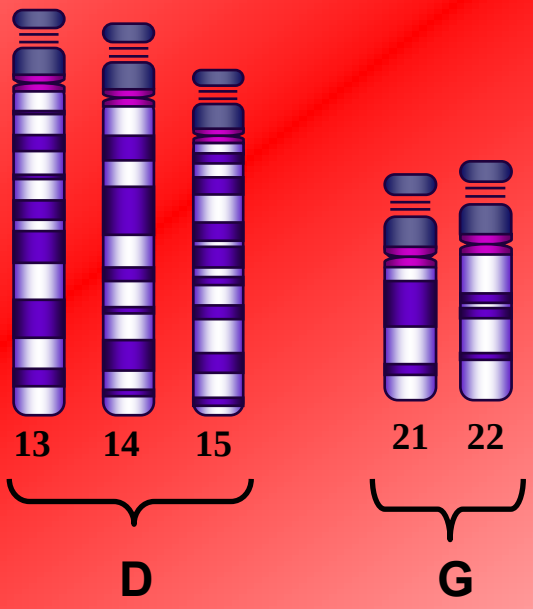
TELOMERE

- Series of short tandem repeats at the end of chromatids.
- Prevents chromosomes from shortening with each replication cycle
- Protects chromosome from nuclease digestion



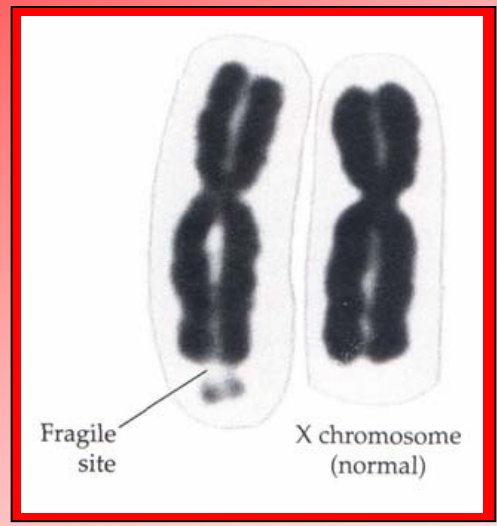
NORs [nucleolar organizer regions]

- Region close centromere of human chromosomes 13, 14, 15, 21 a 22
- Can be identified as secondary constrictions on metaphase chromosomes
- Contains gene coding rRNA
- **Found in all individuals**



Fragile sites

- Weak spots where metaphase chromosomes tend to break
- Look like nonstaining gaps or constriction
- Unlike NORs **does not occur in all individuals**
- Best known on the long arm of X chromosome



KARYOTYPE

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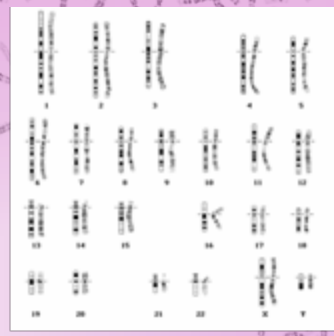
- All eukaryotic cells store their heredity information in chromosomes
- Eukaryotic organisms differs by chromosome number and chromosome morphology
- A simplest way to examine chromosomes is look at a karyotype



KARYOTYPE

Is organized profile of metaphase chromosomes of individual cell

Karyotype is specific to an individual or to related group [species]

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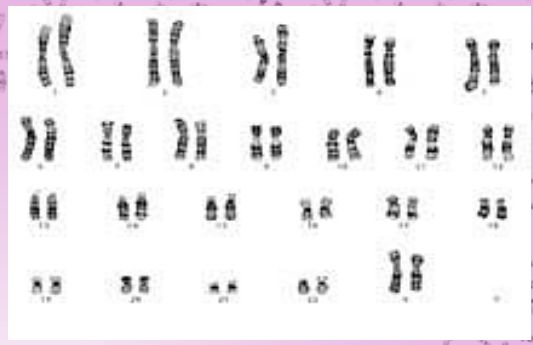


KARYOTYPE
include information about:

- chromosome **number**
- chromosome **size**
- chromosome **shape [morphology]**
- composition of the **sex chromosomes**
- some chromosomal **abnormalities**

CELL MATERIAL for KARYOTYPE:

Tissue source of cell:

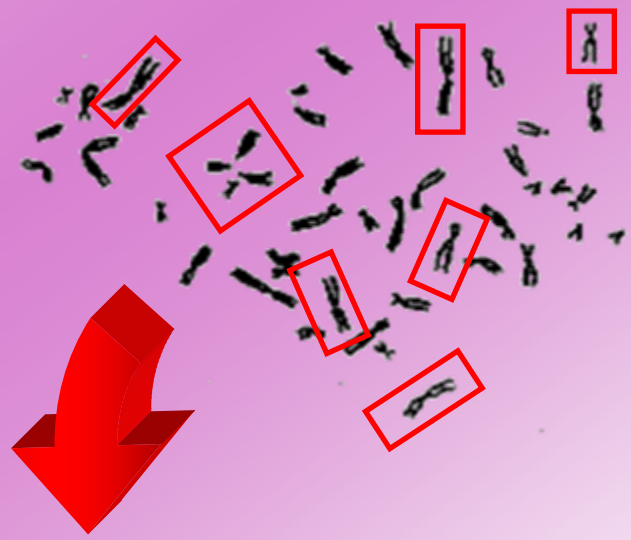
blood – lymphocytes
amniotic fluid
bone marrow
skin

Cell culture pretreatment:

- **stimulation of cell proliferation** [using mitogen like chemicals]
- **stop cell division** at a stage when chromosomes are most condensed and clearly distinguishable
  [using colchicine –as a spindle arresting agent to accumulate metaphase]

MAKING a KARYOTYPE:

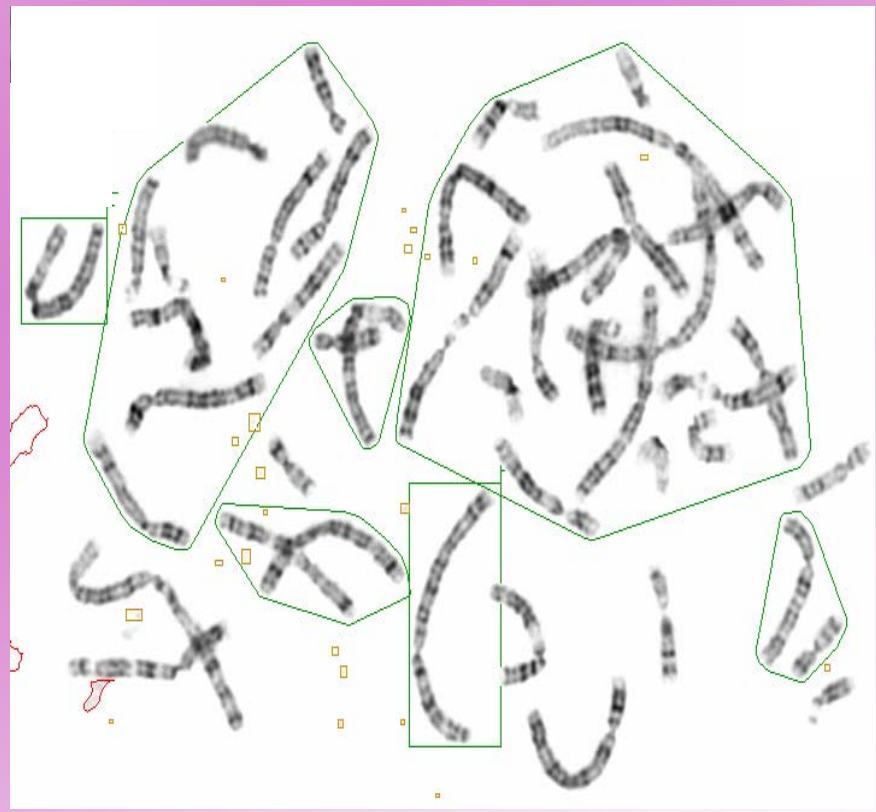
- Metaphase cells are fixed and stained on microscope slide
- Scanning for "good looking" chromosome spreads [not too compact or overlapping]
- Taking picture through a microscope



- Cutting out images of each chromosome and arranging them in order
- Alternatively, a digital image of chromosomes can be cut and paste using a computer

Human metaphase spreads: different tissue

Bone marrow

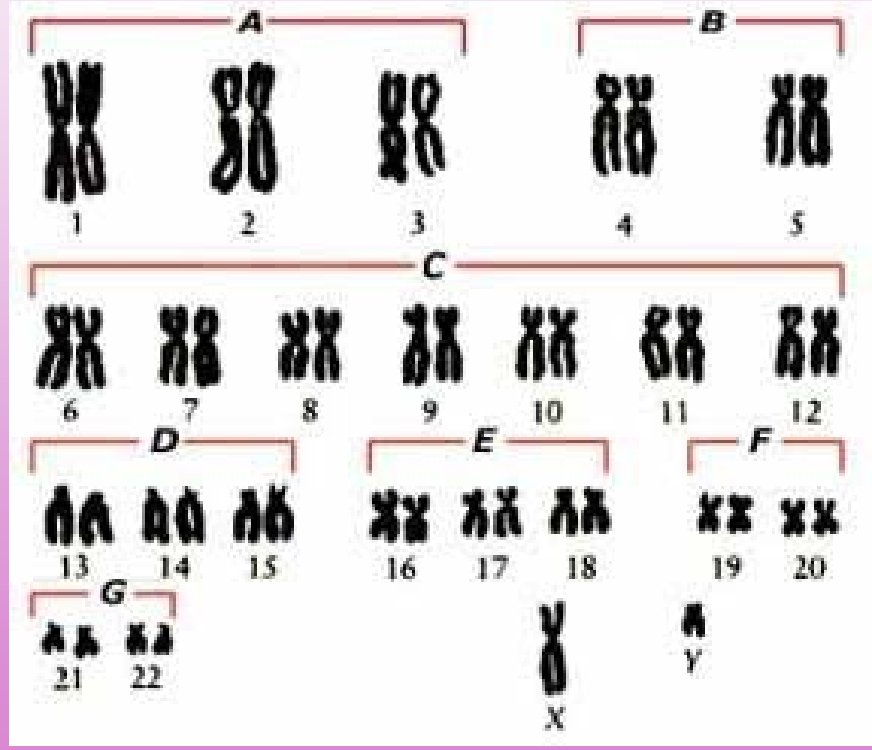


Blood

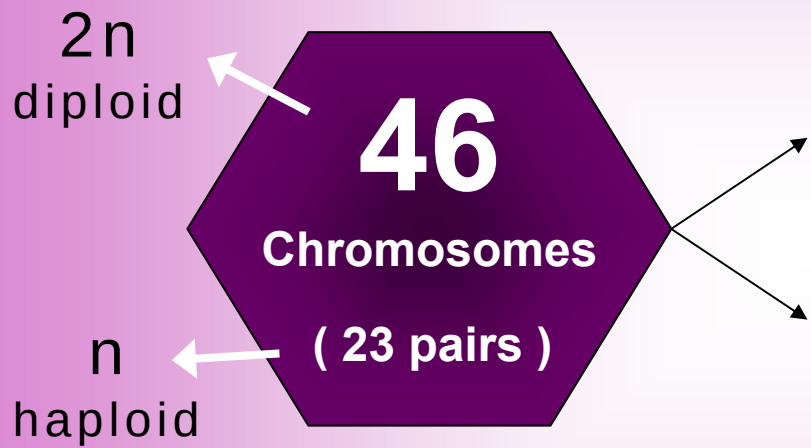
KARYOTYPE ARRANGEMENT

In karyotype, chromosomes are arranged according to:

- **Size**
  chromosomes are arranged and numbered from largest to smallest, with the short p-arm on the top [p=petit]
- **Centromere location**
- **Banding patterns**



Human karyotype :



44 autosomes [22 pairs]

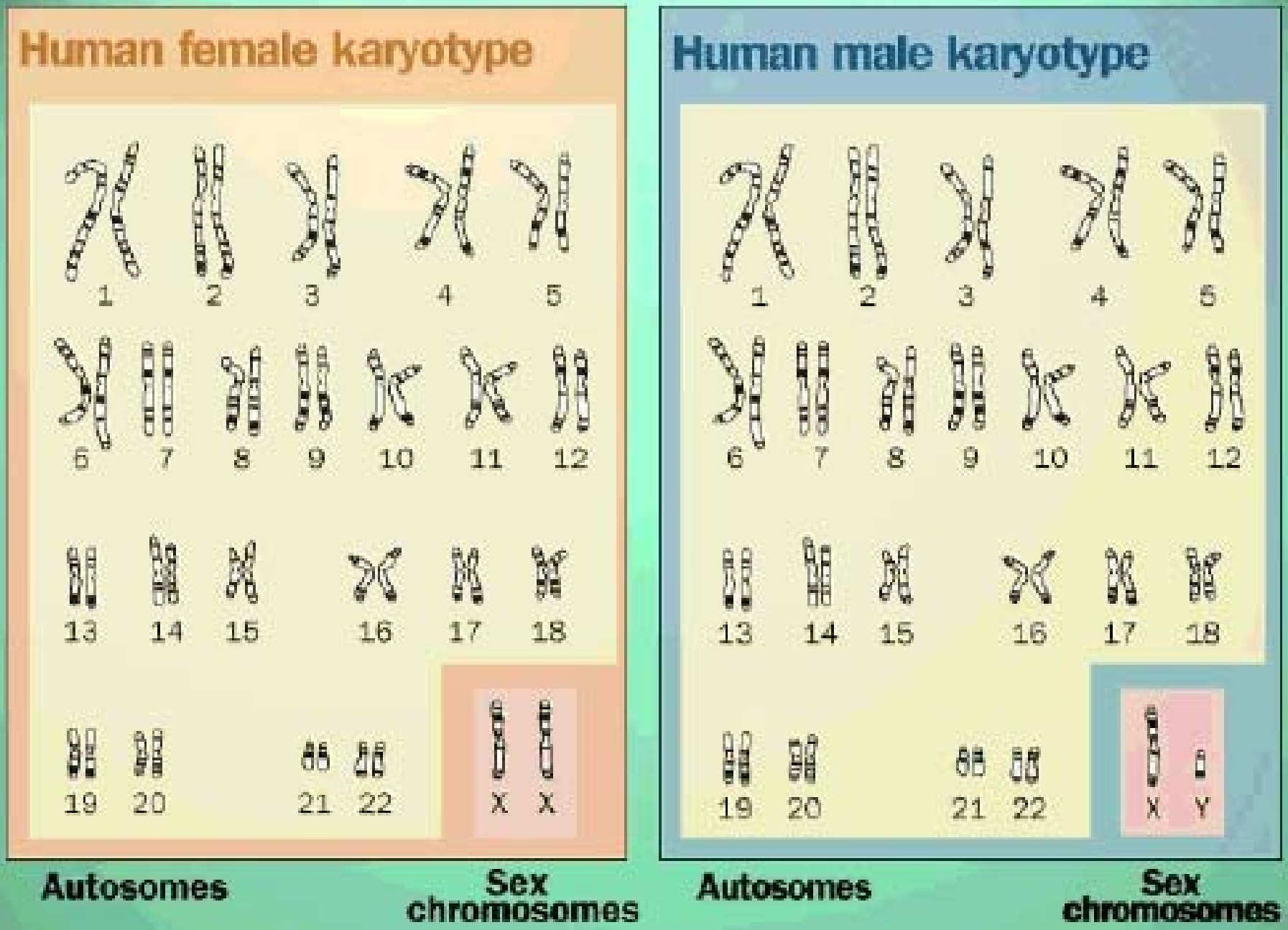
2 sex chromosomes "X", "Y"

<u>Karyotype is presented in standard form:</u>

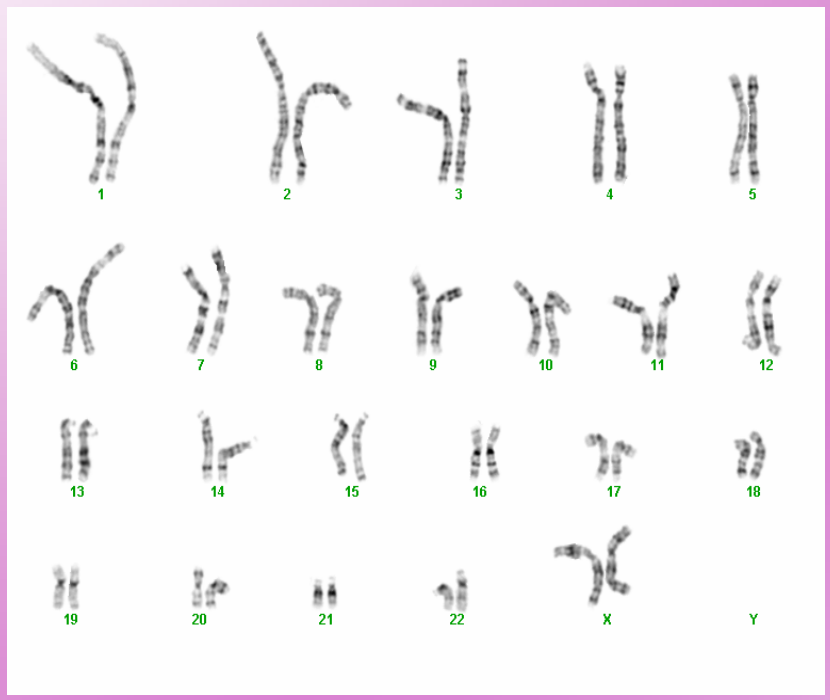
Total number of chromosomes is given, followed by comma and sex chromosomes constitution:

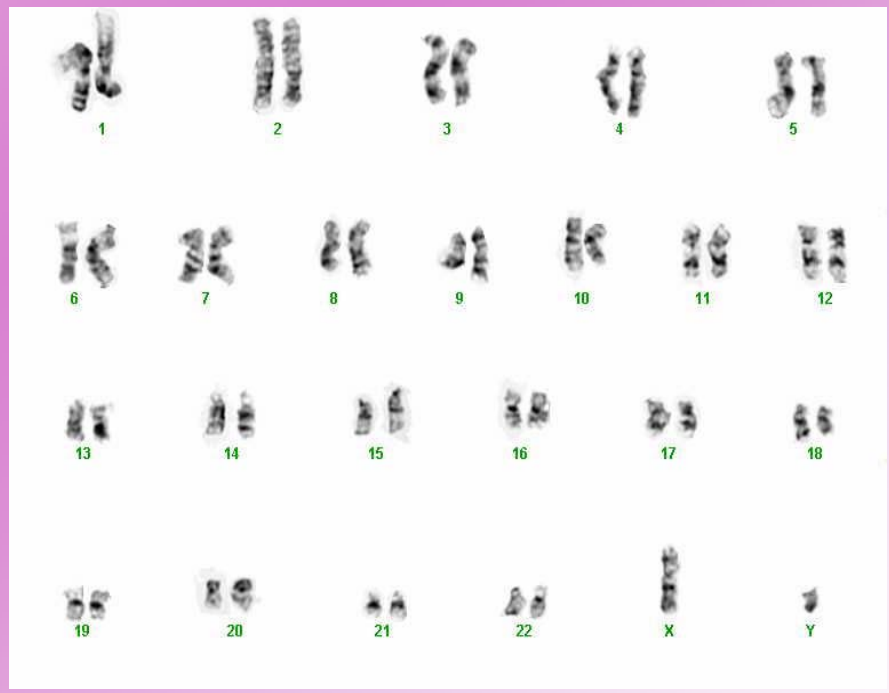
46, XX human female

46, XY human male


Human female karyotype
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
X
Autosomes
Sex chromosomes
Human male karyotype
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Autosomes
Sex chromosomes

Samples of human karyotype





Blood

Bone marrow

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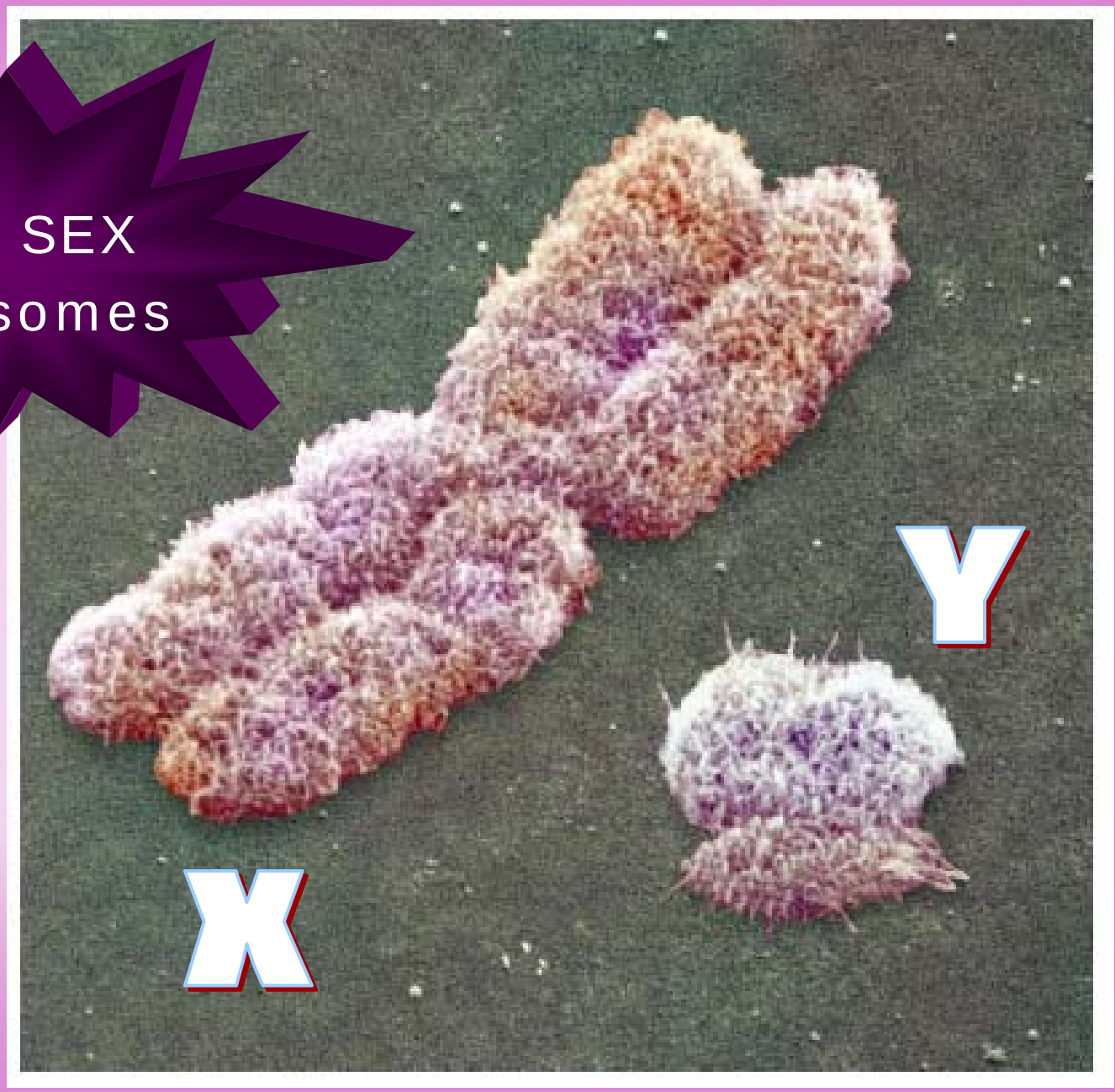
Human SEX chromosomes



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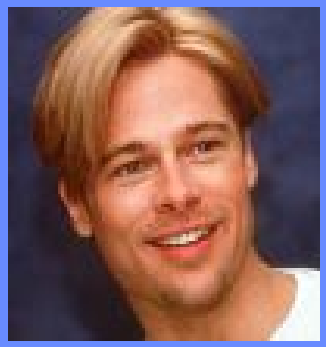
SEX determination

male



X Y

heterogametic

X X

female

homogametic

Sex Determination
General Rule in Mammals:

If a Y chromosome is present,
it will be a male.

If a Y chromosome is not
present, it will be a female.

Why Y chromosome determinates male sex?

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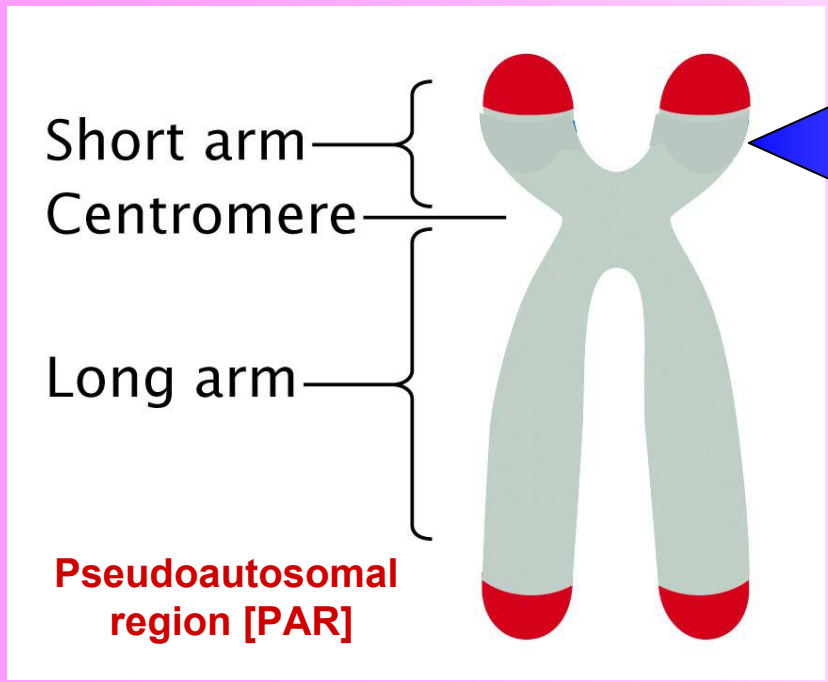
It is not the entire Y chromosome, but just <u>one gene</u> that triggers development of the testes and via hormones maleness

Y chromosome

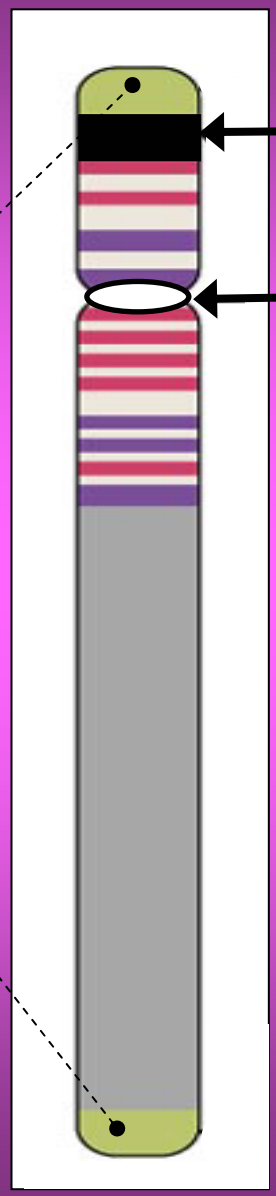


"<u>S</u>ex-determining <u>R</u>egion <u>Y</u>"

SRY gene

[encode testes determination factor TDF]

PAR = regions of homology on the X and Y are essential for pairing in meiosis.



SRY (sex-determine region)

MSY
(Male-Specific region of the Y)

centromere

Pseudoautosomal region (PAR)

X homologous genes

Testis specific genes

Y chromosome

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XY female

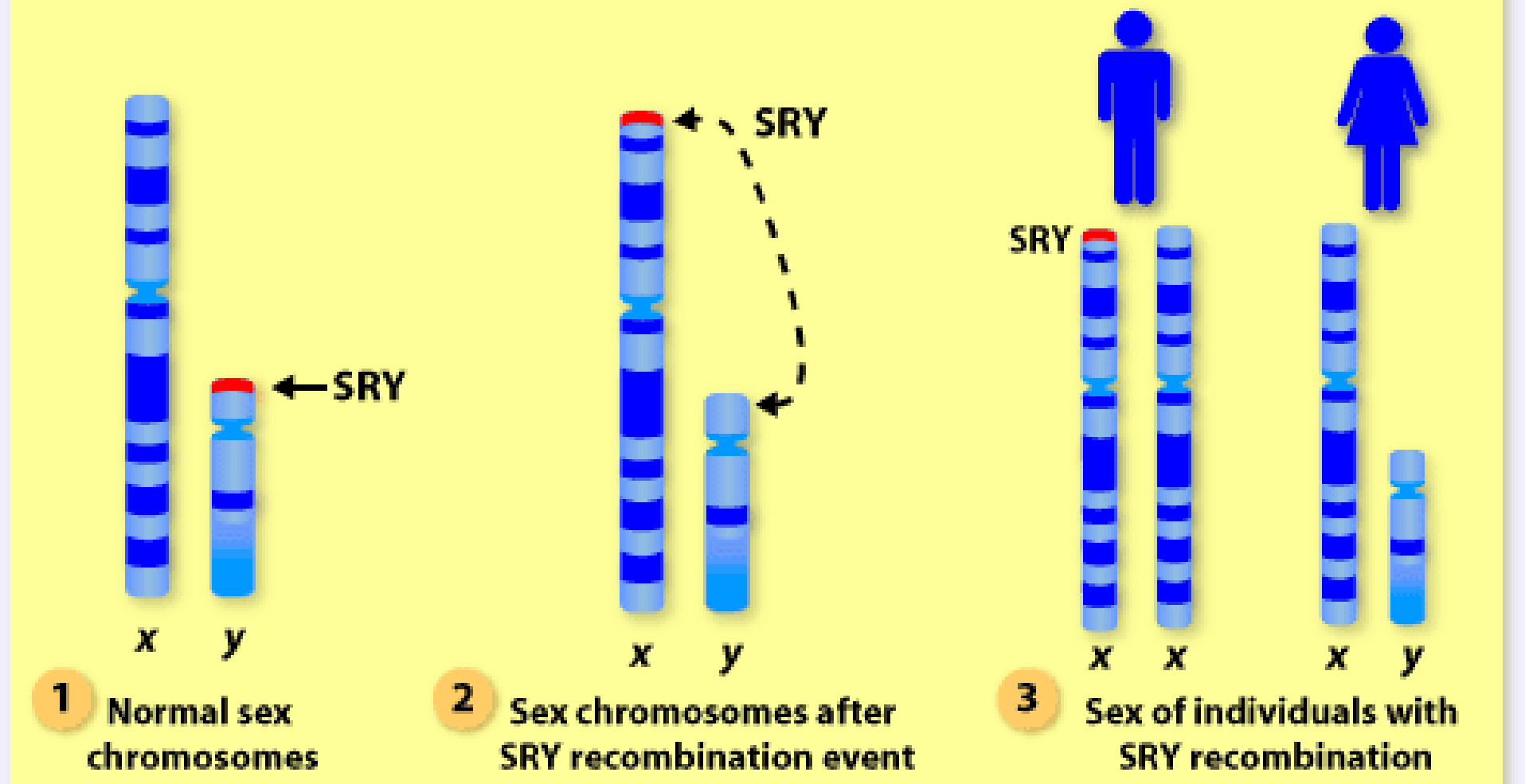
SRY gene on Y chromosome
is mutated or missing

XX male

SRY gene presented on X chromosome

Ivana FELLNEROVÁ, PřF UP Olomouc

SRY (Sex determining Region on the Y) Recombination



Females have two copies of every X-linked gene; males have only one.

How is this difference compensated for?

It happened by the process called:

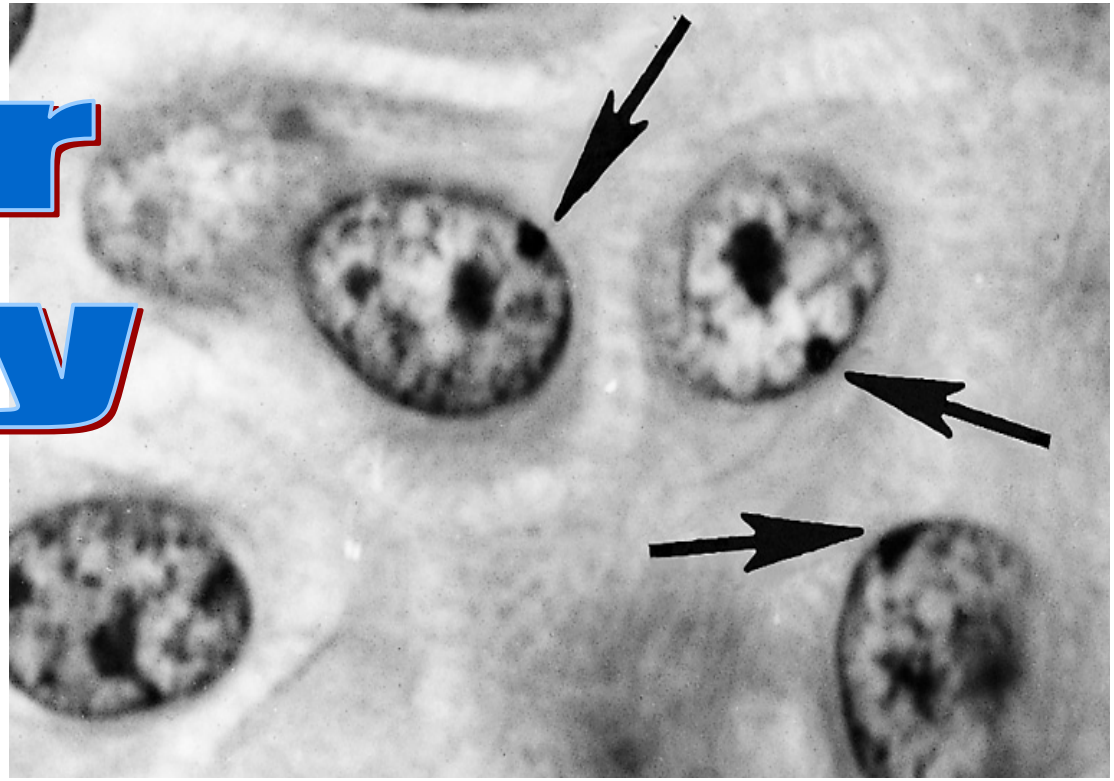
DOSAGE COMPENSATION

X-chromosome inactivation in females

Inactive X-chromosome forms:

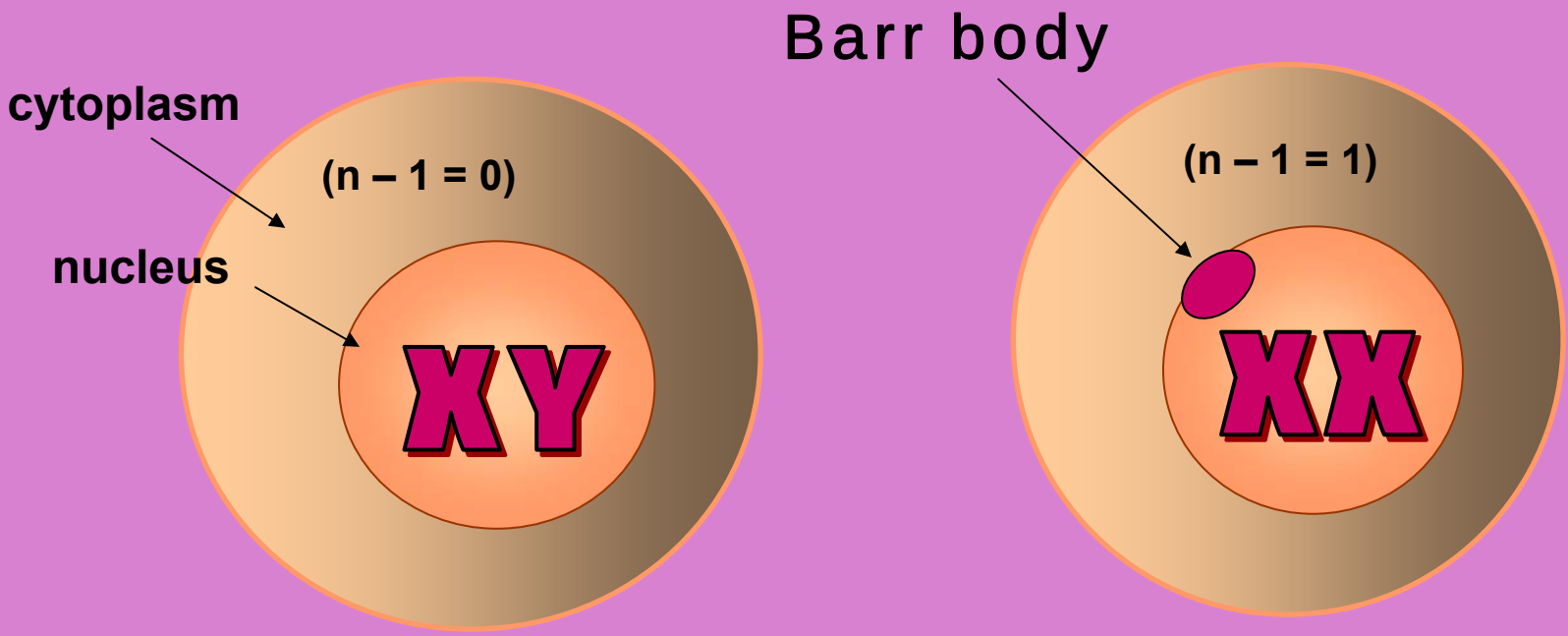
Barr body

Named after its Discoverer Murray Barr



Barr bodies are normally found only in female somatic cells

Barr body math



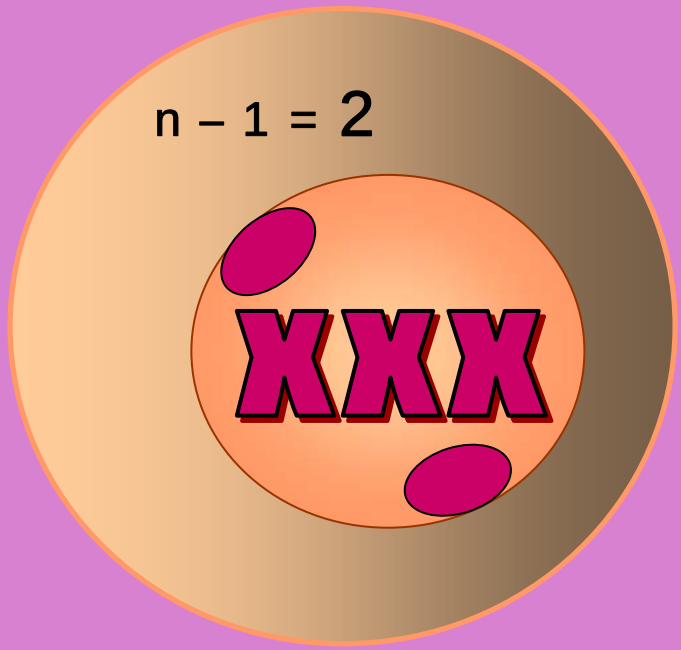
MALE: 46, XY

FEMALE: 46, XX

Multiple X chromosomes

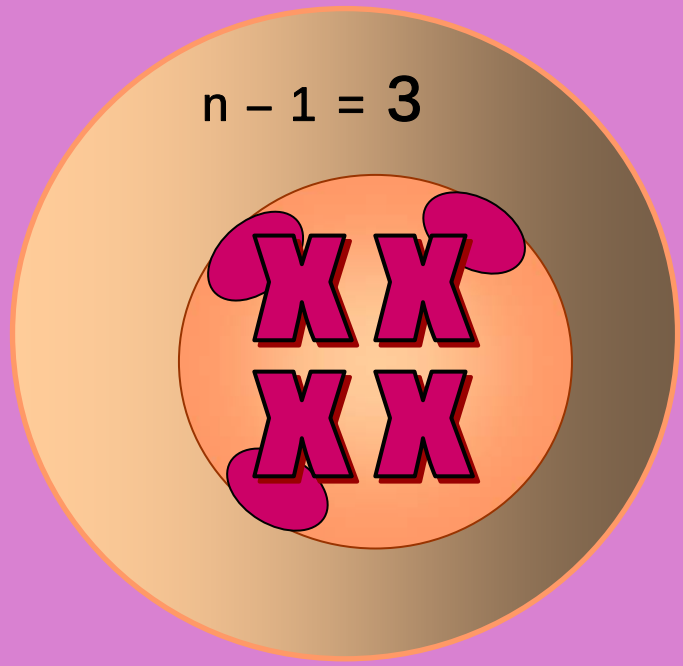
In cells with more than two X chromosomes, only one X remains genetically active and all the others become inactivated

A woman with the chromosome constitution 47, XXX should have 2 Barr bodies in each cell



FEMALE: 47, XXX

A woman with the chromosome constitution 48, XXXX should have 3 Barr bodies in each cell



FEMALE: 48, XXXX