

Chromosomes

What Exactly is a chromosome?

Chromosomes are the **rod-shaped, filamentous bodies** present in the **nucleus**, which become visible **during cell division**.

They are the **carriers of the gene** or unit of heredity.

Chromosome are **not visible** in active nucleus due to their **high water content**, but are clearly seen during cell division.

- Chromosomes were first described by **Strausberger** in **1875**.
- The term "Chromosome", however was first used by **Waldeyer** in **1888**.
- They were given the name chromosome (Chromo = colour; Soma = body) due to their marked **affinity for basic dyes**.
- Their number can be counted easily only during **mitotic metaphase**.

- Chromosomes are composed of **thin chromatin** threads called **Chromatin fibers**.
- These fibers undergo **folding, coiling** and **supercoiling** during prophase so that the chromosomes become progressively thicker and smaller.
- Therefore, chromosomes become readily observable under light microscope.
- At the end of cell division, on the other hand, the fibers uncoil and extend as fine chromatin threads, which are not visible at light microscope

Number of chromosomes

- Normally, all the individuals of a **species have the same number** of chromosomes.
- Closely related species usually have similar chromosome numbers.
- Presence of a whole sets of chromosomes is called **euploidy**.
- It includes haploids, diploids, triploids, tetraploids etc.
- Gametes normally contain only one set of chromosome - this number is called **Haploid**
- Somatic cells usually contain two sets of chromosome - **2n : Diploid**

$3n$ - triploid

$4n$ - tetraploid

The condition in which the chromosomes sets are present in a multiples of "n" is **Polyploidy**

When a change in the chromosome number does not involve entire sets of chromosomes, but only a few of the chromosomes - is **Aneuploidy**.

- **Monosomics ($2n-1$)**
- **Trisomics ($2n+1$)**
- **Nullisomics ($2n-2$)**
- **Tetrasomics ($2n+2$)**

Organism	No. chromosomes
■ Human	46
■ Chimpanzee	48
■ Dog	78
■ Horse	64
■ Chicken	78
■ Goldfish	94
■ Fruit fly	8
■ Mosquito	6
■ Nematode	11(m), 12(f)
■ Horsetail	216
■ Sequoia	22
■ Round worm	2

Organism	No. chromosomes
■ Onion	16
■ Mold	16
■ Carrot	20
■ Tomato	24
■ Tobacco	48
■ Rice	24
■ Maize	20
■ <i>Haploppus gracilis</i>	4
■ <i>Crepis capillaris</i>	6

- On the extreme, **round worm** shows only **two chromosomes**, while the other extreme is represented by **Protozoa** having **300 or more chromosomes**.
- However, most organisms have numbers between **12 to 50**.
- **3-8** in fungi
- From **8 – 16** in Angiosperms (Most common number being **12**).

Chromosome Size

- In contrast to other cell organelles, the size of chromosomes shows a remarkable variation depending upon the stages of cell division.
- **Interphase:** chromosome are longest & thinnest
- **Prophase:** there is a progressive decrease in their length accompanied with an increase in thickness
- **Anaphase:** chromosomes are smallest.
- **Metaphase:** Chromosomes are the most easily observed and studied during metaphase when they are very thick, quite short and well spread in the cell.
- **Therefore, chromosomes measurements are generally taken during mitotic metaphase.**

The size of the chromosomes in mitotic phase of animal and plants sp generally varies between **0.5 μ and 32 μ** in length, and between **0.2 μ and 3.0 μ** in diameter.

The longest metaphase chromosomes found in *Trillium* - **32 μ** .

The giant chromosomes found in **diptera** and they may be as long as **300 μ** and up to **10 μ** in diameter.

In general, **plants have longer chromosomes** than animal and species having lower chromosome numbers have long chromosomes than those having higher chromosome numbers

Among plants, **dicots** in general, have a higher number of chromosome than monocots.

Chromosomes are longer in monocot than dicots.

- In order to understand chromosomes and their function, we need to be able to discriminate among different chromosomes.
- First, chromosomes differ greatly in size
- Between organisms the size difference can be over 100-fold, while within a sp, some chromosomes are often 10 times as large as others.
- In a species **Karyotype**, a pictorial or photographic representation of all the different chromosomes in a cell of an individual, chromosomes are usually ordered by size and numbered from largest to smallest.

Can distinguish chromosomes by “painting” – using DNA hybridization + fluorescent probes – during mitosis

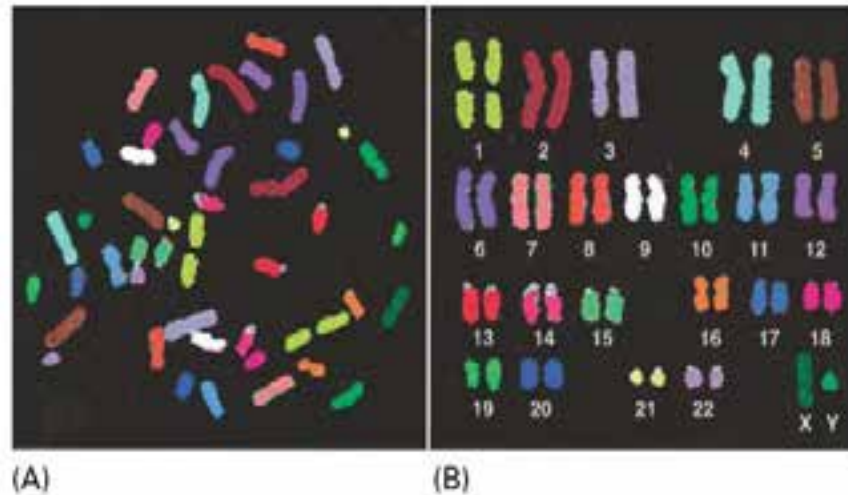
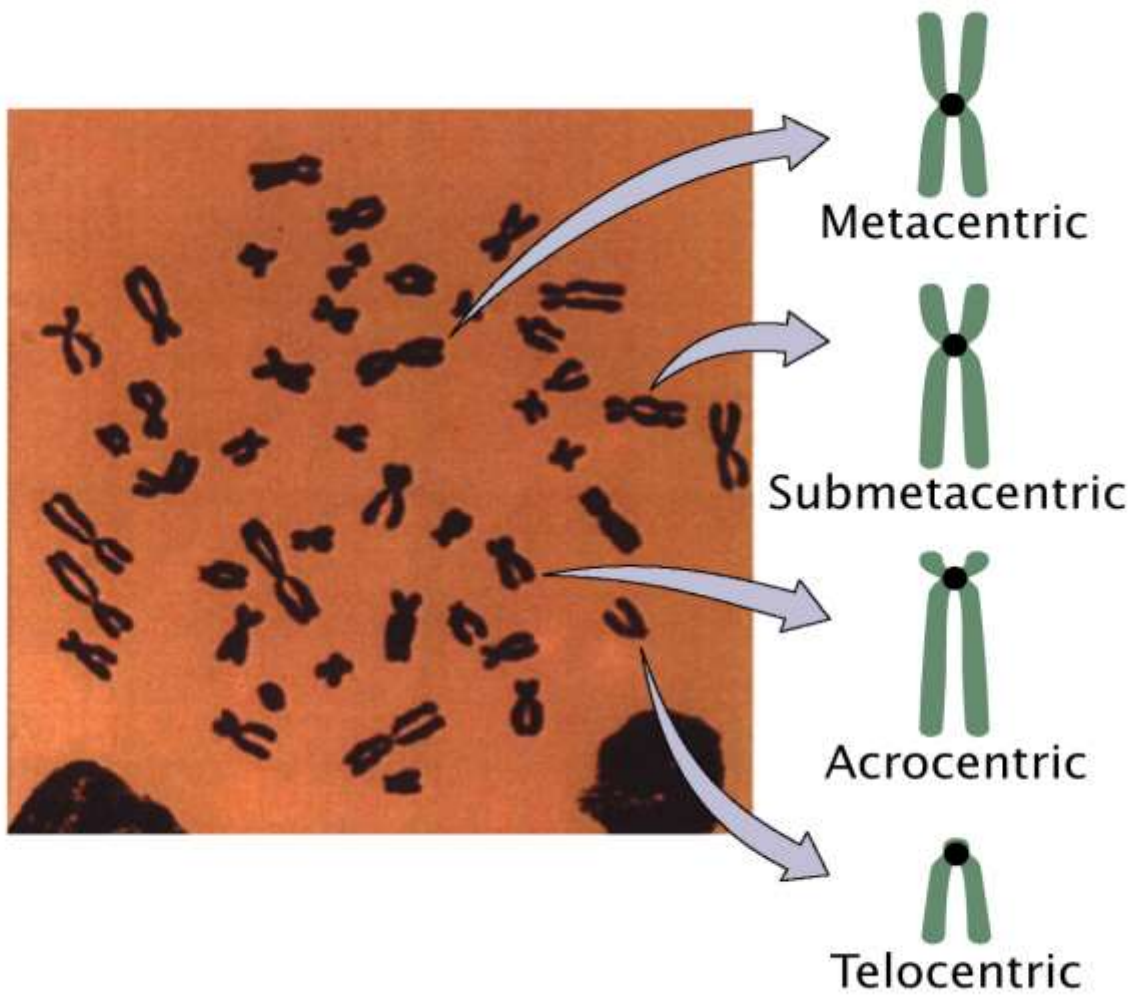


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- **Karyotype**: is the general morphology of the somatic chromosome. Generally, karyotypes represent by arranging in the descending order of size keeping their centromeres in a straight line.
- **Idiotype**: the karyotype of a species may be represented diagrammatically, showing all the morphological features of the chromosome; such a diagram is known as **Idiotype**.

- Chromosomes may differ in the position of the **Centromere**, the place on the chromosome where spindle fibers are attached during cell division.
- In general, if the centromere is near the middle, the chromosome is **metacentric**
- If the centromere is toward one end, the chromosome is **acrocentric** or **submetacentric**
- If the centromere is very near the end, the chromosome is **telocentric**.



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- The centromere divides the chromosome into two arms, so that, for example, an acrocentric chromosome has one short and one long arm,
- While, a metacentric chromosome has arms of equal length.
- All house mouse chromosomes are telocentric, while human chromosomes include both metacentric and acrocentric, but no telocentric.

Autosomal pair**Sex chromosome**

	Diploid (2n)	Autosomal pair		Sex chromosome	
		No. of metacentrics	No. of acrocentric or telocentric	X	Y
Cat	38	16	2	M	M
Dog	78	0	38	M	A
Pig	38	12	6	M	M
Goat	60	0	29	A	M
Sheep	54	3	23	A	M
Cow	60	0	29	M	M
Horse	64	13	18	M	A

M – Metacentric; A – Acrocentric

Euchromatin and Heterochromatin

- Chromosomes may be identified by regions that stain in a particular manner when treated with various chemicals.
- Several different chemical techniques are used to identify certain chromosomal regions by staining them so that they form **chromosomal bands**.
- For example, darker bands are generally found near the centromeres or on the ends (telomeres) of the chromosome, while other regions do not stain as strongly.
- The position of the dark-staining areas are **heterochromatic region** or **heterochromatin**.
- Light staining areas are **euchromatic region** or **euchromatin**.

- Heterochromatin is classified into two groups:
(i) Constitutive and (ii) Facultative.
- Constitutive heterochromatin remains permanently in the heterochromatic stage, i.e., it does not revert to the euchromatic stage.
- In contrast, facultative heterochromatin consists of euchromatin that takes on the staining and compactness characteristics of heterochromatin during some phase of development.

Satellite DNAs

When the DNA of a prokaryote, such as *E. coli*, is isolated, fragmented and centrifuged to equilibrium in a Cesium chloride (CsCl) density gradient, the DNA usually forms a single band in the gradient.

On the other hand, CsCl density-gradient analysis of DNA from eukaryotes usually reveals the presence of one large band of DNA (usually called “**Mainband**” DNA) and one to several small bands.

These small bands are referred to as “**Satellite DNAs**”.

For e.g., in *Drosophila virilis*, contain three distinct satellite DNAs.

Prokaryotic and Eukaryotic Chromosomes

- Not only the genomes of eukaryotes are more complex than prokaryotes, but **the DNA of eukaryotic cell** is also organized differently from that of prokaryotic cells.
- The genomes of prokaryotes are contained in **single chromosomes**, which are usually **circular** DNA molecules.
- In contrast, the genomes of eukaryotes are composed of **multiple chromosomes**, each containing a **linear molecular** of DNA.

- Although the **numbers and sizes** of chromosomes **vary** considerably between different species, their basic structure is the same in all eukaryotes

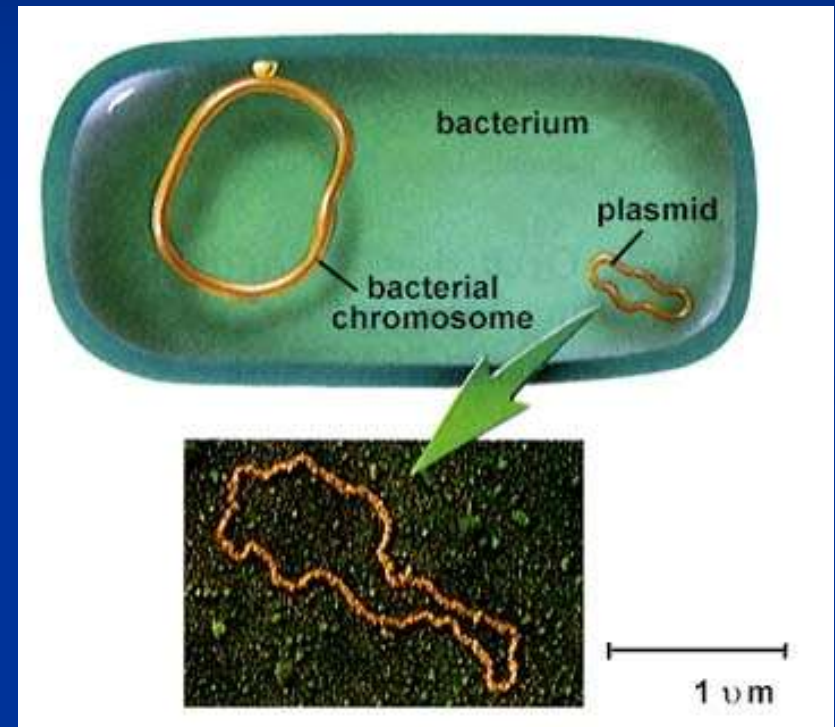
Organism	Genome Size (Mb)^a	Chromosome number^a
<i>Arabidopsis thaliana</i>	70	5
Corn	5000	10
Onion	15,000	8
Lily	50,000	12
Fruit fly	165	4
Chicken	50,000	39
Mouse	1,200	20
Cow	3000	30
Human	3000	23

a – both genome size and chromosome numbers are for haploid cells

- The DNA of eukaryotic cell is **tightly bound** to small **basic proteins (histones)** that package the DNA in an orderly way in the cell nucleus.
- This task is substantial (necessary), given the DNA content of most eukaryotes
- For e.g., the total extended **length of DNA in a human cell is nearly 2 m**, but this must be fit into a nucleus with a diameter of only **5 to 10 μ m**.
- Although DNA packaging is also a problem in bacteria, the mechanism by which prokaryotic DNA are packaged in the cell appears distinct from that eukaryotes and is not well understood.

Prokaryotic chromosome

- The prokaryotes usually have **only one chromosome**, and it bears little morphological resemblance to eukaryotic chromosomes.
- Among prokaryotes there is considerable variation in **genome length bearing genes**.
- The genome length is smallest in **RNA viruses**
- In this case, the organism is provided with only a **few genes** in its chromosome.
- The number of gene may be as high as **150** in some larger **bacteriophage** genome.



- In *E.coli*, about **3000 to 4000 genes** are organized into its one circular chromosome.
- The chromosome exists as a **highly folded and coiled** structure dispersed throughout the cell.
- The folded nature of chromosome is due to the incorporation of RNA with DNA.
- There are about **50 loops** in the chromosome of E.coli.
- These loops are highly **twisted** or **supercoiled** structure with about **four million nucleotide pairs**.
- Its molecular weight is about **2.8×10^9**
- During replication of DNA, the coiling must be relaxed.
- **DNA gyrase** is necessary for the unwinding the coils.

Bacterial Chromosome

- Single, circular DNA molecule located in the nucleoid region of cell

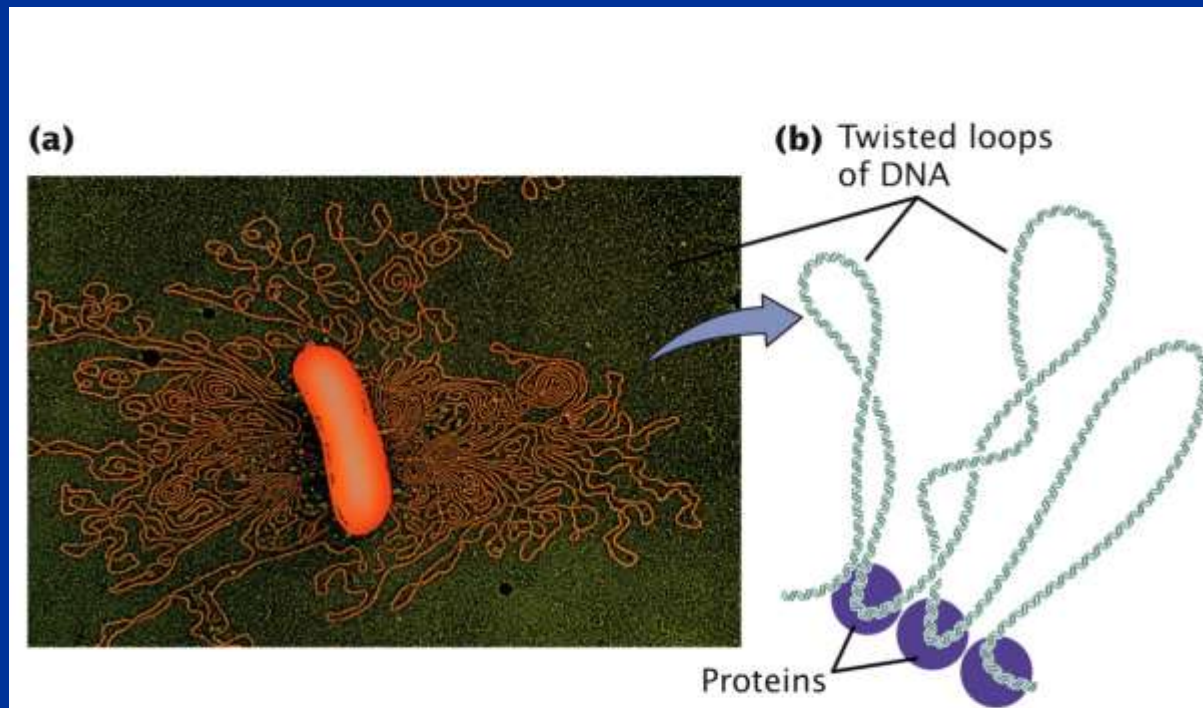


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Supercoiling

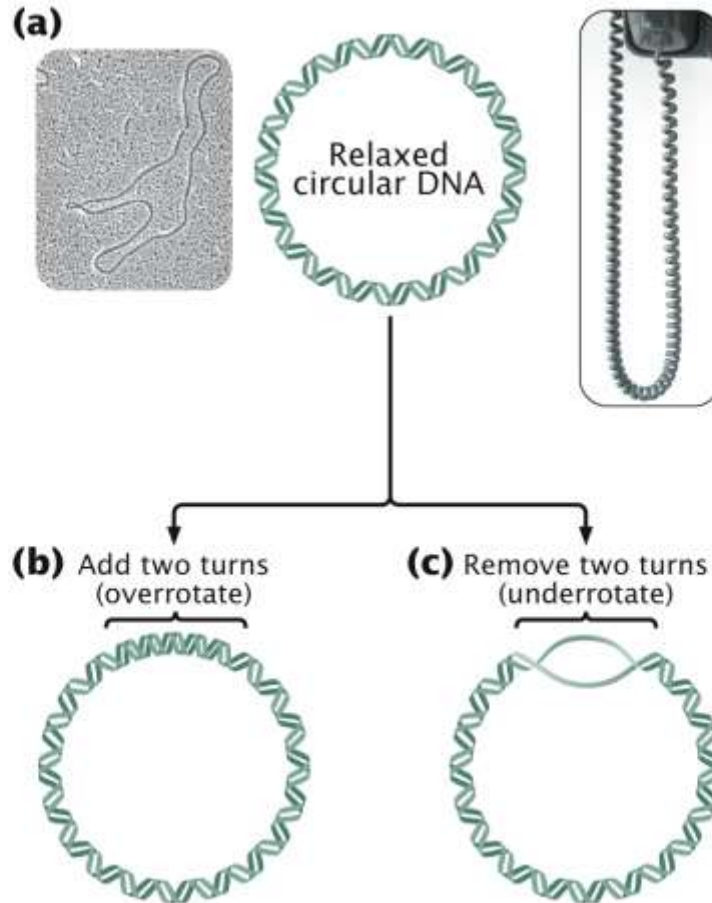
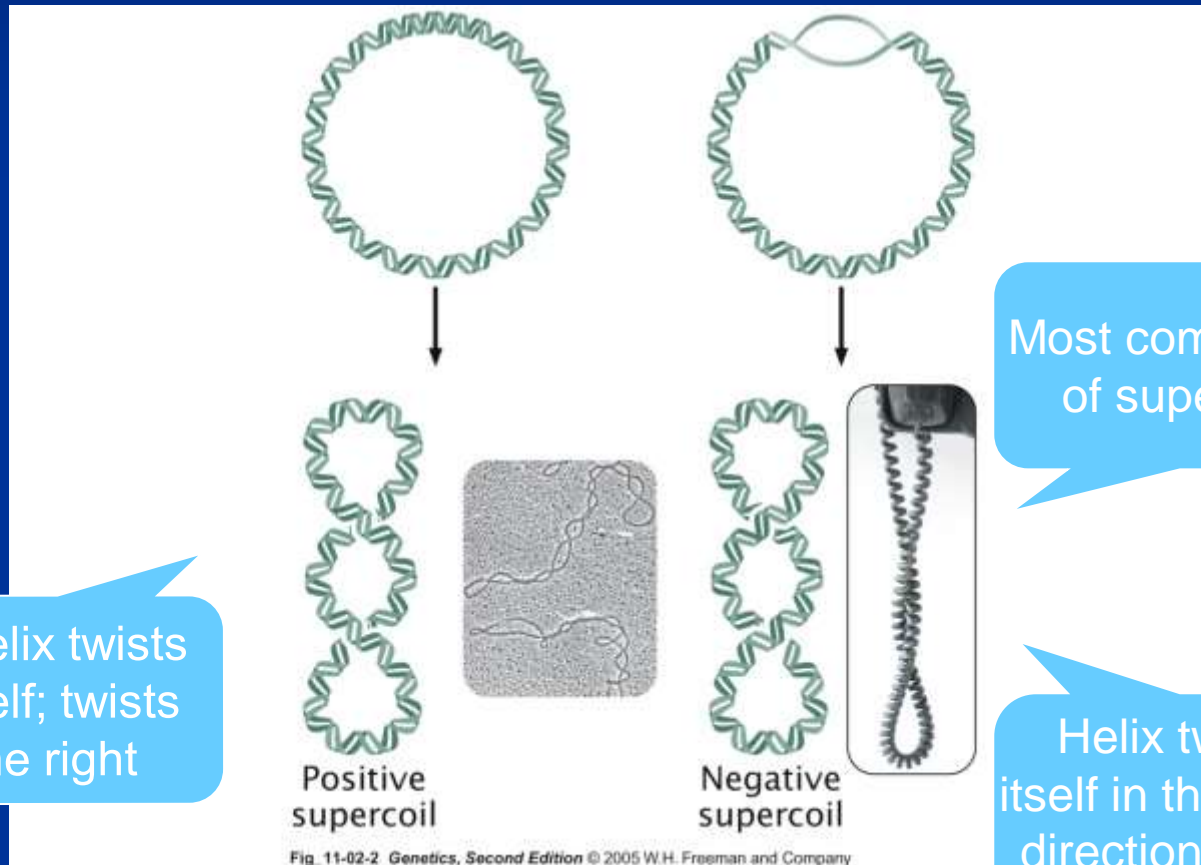


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Supercoiling



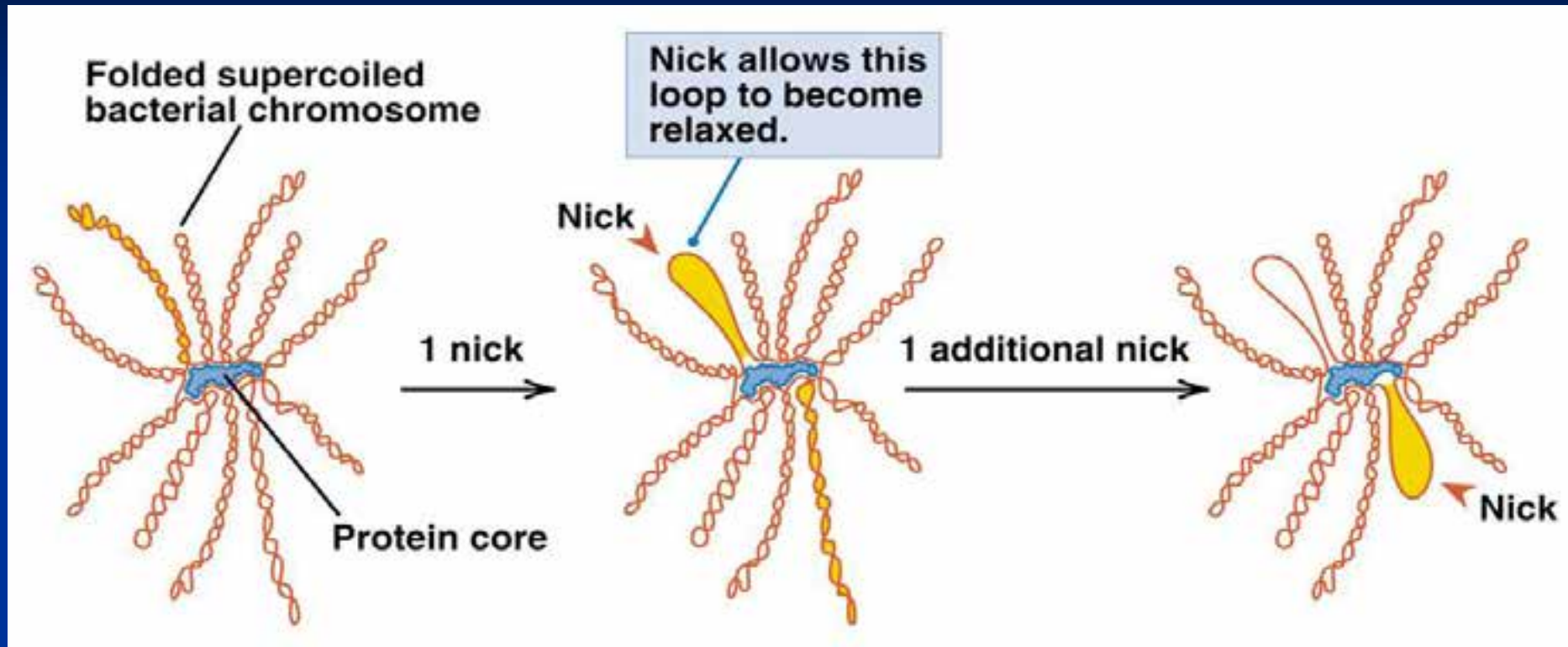
The helix twists on itself; twists to the right

Most common type of supercoiling

Helix twists on itself in the opposite direction; twists to the left

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Mechanism of folding of a bacterial chromosome



There are many supercoiled loops (~100 in *E. coli*) attached to a central core. Each loop can be independently relaxed or condensed.

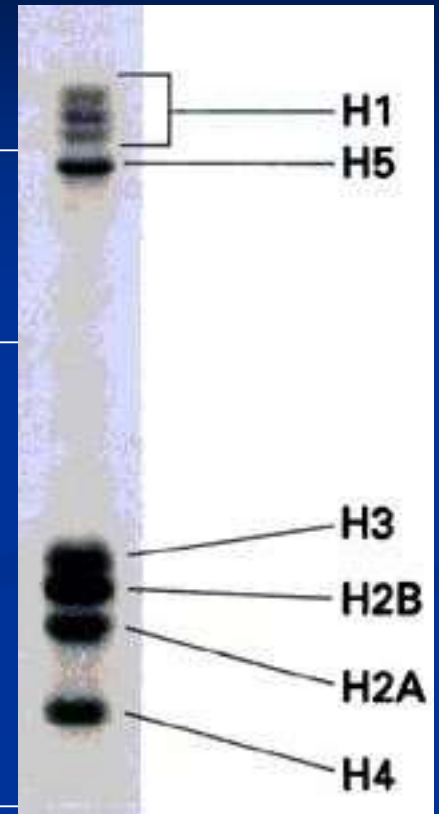
Topoisomerase enzyme – (Type I and II) that introduce or remove supercoiling.

Chromatin

- The complexes between eukaryotic DNA and proteins are called **Chromatin**, which typically contains about twice as much protein as DNA.
- The major proteins of chromatin are the **histones** – small proteins containing a high proportion of basic aminoacids (**arginine and lysine**) that facilitate binding negatively charged DNA molecule .
- There are **5 major types** of histones: **H1, H2A, H2B, H3,** and **H4** – which are very similar among different sp of eukaryotes.
- The histones are extremely abundant proteins in eukaryotic cells.
- Their mass is approximately equal to that of the cell's DNA

The major histone proteins:

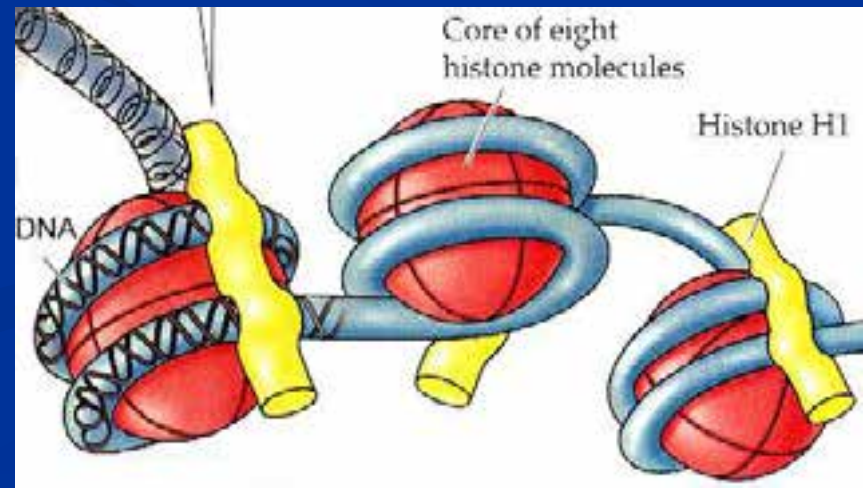
Histone	Mol. Wt	No. of Amino acid	Percentage Lys + Arg
H1	22,500	244	30.8
H2A	13,960	129	20.2
H2B	13,774	125	22.4
H3	15,273	135	22.9
H4	11,236	102	24.5



The DNA double helix is bound to proteins called **histones**. The histones have **positively charged** (basic) amino acids to bind the negatively charged (acidic) DNA. Here is an SDS gel of histone proteins, separated by size

- In addition, chromatin contains an approximately equal mass of a wide variety of **non-histone chromosomal proteins**.
- There are more than a **thousand different types** of these proteins, which are involved in a range of activities, including **DNA replication** and **gene expression**.
- The DNA of prokaryotes is similarly associated with proteins, some of which presumably function as histones do, packing the DNA within the bacterial cell.
- Histones, however are unique feature of eukaryotic cells and are responsible for distinct structural organization of eukaryotic chromatin

- The basic structural unit of chromatin, the **nucleosome**, was described by **Roger Kornberg** in **1974**.
- Two types of experiments led to Kornberg's proposal of the nucleosome model.
- First, **partial digestion of chromatin with micrococcal nuclease** (an enzyme that degrades DNA) was found to yield DNA fragments approximately **200 base pairs long**.
- In contrast, a similar digestion of naked DNA (not associated with protein) yielded a **continuous smear randomly sized fragments**.
- These results suggest that the binding of proteins to DNA in **chromatin protects** the regions of DNA from nuclease digestion, so that enzyme can attack DNA only at sites separated by approximately 200 base pairs.



- Electron microscopy revealed that chromatin fibers have a beaded appearance, with the beads spaced at intervals of approximately 200 base pairs.
- Thus, both nuclease digestion and the electron microscopic studies suggest that chromatin is composed of repeating 200 base pair unit, which were called **nucleosome**.

individual nucleosomes = “beads on a string”

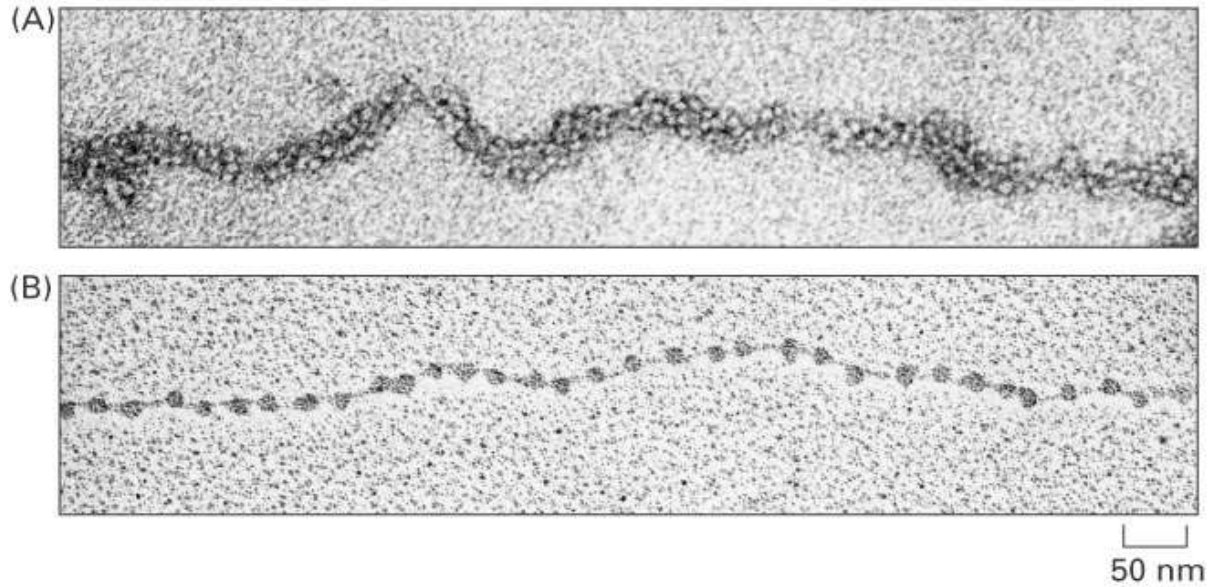


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- Detailed analysis of these nucleosome core particles has shown that they contain 146 base pairs of DNA wrapped 1.75 times around a histone core consisting of two molecules each of H2A, H2B, H3, and H4 (the core histones).
- One molecule of the fifth histone H1, is bound to the DNA as it enters and exists each nucleosome core particle.
- This forms a chromatin subunit known as **chromatosome**, which consist of 166 base pairs of DNA wrapped around histone core and held in place by H1 (a linker histone)

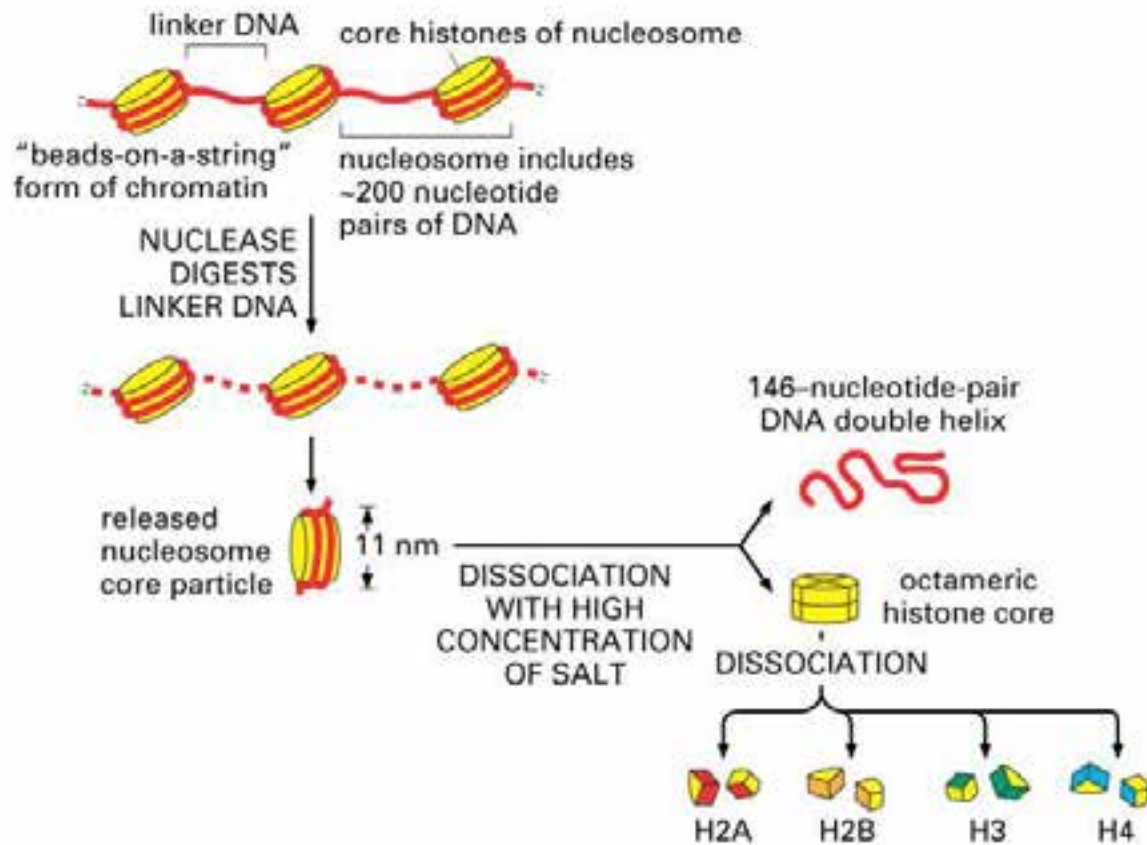
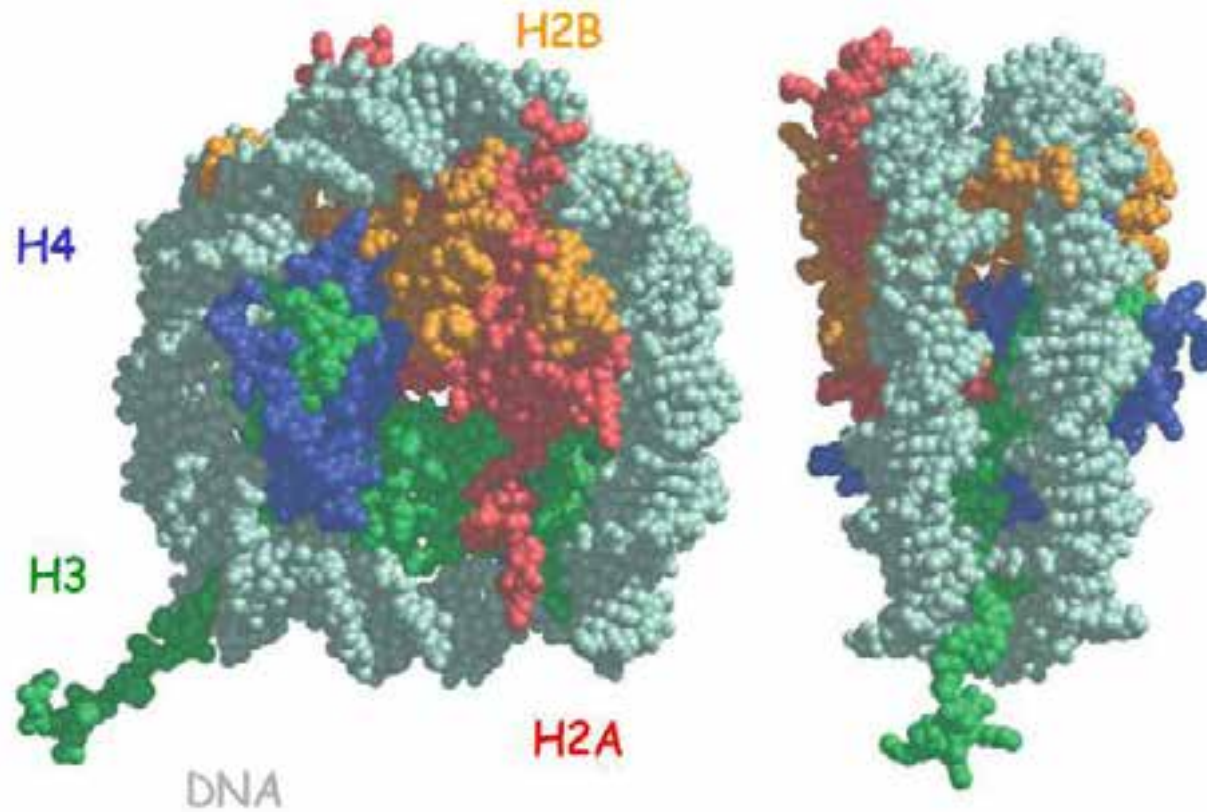
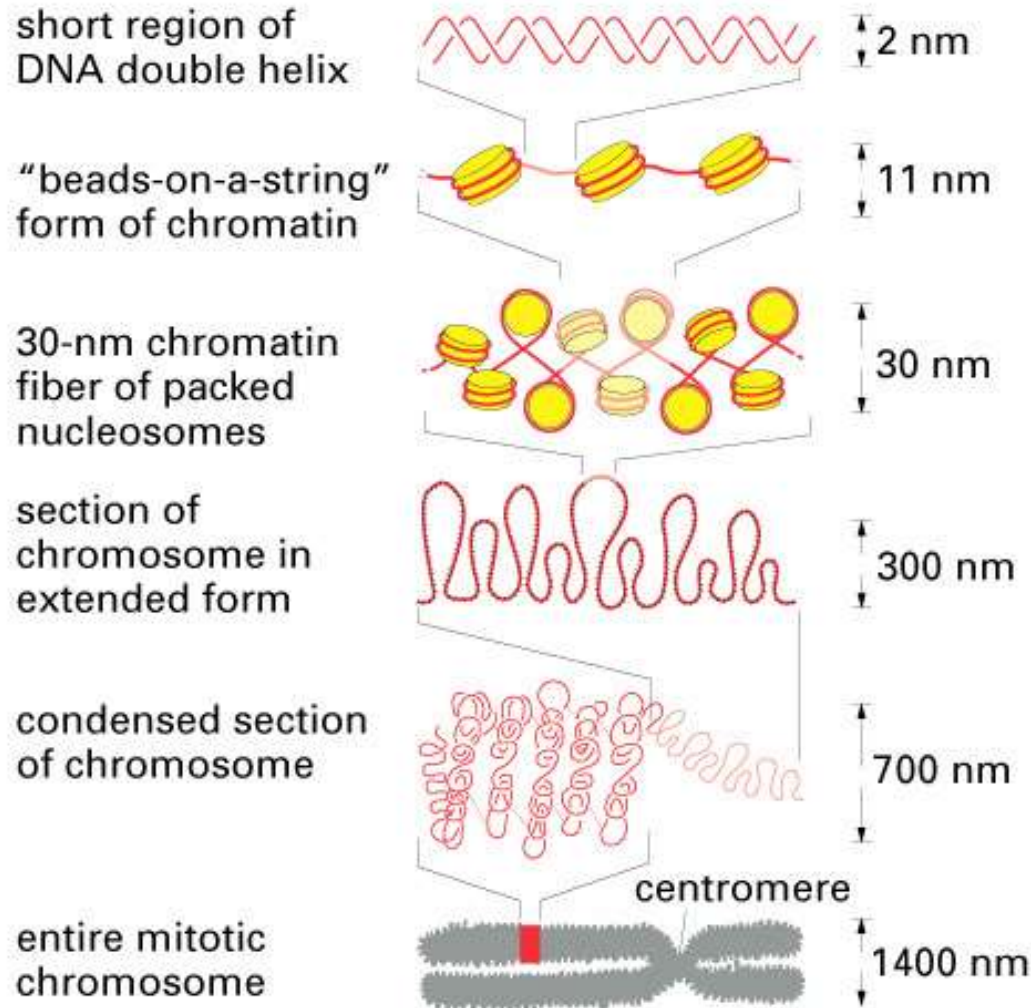


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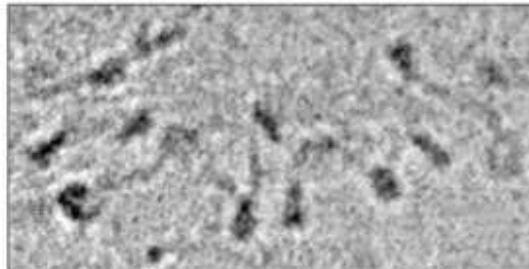
Nucleosome Core Particle

19

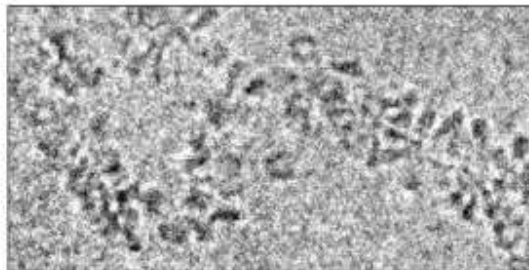




NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

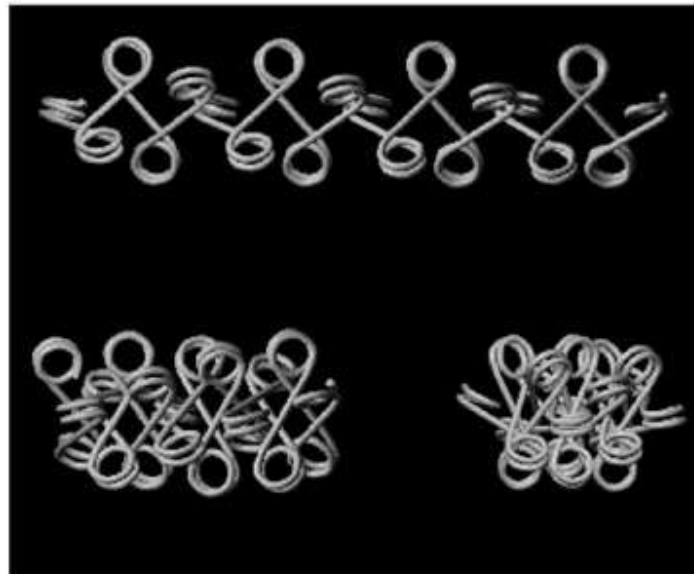


(A)



(B)

50 nm



(C)

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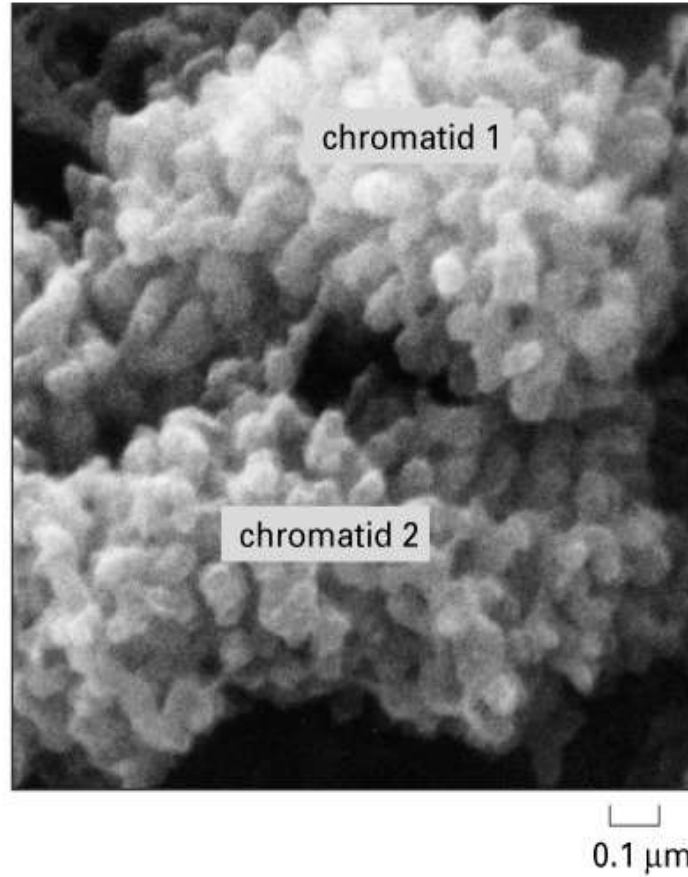
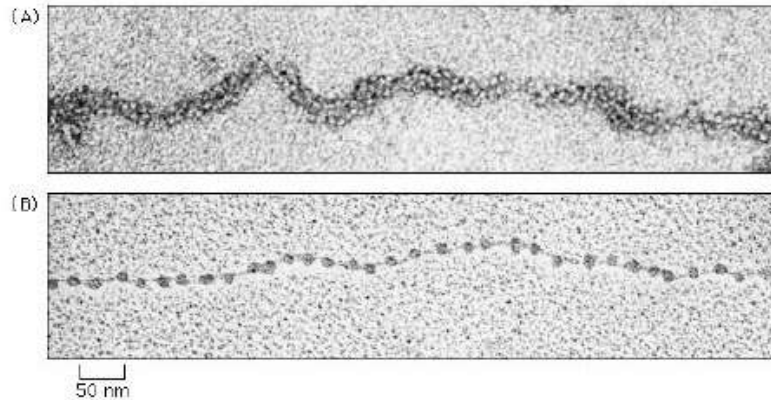


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ELECTRON MICROGRAPHS

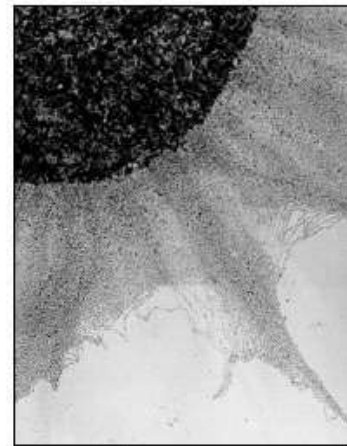
NUCLEOSOMES



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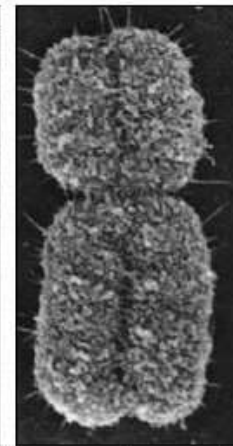
INTERPHASE CHROMATIN



(A)

10 μm

MITOTIC CHROMATIN



(B)

1 μm

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Centromeres and Telomeres

- Centromeres and telomeres are two essential features of all eukaryotic chromosomes.
- Each provide a unique function i.e., **absolutely necessary for the stability of the chromosome.**
- Centromeres are required for the segregation of the centromere during meiosis and mitosis.
- Telomeres provide terminal stability to the chromosome and ensure its survival

Centromere

- The region where two sister chromatids of a chromosome appear to be joined or “**held together**” during mitotic metaphase is called **Centromere**
- When chromosomes are stained they typically show a **dark-stained** region that is the centromere.
- Also termed as **Primary constriction**
- During **mitosis**, the centromere that is shared by the sister chromatids must divide so that the chromatids can migrate to opposite poles of the cell.
- On the other hand, during the first meiotic division the centromere of sister chromatids must remain intact
- whereas during meiosis II they must act as they do during mitosis.
- **Therefore the centromere is an important component of chromosome structure and segregation.**

- As a result, centromeres are the first parts of chromosomes to be seen moving towards the opposite poles during anaphase.
- The remaining regions of chromosomes lag behind and appear as if they were being pulled by the centromere.

Kinetochores

- Within the centromere region, most species have several locations where spindle fibers attach, and these sites consist of DNA as well as protein.
- The actual location where the attachment occurs is called the **kinetochore** and is composed of both DNA and protein.
- The DNA sequence within these regions is called **CEN DNA**.

- Typically CEN DNA is about 120 base pairs long and consists of several sub-domains, **CDE-I**, **CDE-II** and **CDE-III**.
- Mutations in the first two sub-domains have no effect upon segregation,
- but a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation.
- Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere.

- The protein component of the kinetochore is only now being characterized.
- A complex of three proteins called **Cbf-III** binds to normal CDE-III regions but can not bind to a CDE-III region with a point mutation that prevents mitotic segregation.

Telomere

- The two ends of a chromosome are known as telomeres.
- It required for the replication and stability of the chromosome.
- When telomeres are damaged or removed due to chromosome breakage, the damaged chromosome ends can readily fuse or unite with broken ends of other chromosome.
- Thus it is generally accepted that structural integrity and individuality of chromosomes is maintained due to telomeres.

- McClintock noticed that if two chromosomes were broken in a cell, the end of one could attach to the other and vice versa.
- What she never observed was the attachment of the broken end to the end of an unbroken chromosome.
- Thus the ends of broken chromosomes are sticky, whereas the normal end is not sticky, suggesting the ends of chromosomes have unique features.

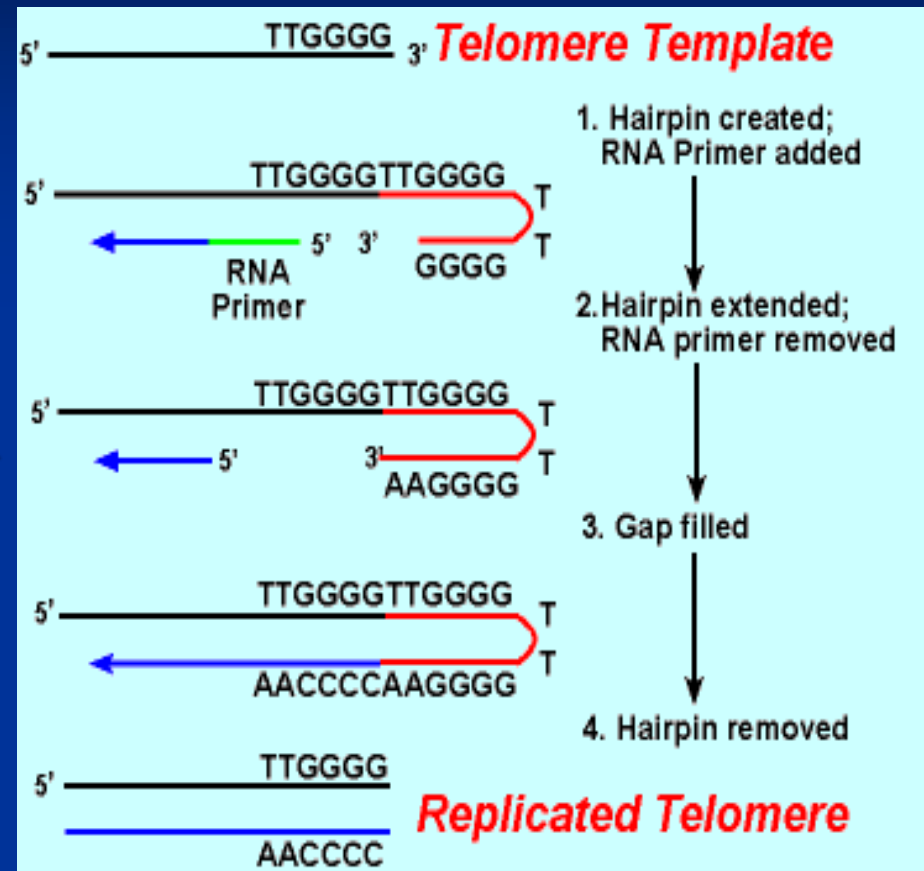
Telomere Repeat Sequences

until recently, little was known about molecular structure of telomeres. However, during the last few years, telomeres have been isolated and characterized from several sp.

Species	Repeat Sequence
<i>Arabidopsis</i>	TTTAGGG
Human	TTAGGG
<i>Oxytricha</i>	TTTTGGGG
Slime Mold	TAGGG
<i>Tetrahymena</i>	TTGGGG
Trypanosome	TAGGG

Tetrahymena - protozoa organism.

- The telomeres of this organism end in the sequence 5'-TTGGGG-3'.
- The **telomerase** adds a series of 5'-TTGGGG-3' repeats to the ends of the lagging strand.
- A hairpin occurs when unusual base pairs between guanine residues in the repeat form.
- Finally, the hairpin is removed at the 5'-TTGGGG-3' repeat.
- Thus the end of the chromosome is faithfully replicated.



RNA Primer - Short stretches of ribonucleotides (RNA substrates) found on the lagging strand during DNA replication. Helps initiate lagging strand replication

Staining and Banding chromosome

Staining procedures have been developed in the past two decades and these techniques help to study the karyotype in plants and animals.

1. **Feulgen Staining:**

Cells are subjected to a mild hydrolysis in 1N HCl at 60⁰C for 10 minutes.

This treatment produces a free aldehyde group in deoxyribose molecules.

When **Schiff's reagent** (basic fuchsin bleached with sulfurous acid) to give a deep pink colour.

Ribose of RNA will not form an aldehyde under these conditions, and **the reaction is thus specific for DNA**

2. Q banding:

The Q bands are the **fluorescent bands** observed after **quinacrine mustard staining** and observation with UV light.

The distal ends of each chromatid are not stained by this technique.

The Y chromosome become brightly fluorescent both in the interphase and in metaphase.

3. R banding:

The R bands (from **reverse**) are those located in the zones that do not fluoresce with the quinacrine mustard, that is they are between the Q bands and can be visualized as green.

4. G banding:

The G bands (from **Giemsa**) have the same location as Q bands and do not require fluorescent microscopy.

Many techniques are available, each involving some pretreatment of the chromosomes.

In **ASG (Acid-Saline-Giemsa)** cells are incubated in citric acid and NaCl for one hour at 60°C and are then treated with the Giemsa stain.

5. C banding:

The C bands correspond to **constitutive heterochromatin**.

The heterochromatin regions in a chromosome distinctly differ in their stainability from euchromatic region.

VARIATION IN STRUTURE OF CHROMOSOME

Chromosomal Aberrations

- The somatic ($2n$) and gametic (n) chromosome numbers of a species ordinarily remain constant.
- This is due to the extremely precise mitotic and meiotic cell division.
- Somatic cells of a diploid species contain two copies of each chromosome, which are called homologous chromosome.
- Their gametes, therefore contain only one copy of each chromosome, that is they contain one chromosome complement or genome.
- Each chromosome of a genome contains a definite numbers and kinds of genes, which are arranged in a definite sequence.

Chromosomal Aberrations

- Sometime due to mutation or spontaneous (without any known causal factors), variation in chromosomal number or structure do arise in nature. - Chromosomal aberrations.
- Chromosomal aberration may be grouped into two broad classes:
 1. Structural and 2. Numerical

Structural Chromosomal Aberrations

- Chromosome structure variations result from **chromosome breakage**.
- Broken chromosomes tend to **re-join**; if there is more than one break, rejoining occurs at **random** and not necessarily with the correct ends.
- The result is structural changes in the chromosomes.
- Chromosome breakage is caused by **X-rays, various chemicals**, and can also occur spontaneously.

■ There are **four** common type of structural aberrations:

1. Deletion or Deficiency
2. Duplication or Repeat
3. Inversion, and
4. Translocation.

- Consider a normal chromosome with genes in alphabetical order: **a b c d e f g h i**

1. Deletion: part of the chromosome has been removed: **a b c g h i**

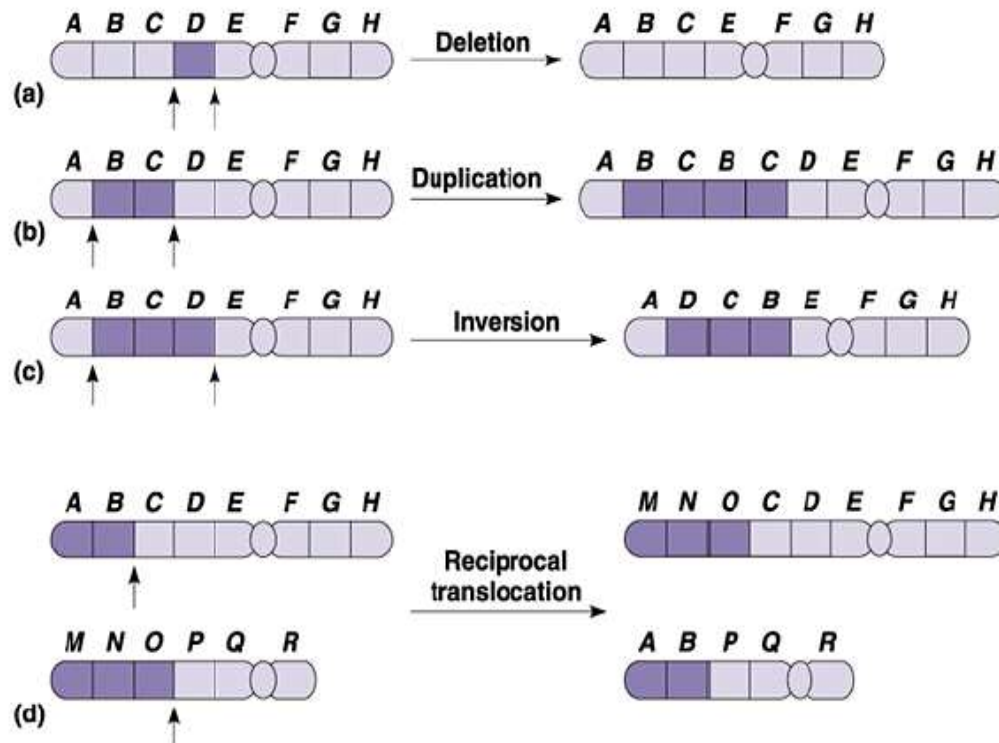
2. Duplication: part of the chromosome is duplicated:
a b c d e f d e f g h i

3. Inversion: part of the chromosome has been re-inserted in reverse order: **a b c f e d g h i**

ring: the ends of the chromosome are joined together to make a ring

4. translocation: parts of two non-homologous chromosomes are joined:

If one normal chromosome is **a b c d e f g h i**
and the other chromosome is **u v w x y z**,
then a translocation between them would be
a b c d e f x y z and **u v w g h i**.



Deletion or deficiency

Loss of a chromosome segment is known as deletion or deficiency

It can be **terminal deletion** or **interstitial or intercalary** deletion.

A single break near the end of the chromosome would be expected to result in **terminal deficiency**.

If two breaks occur, a section may be deleted and an **intercalary deficiency** created.

Terminal deficiencies might seem less complicated.

But majority of deficiencies detected are intercalary type within the chromosome.

Deletion was the first structural aberration detected by Bridges in 1917 from his genetic studies on X chromosome of *Drosophila*.

- Deletion generally produce striking **genetic and physiological effects**.
- When homozygous, most deletions are lethal, because most genes are necessary for life and a homozygous deletion would have zero copies of some genes.
- When heterozygous, the genes on the normal homologue are **hemizygous**: there is only 1 copy of those genes.
- Crossing over is absent in deleted region of a chromosome since this region is present in only one copy in deletion heterozygotes.
- In *Drosophila*, several deficiencies induced the mutants like Blond, Pale, Beaded, Carved, Notch, Minute etc.

Deletion in Prokaryotes:

Deletions are found in prokaryotes as well, e.g., E.coli, T4 phage and Lambda phage.

In E.coli, deletions of up to 1 % of the bacterial chromosome are known.

In lambda phage, however 20% of the genome may be missing in some of the deletions.

Deletion in Human:

Chromosome deletions are usually lethal even as heterozygotes, resulting in zygotic loss, stillbirths, or infant death.

Sometimes, infants with small chromosome deficiencies however, survive long enough to permit the abnormal phenotype they express.

Cri-du-chat (Cat cry syndrome):

The name of the syndrome came from a catlike mewing cry from small weak infants with the disorder.

Other characteristics are microcephaly (small head), broad face and saddle nose, physical and mental retardation.

Cri-du-chat patients die in infancy or early childhood.

The chromosome deficiency is in the short arm of **chromosome 5**.

Myelocytic leukemia

Another human disorder that is associated with a chromosome abnormality is chronic myelocytic leukemia.

A deletion of **chromosome 22** was described by P.C.Nowell and Hungerford and was called **“Philadelphia” (Ph’)** chromosome after the city in which the discovery was made.

Duplication

The presence of an additional chromosome segment, as compared to that normally present in a nucleus is known as **Duplication**.

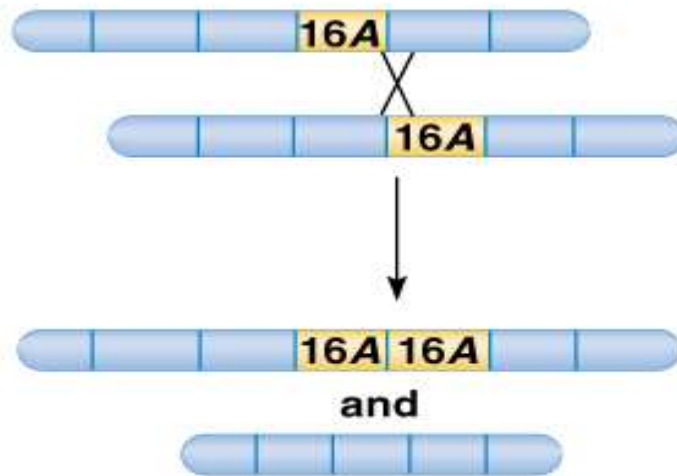
- In a diploid organism, presence of a chromosome segment in more than two copies per nucleus is called duplication.
- Four types of duplication:
 1. Tandem duplication
 2. Reverse tandem duplication
 3. Displaced duplication
 4. Translocation duplication

- The extra chromosome segment may be located immediately after the normal segment in precisely the same orientation forms the **tandem**
- When the gene sequence in the extra segment of a tandem in the reverse order i.e, inverted , it is known as **reverse tandem duplication**
- In some cases, the extra segment may be located in the same chromosome but away from the normal segment – termed as **displaced duplication**
- The additional chromosome segment is located in a non-homologous chromosome is **translocation duplication.**

Origin

- Origin of duplication involves **chromosome breakage** and **reunion of chromosome** segment with its homologous chromosome.
- As a result, one of the two homologous involved in the production of a duplication ends up with a **deficiency**, while the **other has a duplication for the concerned segment**.
- Another phenomenon, known as **unequal crossing over**, also leads to exactly the same consequences for small chromosome segments.
- For e.g., duplication of the band **16A of X** chromosome of **Drosophila produces Bar eye**.
- This duplication is believed to originate due to unequal crossing over between the two normal X chromosomes of female.

a)

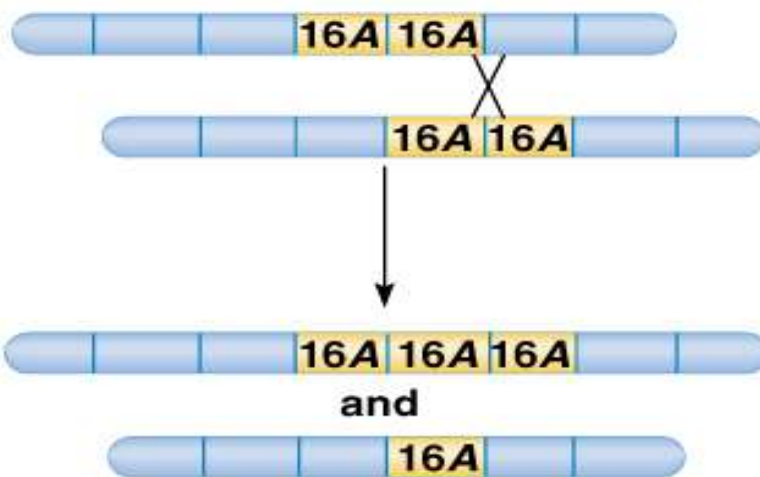


Unequal crossing-over

Duplication—2 copies of 16A (*Bar*)

Deletion

b)



Unequal crossing-over

Three copies of 16A (double-*Bar*)

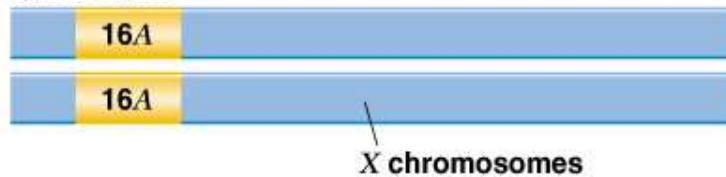
One copy of 16A (normal)

Genotype

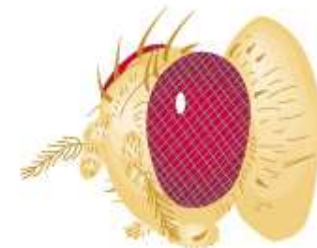
Polytene bands

Phenotype

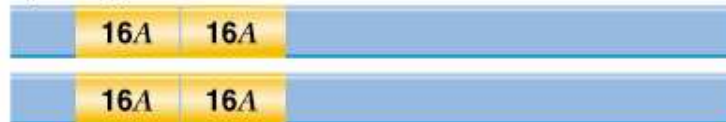
a) Wild type



$\frac{E}{F}$
15
16
 $\frac{A}{B}$



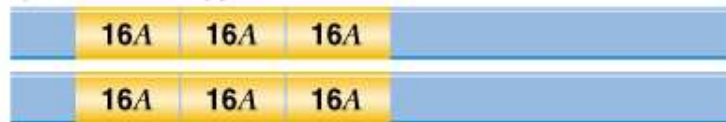
b) *Bar* type



16
 $\frac{A}{A}$
16
 $\frac{A}{A}$



c) Double*Bar* type



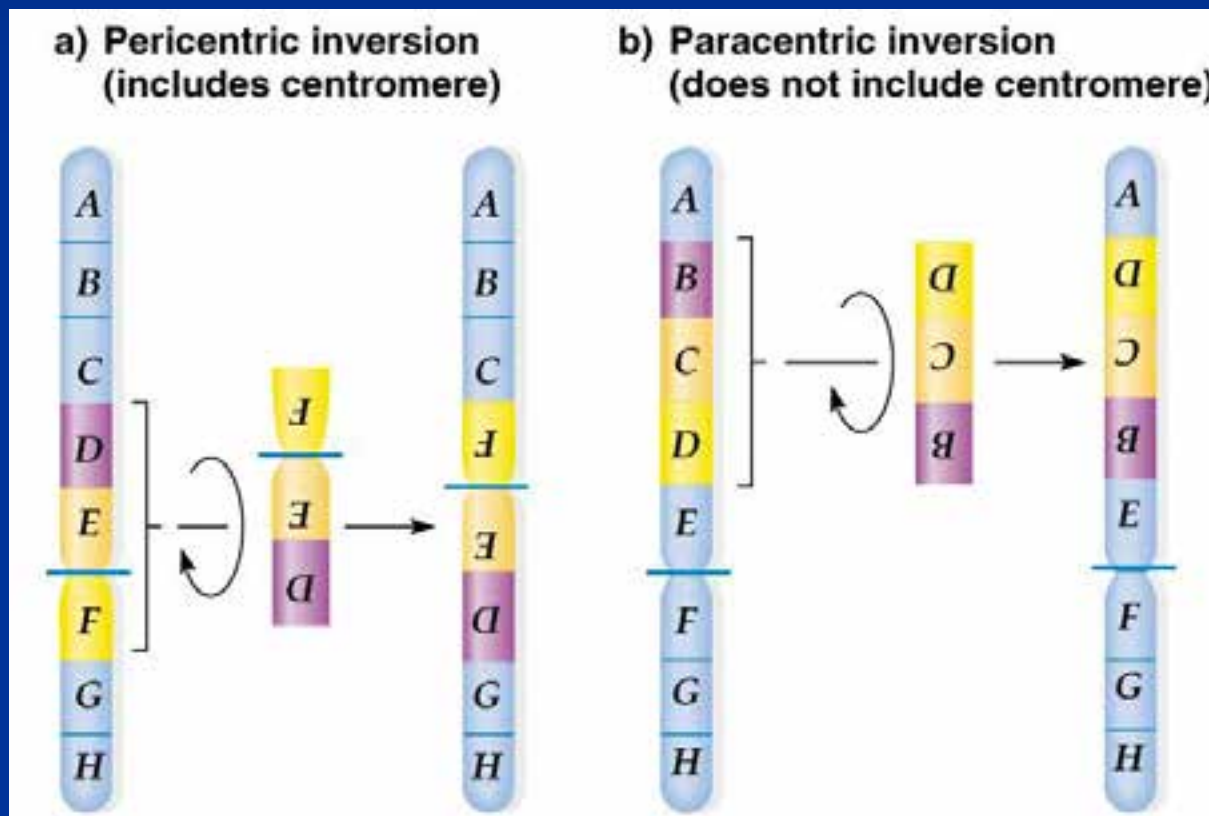
16
 $\frac{A}{A}$
16
 $\frac{A}{A}$
16
 $\frac{A}{A}$



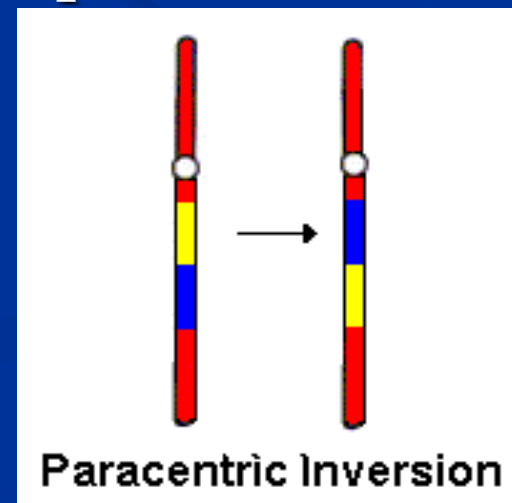
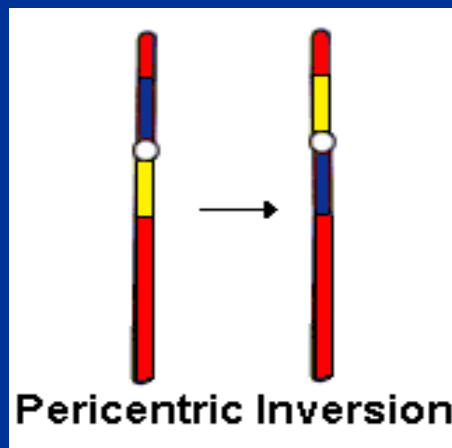
Inversion

- When a segment of chromosome is oriented in the reverse direction, such segment said to be **inverted** and the phenomenon is termed as **inversion**.
- The existence of inversion was first detected by **Strutevant** and **Plunkett** in 1926.
- Inversion occur when parts of chromosomes become detached , turn through 180^0 and are reinserted in such a way that the genes are in reversed order.
- For example, a certain segment may be broken in two places, and the breaks may be in close proximity because of chance loop in the chromosome.
- When they rejoin, the wrong ends may become connected.
- The part on one side of the loop connects with broken end different from the one with which it was formerly connected.
- This leaves the other two broken ends to become attached.
- The part within the loop thus becomes turned around or inverted.

- Inversion may be classified into two types:
 - Pericentric - include the centromere
 - Paracentric - do not include the centromere



- An inversion consists of two breaks in one chromosome.
- The area between the breaks is inverted (turned around), and then reinserted and the breaks then unite to the rest of the chromosome.
- If the inverted area includes the centromere it is called a pericentric inversion.
- If it does not, it is called a paracentric inversion.



Inversions in natural populations

- In natural populations, pericentric inversions are much less frequent than paracentric inversions.
- In many sp, however, pericentric inversions are relatively common, e.g., in some **grasshoppers**.
- Paracentric inversions appear to be very frequent in natural populations of *Drosophila*.

Translocation

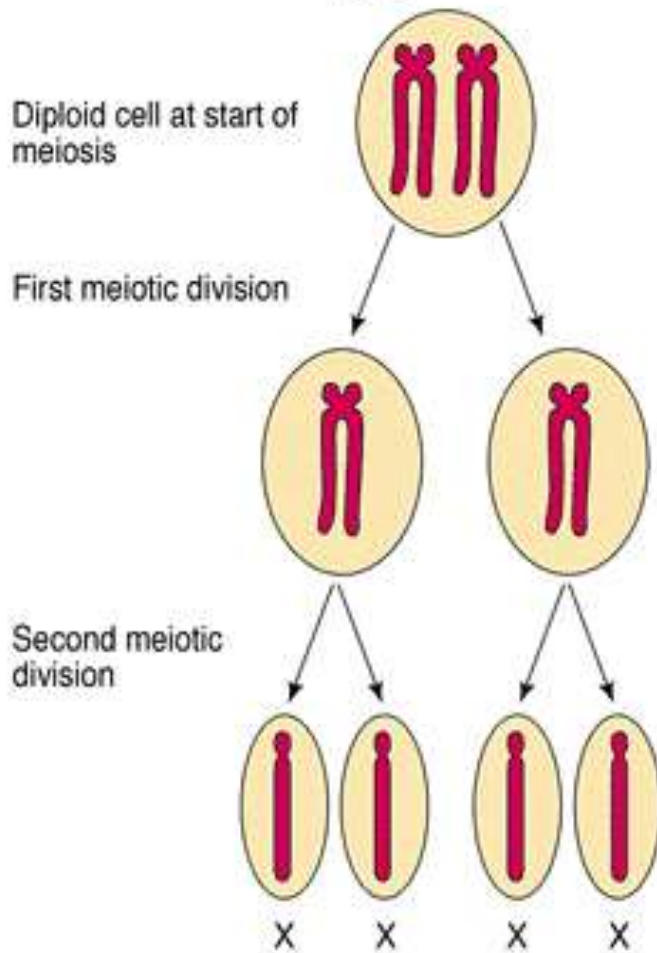
- Integration of a chromosome segment into a nonhomologous chromosome is known as **translocation**.
- Three types:
 1. simple translocation
 2. shift
 3. reciprocal translocation.

- **Simple translocation:** In this case, **terminal segment** of a chromosome is **integrated** at one end of a non-homologous region. Simple translocations are rather **rare**.
- **Shift:** In shift, an **intercalary segment** of a chromosome is **integrated** within a non-homologous chromosome. Such translocations are known in the populations of *Drosophila*, *Neurospora* etc.
- **Reciprocal translocation:** It is produced when two non-homologous chromosomes exchange segments – i.e., segments **reciprocally** transferred.
- Translocation of this type is most common

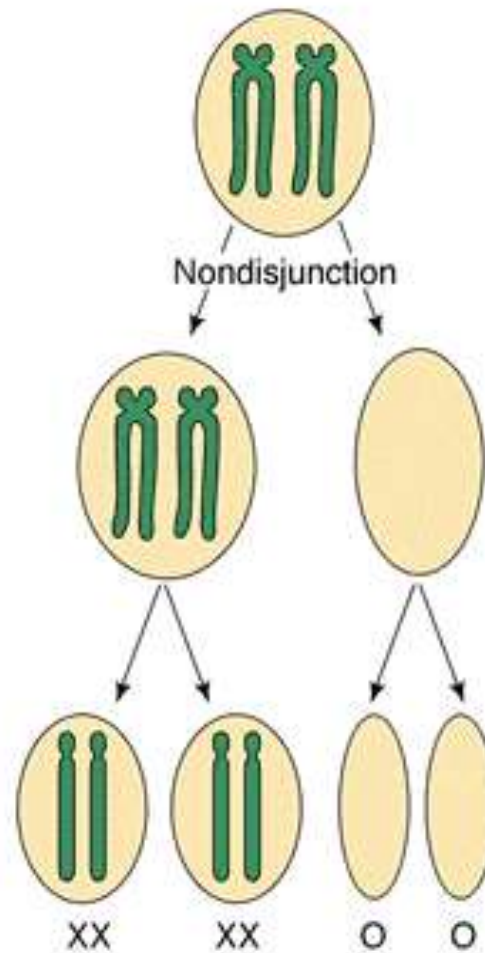
Non-Disjunction

- Generally during gametogenesis the homologous chromosomes of each pair separate out (disjunction) and are equally distributed in the daughter cells.
- But sometime there is an **unequal distribution** of chromosomes in the daughter cells.
- The failure of separation of homologous chromosome is called **non-disjunction**.
- This can occur either during **mitosis** or **meiosis** or **embryogenesis**.

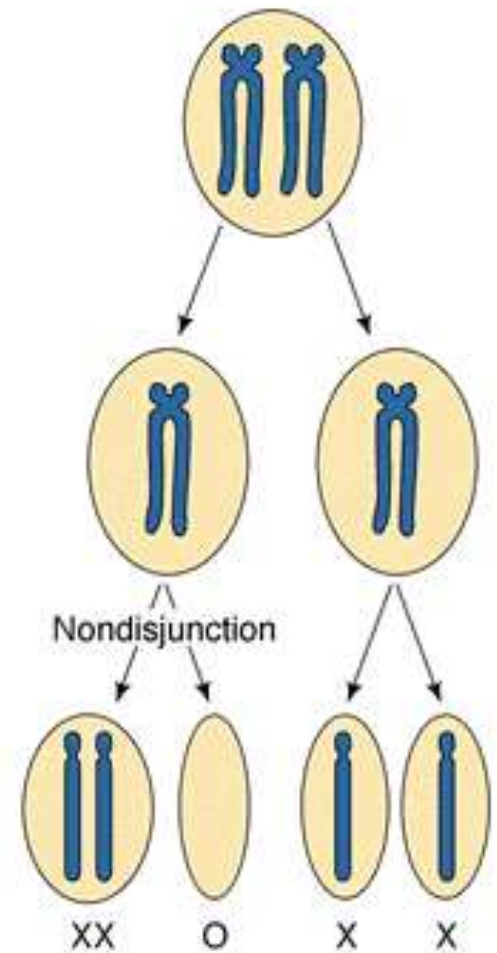
a) Normal X chromosome segregation



b) Nondisjunction in meiosis I



c) Nondisjunction in meiosis II



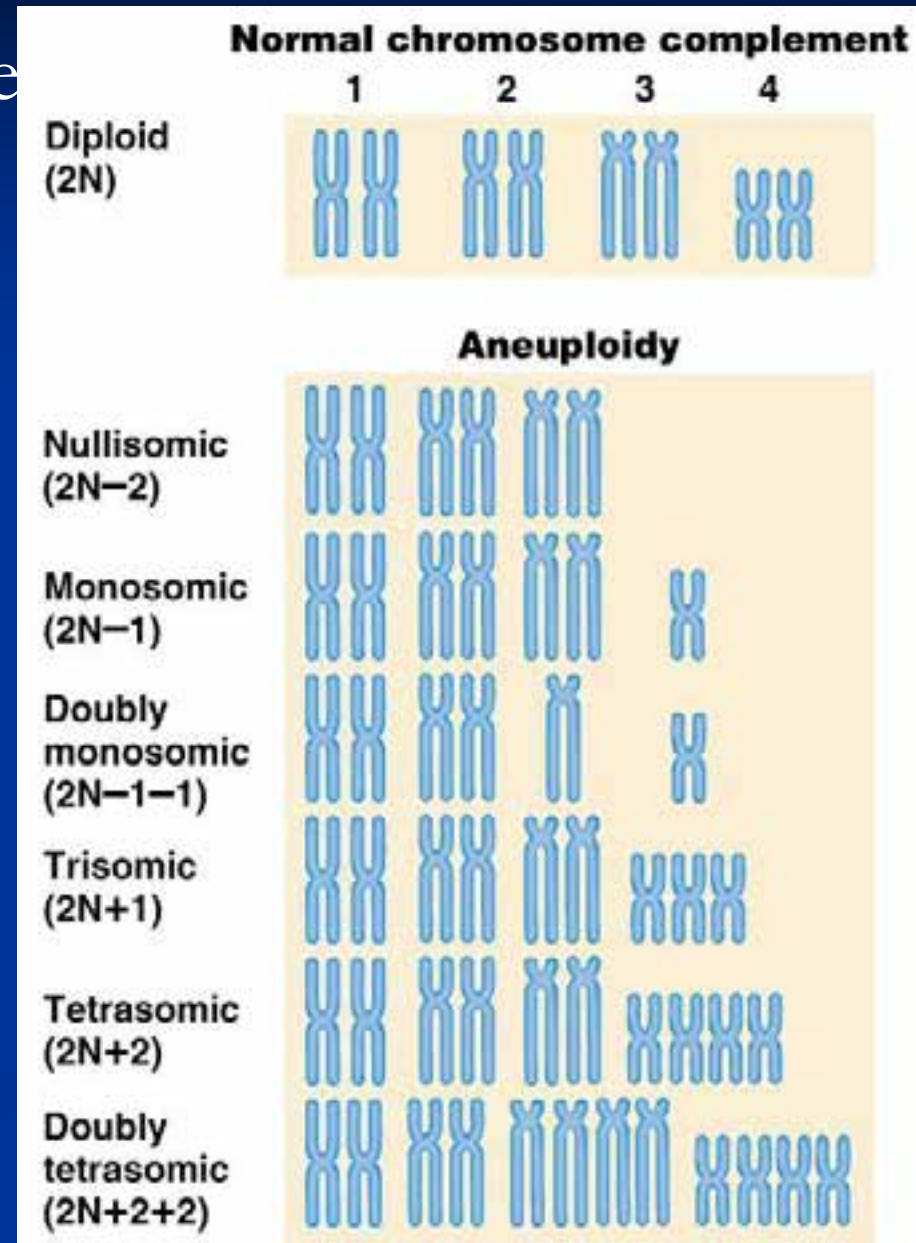
- **Mitotic non-disjunction:** The failure of separation of homologous chromosomes during mitosis is called mitotic non-disjunction.
- It occurs after fertilization.
- May happen during first or second cleavage.
- Here, one blastomere will receive 45 chromosomes, while other will receive 47.
- **Meiotic non-disjunction:** The failure of separation of homologous chromosomes during meiosis is called mitotic non-disjunction
- Occurs during gametogenesis
- Here, one type contain 22 chromosome, while other will be 24.

Variation in chromosome number

- Organism with one complete set of chromosomes is said to be euploid (applies to haploid and diploid organisms).
- Aneuploidy - variation in the number of individual chromosomes (but not the total number of sets of chromosomes).
- The discovery of aneuploidy dates back to 1916 when **Bridges** discovered XO male and XXY female *Drosophila*, which had 7 and 9 chromosomes respectively, instead of normal 8.

More about Aneuploidy

- **Nullisomy** - loss of one homologous chromosome pair. (e.g., Oat)
- **Monosomy** – loss of a single chromosome (Maize).
- **Trisomy** - one extra chromosome. (Datura)
- **Tetrasomy** - one extra chromosome pair.



Uses of Aneuploidy

- They have been used to determine the phenotypic effect of loss or gain of different chromosome
- Used to produce **chromosome substitution** lines. Such lines yield information on the effects of different chromosomes of a variety in the same genetic background.
- They are also used to produce **alien addition** and **alien substitution lines**. These are useful in gene transfer from one species to another.
- Aneuploidy permits the location of a gene as well as of a linkage group onto a specific chromosome.

Trisomy in Humans

Down Syndrome

- The best known and most common chromosome related syndrome.
- Formerly known as “**Mongolism**”
- 1866, when a physician named **John Langdon Down** published an essay in England in which he described a set of children with common features who were distinct from other children with mental retardation he referred to as “**Mongoloids.**”
- One child in every 800-1000 births has Down syndrome
- 250,000 in US has Down syndrome.
- The cost and maintaining Down syndrome case in US is estimated at \$ 1 billion per year.

- Patients having Down syndrome will be short in stature (four feet tall) and have an epicanthal fold, broad short skulls, wide nostrils, large tongue, stubby hands
- Some babies may have short necks, small hands, and short fingers.
- They are characterized as low in mentality.
- Down syndrome results if the extra chromosome is number 21.

Amniocentesis for Detecting Aneuploidy

- Chromosomal abnormalities are sufficiently well understood to permit genetic counseling.
- A fetus may be checked in early stages of development by karyotyping the cultured cells obtained by a process called **amniocentesis**.
- A sample of fluid will be taken from mother and fetal cells are cultured and after a period of two to three weeks, chromosomes in dividing cells can be stained and observed.
- If **three No.21 chromosomes** are present, **Down syndrome confirmed**.

- The risk for mothers less than 25 years of age to have the trisomy is about 1 in 1500 births.
- At 40 years of age, 1 in 100 births
- At 45 years 1 in 40 births.

Other Syndromes

Chromosome Nomenclature: 47, +13

Chromosome formula: $2n+1$

Clinical Syndrome: Trisomy-13

Estimated Frequency Birth: 1/20,000

Main Phenotypic Characteristics:

Mental deficiency and deafness, minor muscle seizures, cleft lip, cardiac anomalies

Other Syndromes

Chromosome Nomenclature: 47, +18

Chromosome formula: $2n+1$

Clinical Syndrome: Trisomy-18

Estimated Frequency Birth: 1/8,000

Main Phenotypic Characteristics:

Multiple congenital malformation of many organs, malformed ears, small mouth and nose with general elfin appearance.

90% die in the first 6 months.

Other Syndromes

Chromosome Nomenclature: 45, X

Chromosome formula: $2n - 1$

Clinical Syndrome: Turner

Estimated Frequency Birth: 1/2,500 female

Main Phenotypic Characteristics:

Female with retarded sexual development, usually sterile, short stature, webbing of skin in neck region, cardiovascular abnormalities, hearing impairment.

Other Syndromes

Chromosome Nomenclature: 47, XXY, 48, XXXY, 48,XXYY, 49, XXXXY, 50, XXXXXY

Chromosome formula: $2n+1$; $2n+2$; $2n+2$; $2n+3$; $2n+4$

Clinical Syndrome: Klinefelter

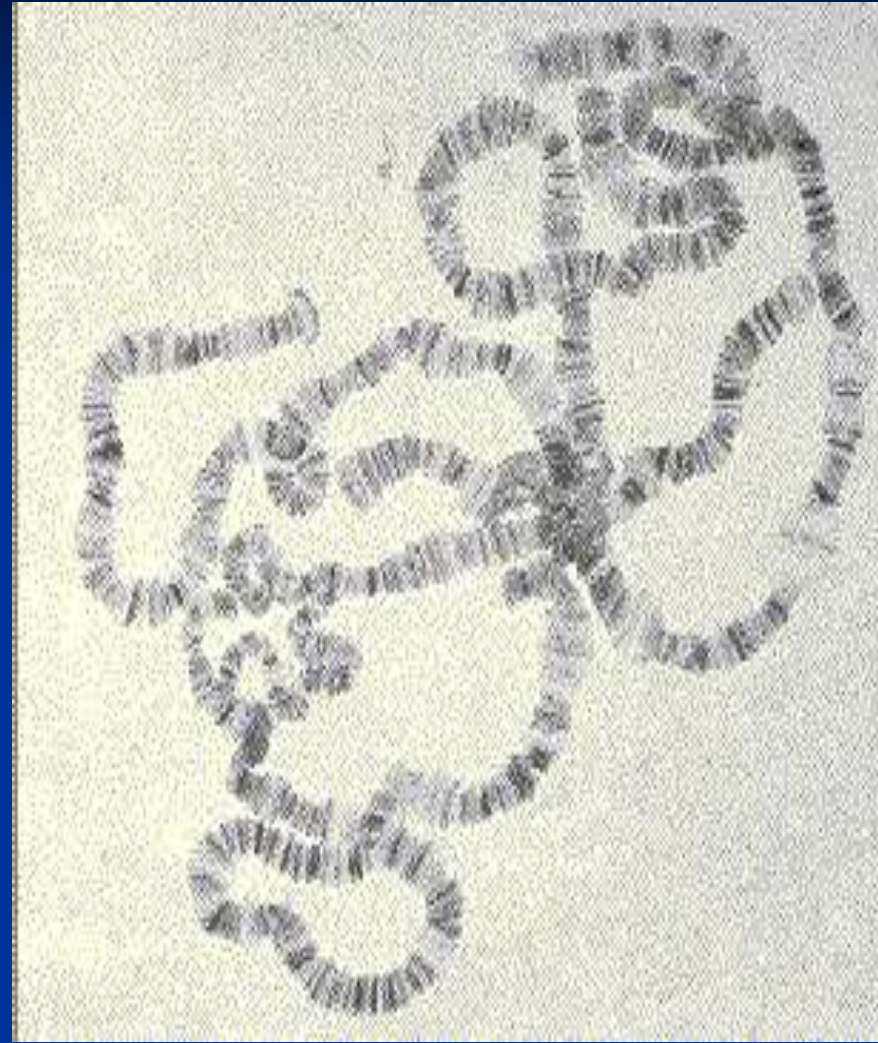
Estimated Frequency Birth: 1/500 male borth

Main Phenotypic Characteristics:

Pitched voice, Male, subfertile with small testes, developed breasts, feminine, long limbs.

Giant chromosomes

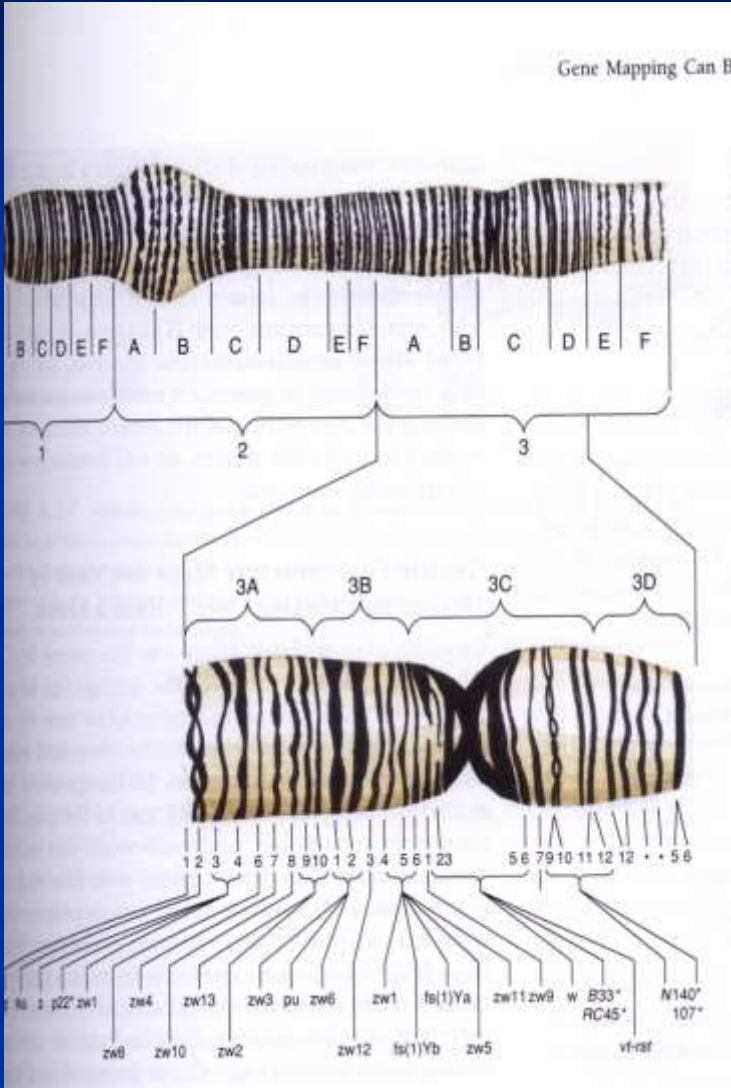
- Found in certain tissues e.g., salivary glands of larvae, gut epithelium, Malphigian tubules and some fat bodies, of some Diptera (**Drosophila**, **Sciara**, **Rhyncosciara**)
- These chromosomes are very long and thick (upto **200 times their size** during mitotic metaphase in the case of *Drosophila*)
- Hence they are known as **Giant chromosomes**.



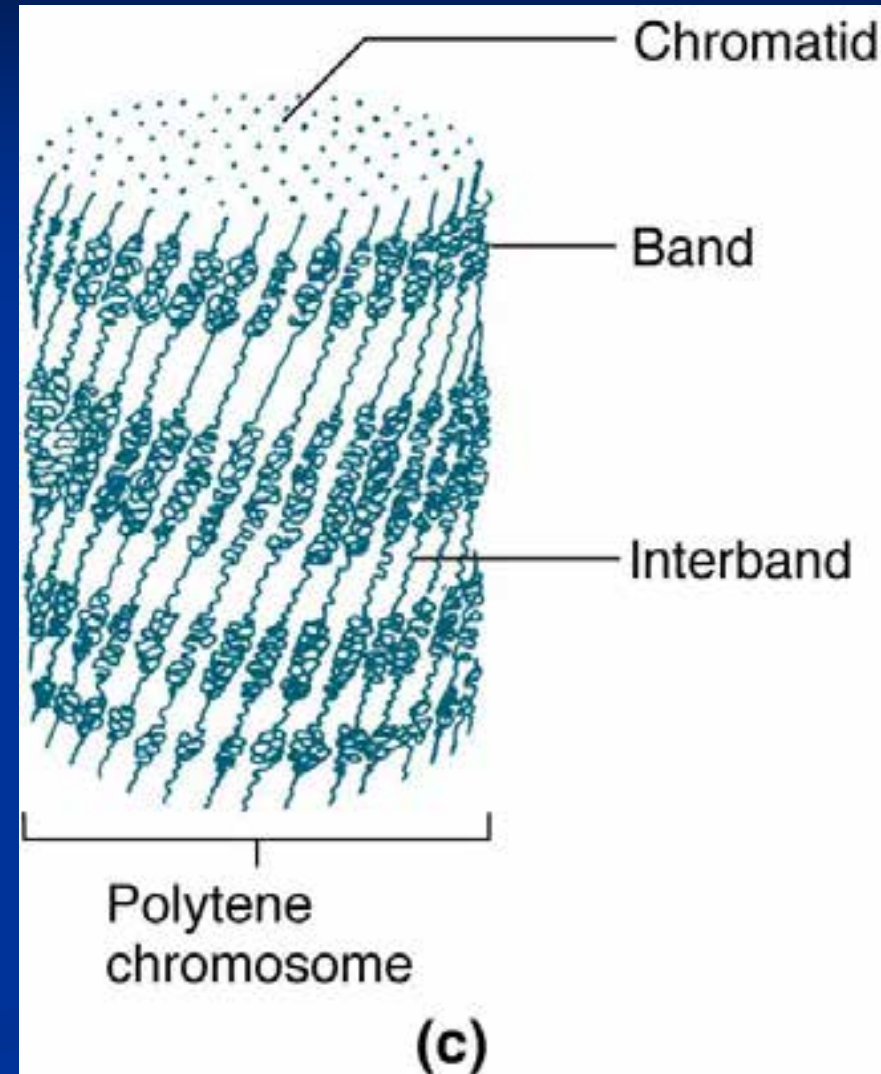
- They are first discovered by **Balbani** in **1881** in dipteran salivary glands and thus also known as **salivary gland chromosomes**.
- But their significance was realized only after the extensive studies by **Painter** during 1930's.
- Giant chromosomes have also been discovered in suspensors of young embryos of many plants, but these do not show the bands so typical of salivary gland chromosomes.

- He described the morphology in detail and discovered the relation between salivary gland chromosomes and germ cell chromosomes.
- Slides of *Drosophila* giant chromosomes are prepared by squashing in **acetocarmine** the salivary glands dissected out from the larvae.
- The total length of *D.melanogater* giant chromosomes is about **2,000 μ** .

- Giant chromosomes are made up of several dark staining regions called “**bands**”.
- It can be separated by relatively light or non-staining “**interband**” regions.
- The bands in *Drosophila* giant chromosome are visible even without staining, but after staining they become very sharp and clear.
- In *Drosophila* about **5000 bands** can be recognized.



- Some of these bands are as thick as 0.5μ , while some may be only 0.05μ thick.
- About 25,000 base-pairs are now estimated for each band.
- All the available evidence clearly shows that each giant chromosome is composed of numerous strands, each strand representing one chromatid.
- Therefore, these chromosomes are also known as “**Polytene chromosome**”, and the condition is referred to as “**Polytene**”



- The numerous strands of these chromosomes are produced **due to repeated replication of the paired chromosomes** without any nuclear or cell division.
- So that the number of strands (chromatids) in a chromosome doubles after every round of DNA replication
- It is estimated that giant chromosomes of **Drosophila** have about **1,024 strands**
- In the case of **Chironomous** may have about **4,096 strands**.
- The bands of giant chromosomes are formed as a result of stacking over one another of the chromomeres of all strands present in them.

- Since chromatin fibers are highly coiled in chromosomes, they stain deeply.
- On the other hand, the chromatin fibers in the interband regions are fully extended, as a result these regions take up very light stain.
- In *Drosophila* the location of many genes is correlated with specific bands in the connected chromosomes.
- In interband region do not have at least functional genes

- During certain stages of development, specific bands and inter band regions are associated with them greatly increase in diameter and produced a structure called **Puffs** or **Balbiani rings**.
- Puffs are believed to be produced due to uncoiling of chromatin fibers present in the concerned chromomeres.
- The puffs are sites of active **RNA synthesis**.



10 μm

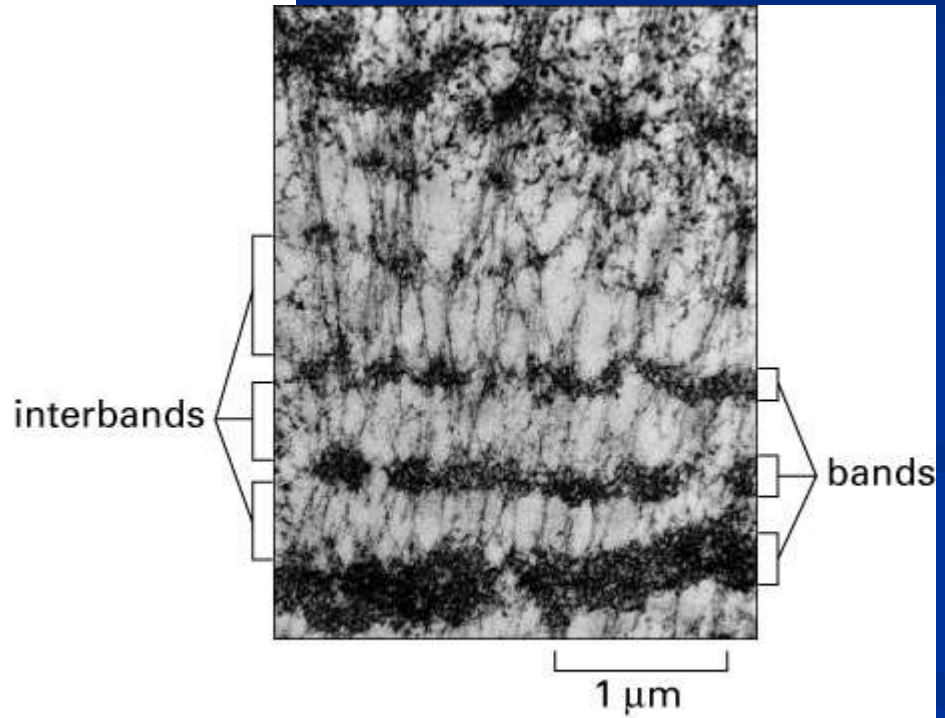
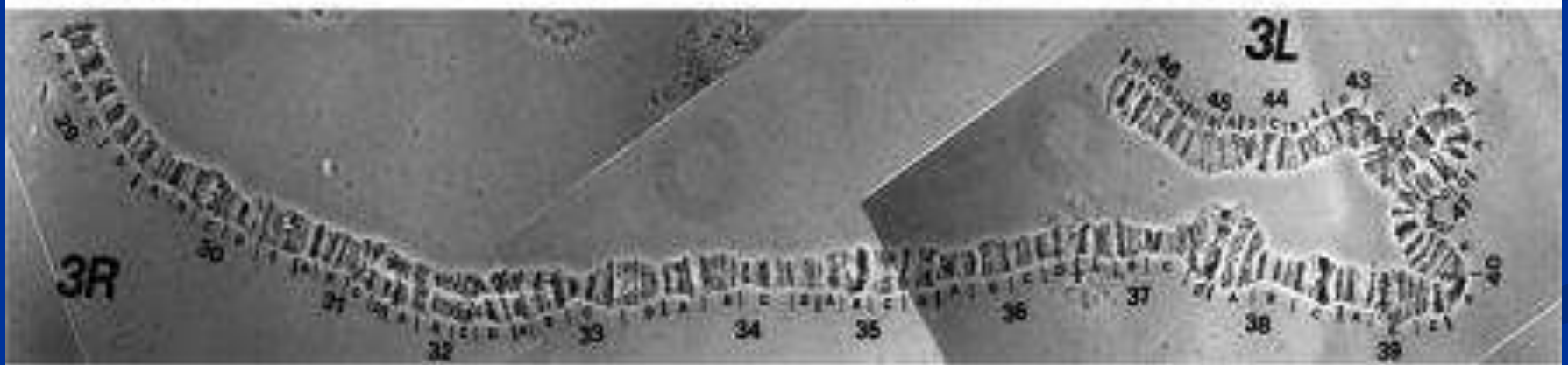
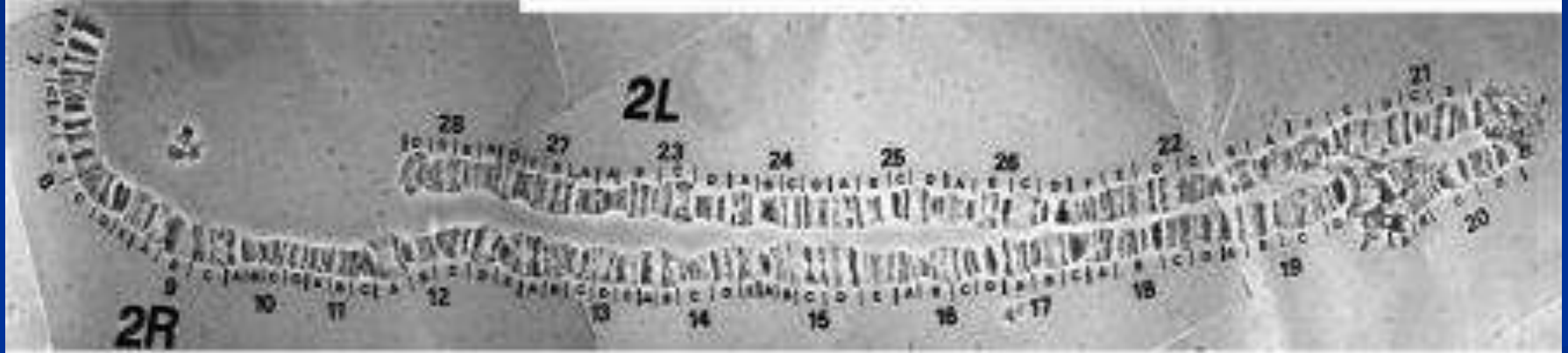


Figure 4-40. Molecular Biology of the Cell, 4th Edition.



Polytene chromosome map of *Anopheles gambiae*

Lampbrush Chromosome

- It was given this name because it is similar in appearance to the brushes used to clean lamp chimneys in centuries past.
- First observed by **Flemming** in 1882.
- The name lampbrush was given by **Ruckert** in 1892.
- These are found in **oocytic** nuclei of vertebrates (sharks, amphibians, reptiles and birds) as well as in invertebrates (Sagitta, sepia, Echinaster and several species of insects).
- Also found in plants – but most experiments in oocytes.

- Lampbrush chromosomes are up to **800 μm** long; thus they provide very favorable material for cytological studies.
- The homologous chromosomes are paired and each has duplicated to produce two chromatids at the lampbrush stage.
- Each lampbrush chromosome contains a central axial region, where the two chromatids are highly condensed
- Each chromosome has several chromomeres distributed over its length.
- From each chromomere, a pair of loops emerges in the opposite directions vertical to the main chromosomal axis.

- One loop represent one chromatid, i.e., one DNA molecule.
- The size of the loop may be ranging the average of 9.5 μm to about 200 μm
- The pairs of loops are produced due to uncoiling of the two chromatin fibers present in a highly coiled state in the chromomeres.

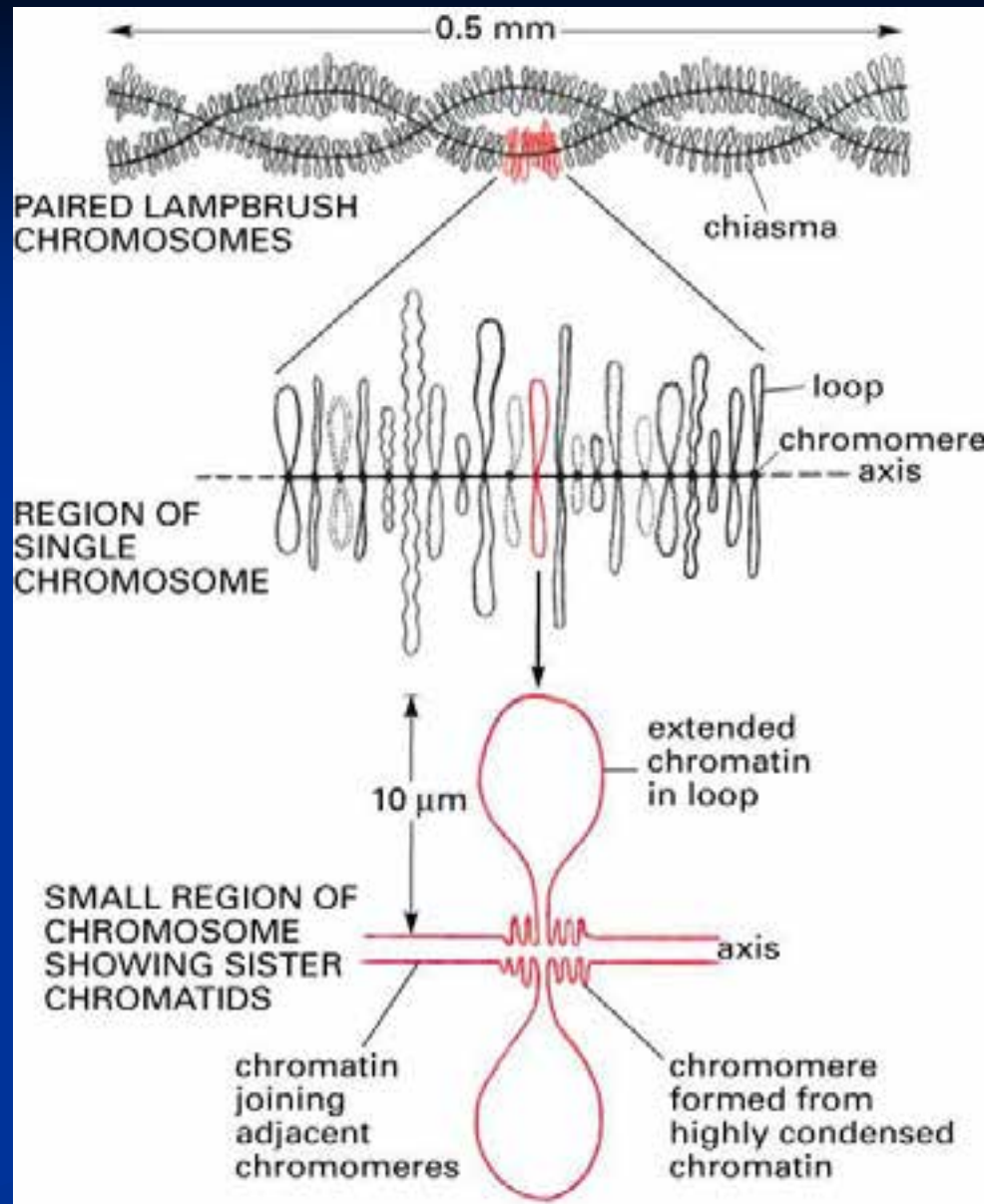
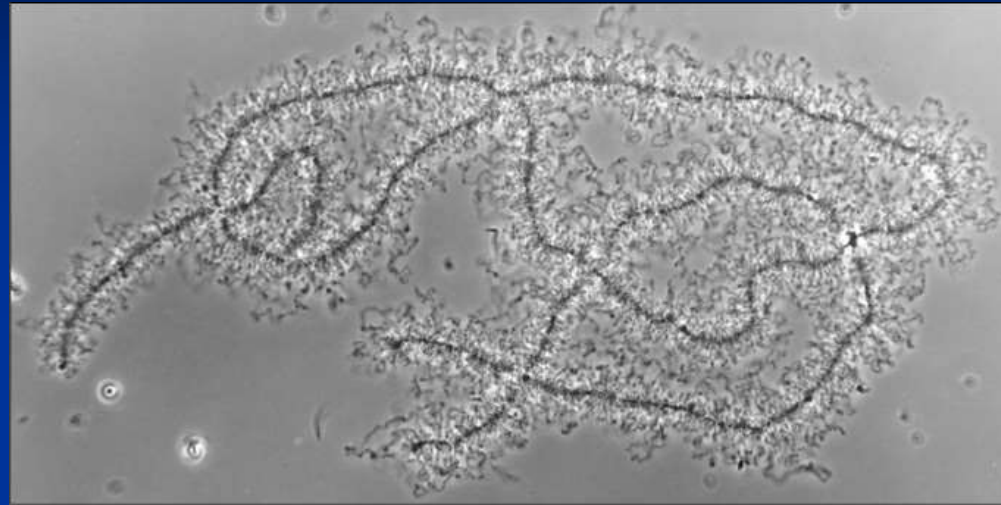


Figure 4-37. Molecular Biology of the Cell, 4th Edition.

- One end of each loop is thinner (thin end) than the other end (thick end).
- There is extensive RNA synthesis at the thin end of the loops, while there is little or no RNA synthesis at the thick end.

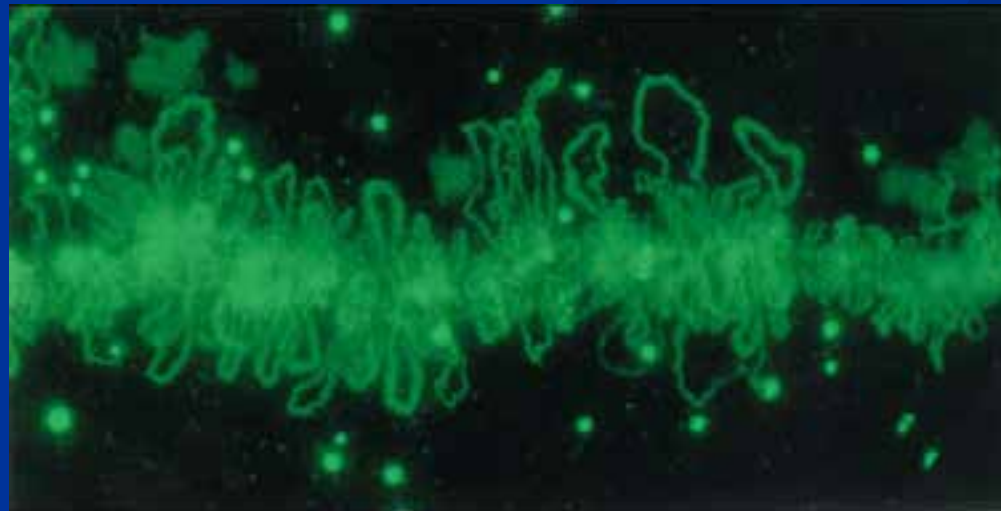
Phase-contrast and fluorescent micrographs of lampbrush chromosomes



(A)

0.1 mm

Figure 4-36 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



(B)

20 μ m

Dosage Compensation

- Sex Chromosomes: females XX, males XY
- Females have two copies of every X-linked gene; males have only one.
- How is this difference in gene dosage compensated for? OR
- How to create equal amount of X chromosome gene products in males and females?

- Levels of enzymes or proteins encoded by genes on the X chromosome are the same in both males and females
- Even though males have 1 X chromosome and females have 2.

- G6PD, glucose 6 phosphate dehydrogenase, gene is carried on the X chromosome
- This gene codes for an enzyme that breaks down sugar
- Females produce the same amount of G6PD enzyme as males
- XXY and XXX individuals produce the same amount of G6PD as anyone else

- In cells with more than two X chromosomes, only one X remains genetically active and all the others become inactivated.
- In some cells the paternal allele is expressed
- In other cells the maternal allele is expressed
- In XXX and XXXX females and XXY males only 1 X is activated in any given cell the rest are inactivated

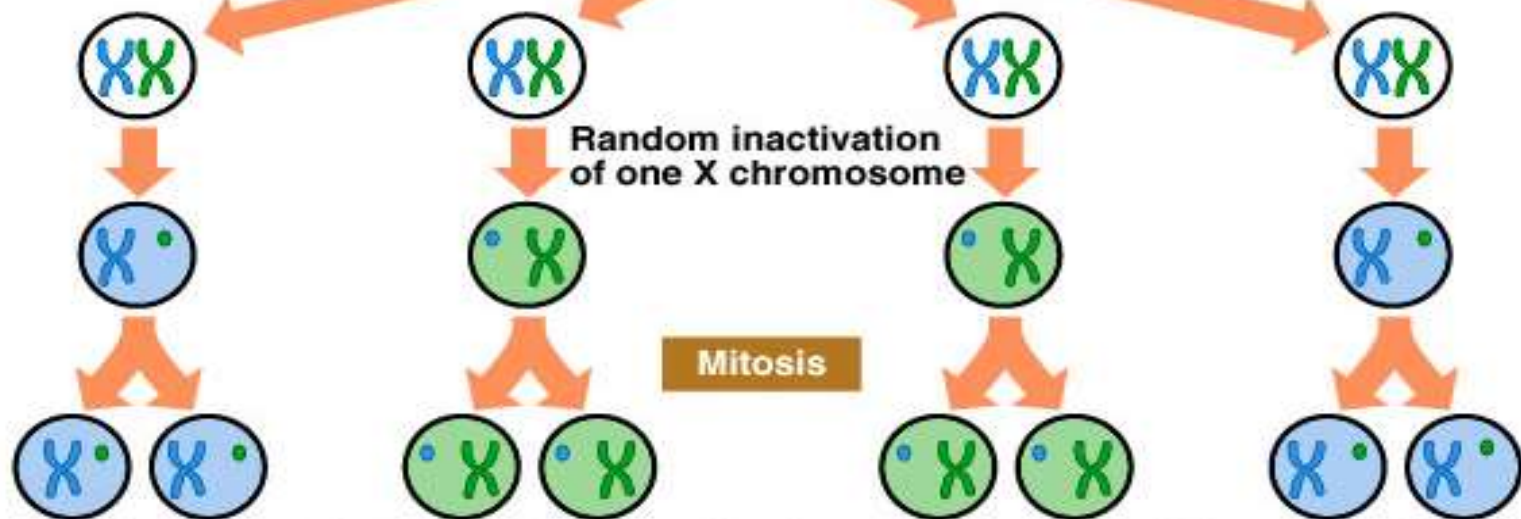
X inactivation

Paternal   Maternal



Fertilized ovum

Mitosis



A

Maternal X inactivated
(Paternal X expressed)

Paternal X inactivated
(Maternal X expressed)

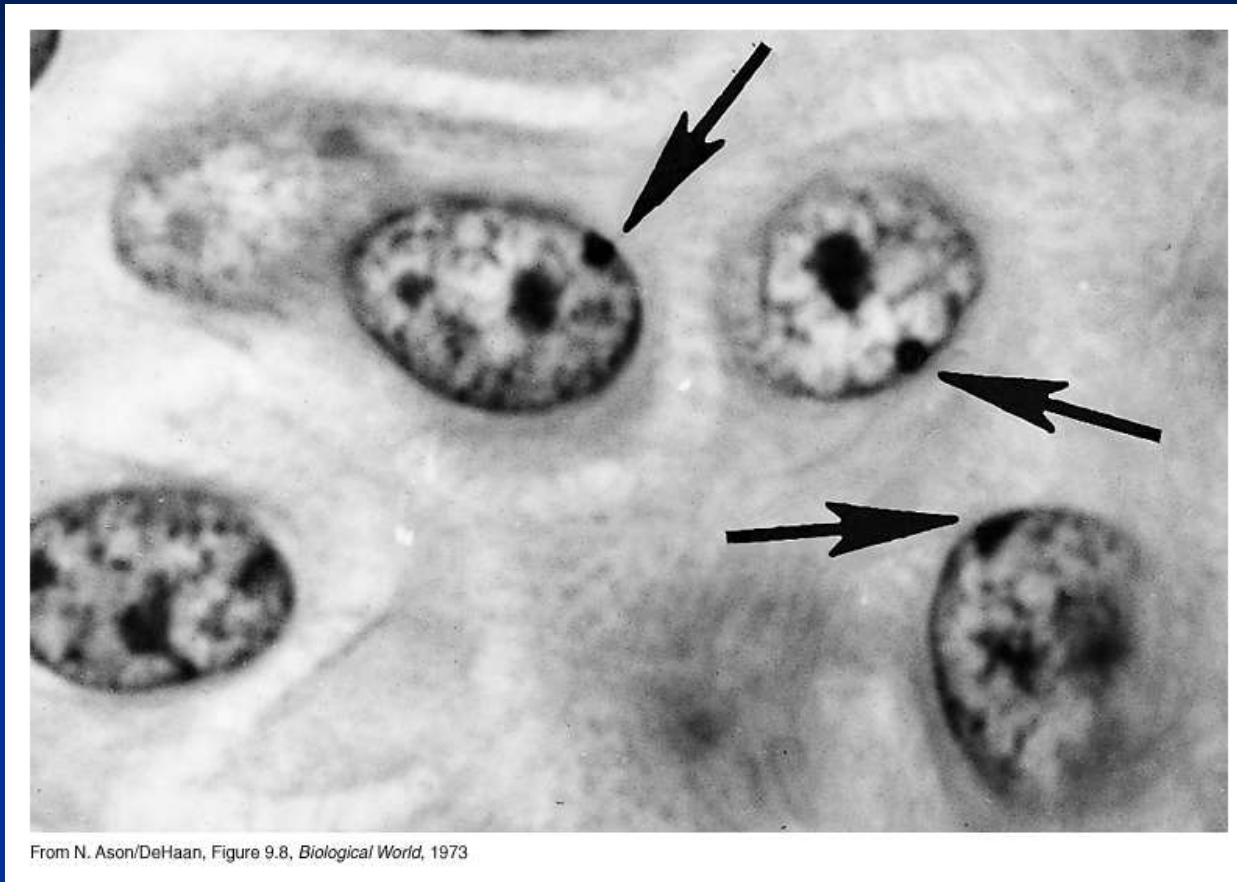
Paternal X inactivated
(Maternal X expressed)

Maternal X inactivated
(Paternal X expressed)

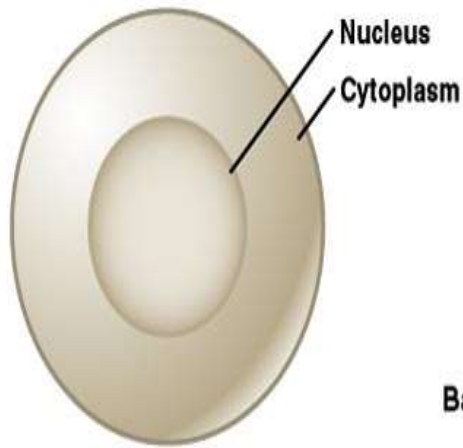
Barr Bodies

- 1940's two Canadian scientists noticed a dark staining mass in the nuclei of cat brain cells
- Found these dark staining spots in female but not males
- This held for cats and humans
- They thought the spot was a tightly condensed X chromosome

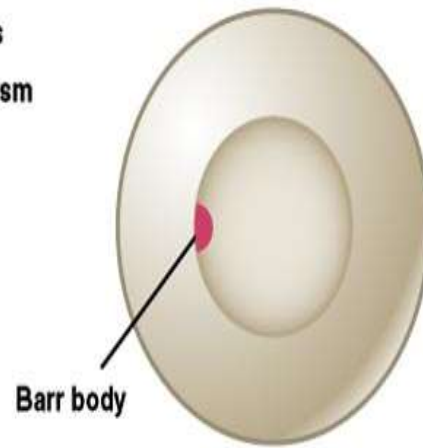
Barr Bodies



Barr bodies represent the inactive X chromosome and are normally found only in female somatic cells.



46, XY
45, X
($N-1=0$)



46, XX
47, XXY
($N-1=1$)



47, XXX
48, XXXY
($N-1=2$)



48, XXXX
49, XXXXY
($N-1=3$)

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

■ XXY individuals are male, but have a Barr body.

■ XO individuals are female but have no Barr bodies.

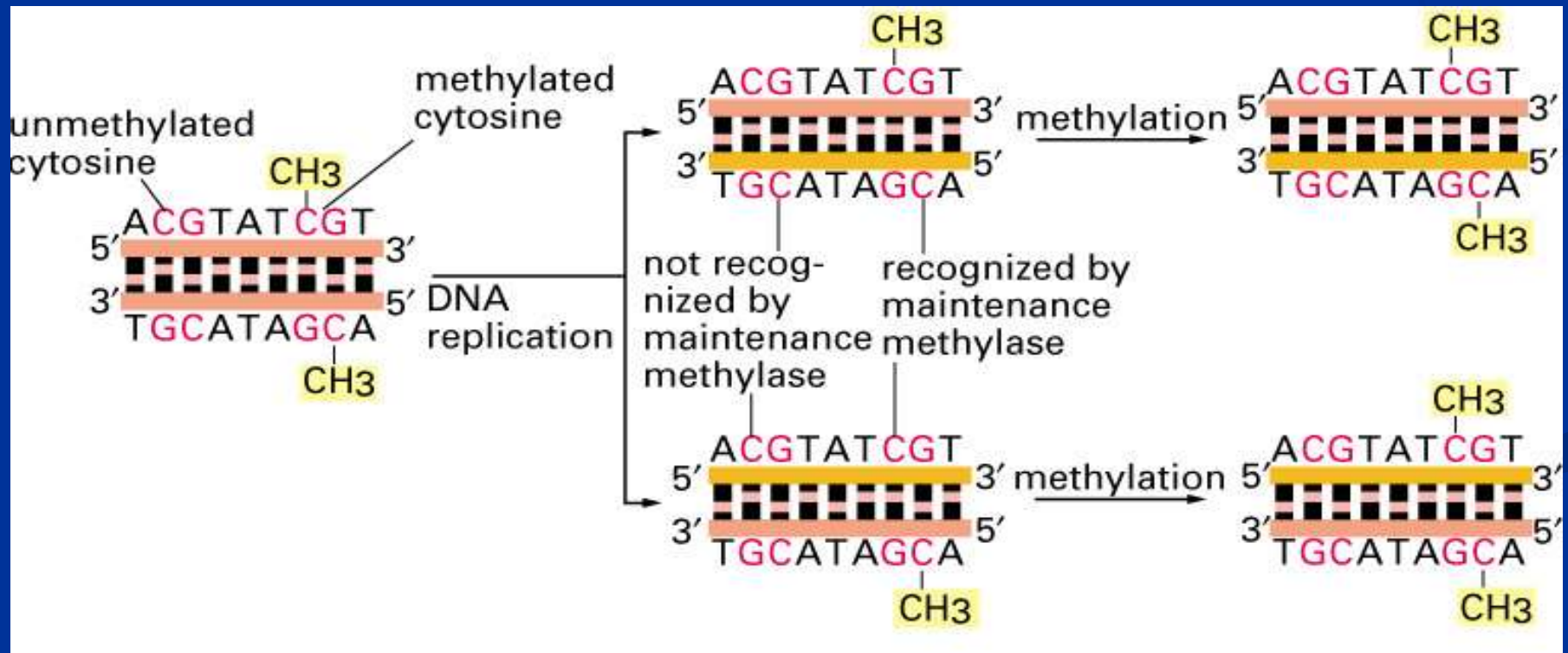
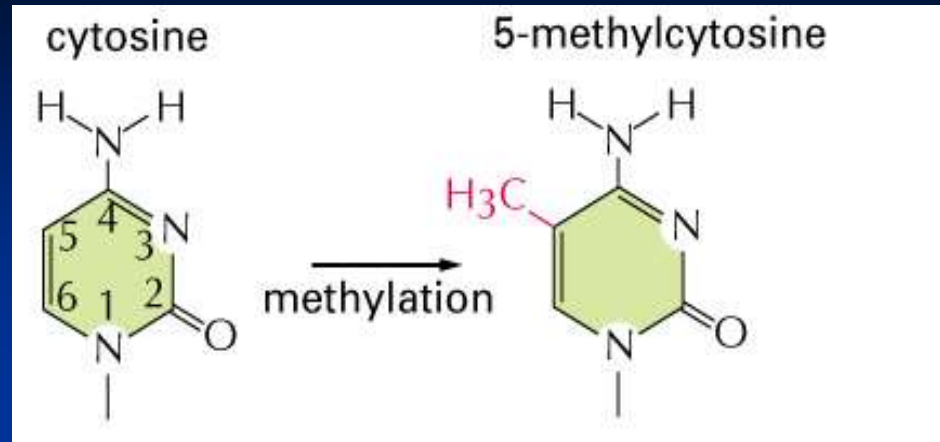
- Which chromosome is inactive is a matter of chance, but once an X has become inactivated, all cells arising from that cell will keep the same inactive X chromosome.
- In the mouse, the inactivation apparently occurs in early in development
- In human embryos, sex chromatin bodies have been observed by the 16th day of gestation.

Mechanism of X-chromosome Inactivation

- A region of the p arm of the X chromosome near the centromere called the X-inactivation center (XIC) is the control unit.
- This region contains the gene for X-inactive specific transcript (*XIST*). This RNA presumably coats the X chromosome that expresses it and then DNA methylation locks the chromosome in the inactive state.

- This occurs about 16 days after fertilization in a female embryo.
- The process is independent from cell to cell.
- A maternal or paternal X is randomly chosen to be inactivated.

- Rollin Hotchkiss first discovered methylated DNA in 1948.
- He found that DNA from certain sources contained, in addition to the standard four bases, a fifth: 5-methyl cytosine.
- It took almost three decades to find a role for it.
- In the mid-1970s, Harold Weintraub and his colleagues noticed that active genes are low in methyl groups or under methylated.
- Therefore, a relationship between under methylation and gene activity seemed likely, as if methylation helped repress genes.



- This would be a valuable means of keeping genes inactive if methylation passed on from parent to daughter cells during cell division.
- Each parental strand retains its methyl groups, which serve as signals to the methylating apparatus to place methyl groups on the newly made progeny strand.
- Thus methylation has two of the requirements for mechanism of determination:
 - 1. It represses gene activity
 - 2. It is permanent.

- Strictly speaking, the DNA is altered, since methyl groups are attached, but because methyl cytosine behaves the same as ordinary cytosine, the genetic coding remain same.
- A striking example of such a role of methylation is seen in the inactivation of the X chromosome in female mammal.
- The inactive X chromosome become heterochromatic and appears as a dark fleck under the microscope – this chromosome said to be lyonized, in honor of Mary Lyon who first postulated the effect in mice.

- An obvious explanation is that the DNA in the lyonized X chromosome is methylated, whereas the DNA in the active, X chromosome is not.
- To check this hypothesis Peter Jones and Lawrence Shapiro grew cells in the presence of drug 5-azacytosine, which prevents DNA methylation.
- This reactivated the lyonized X chromosome.
- Furthermore, Shapiro showed these reactivated chromosomes could be transferred to other cells and still remain active.



Reading assignment

- Grewal and Moazed (2003) “Heterochromatin and epigenetic control of gene expression”
Science 301:798
- Goldmit and Bergman (2004) “Monoallelic gene expression: a repertoire of recurrent themes”
Immunol Rev 200:197