CHROMOSOMES

Lec 4 / 4th biology

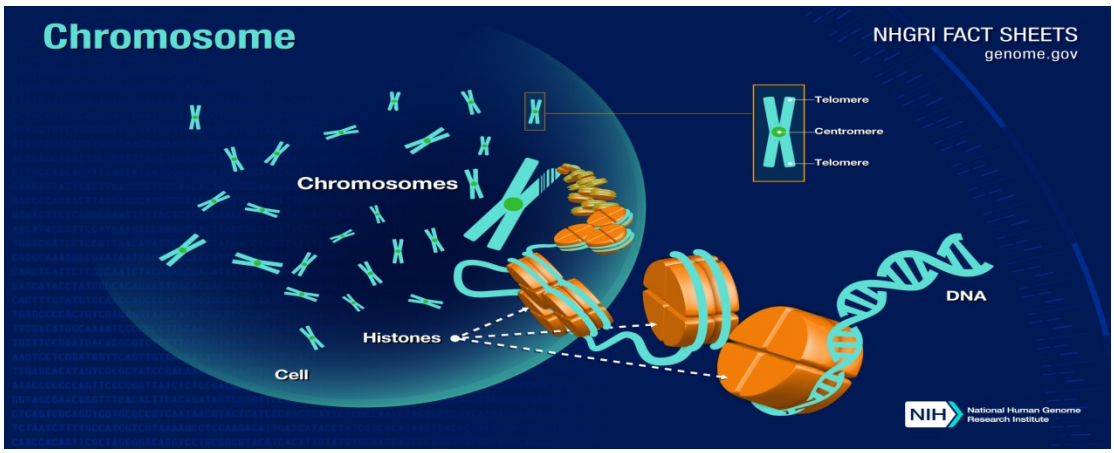
What is a Chromosome?

Chromosomes are thread-like structures located inside the nucleus of animal and plant cells. Each chromosome is made of protein and a single molecule of DNA, Passed from parents to offspring. DNA contains the specific instructions that make each type of living creature unique.

The term chromosome comes from the Greek words for color (chroma) and body (soma). Scientists gave this name to chromosomes because they are cell structures, or bodies, that are strongly stained by some colorful dyes used in research.

What do Chromosomes do?

The unique structure of chromosomes keeps DNA tightly wrapped around spool-like proteins, called **Histones**. Without such packaging, DNA molecules would be too long to fit inside cells. For example, if all of the DNA molecules in a single human cell were unwound from their histones and placed end-to-end, they would stretch 6 feet. (fig.1)

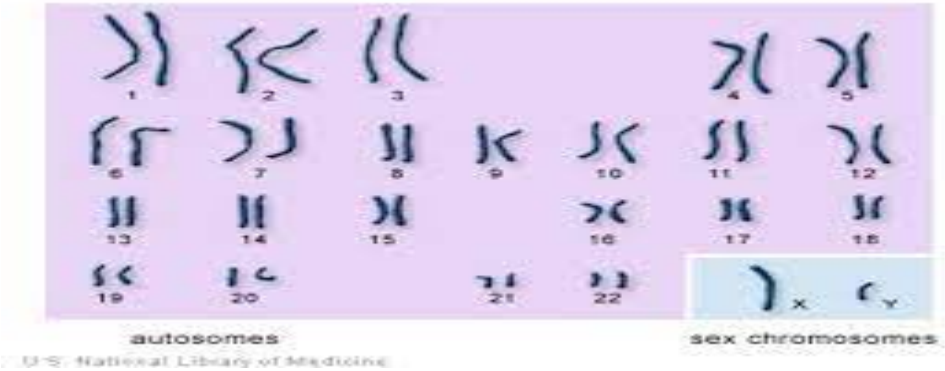


For an organism to grow and function properly, cells must constantly divide to produce new cells to replace old, worn-out cells. During cell division, it is essential that DNA remains intact and evenly distributed among cells. Chromosomes are a key part of the process that ensures DNA is accurately copied and distributed in the vast majority of cell divisions. mistakes do occur on rare occasions.

Changes in the number or structure of chromosomes in new cells may lead to serious problems, For example, in humans, one type of leukemia and some other cancers are caused by defective chromosomes made up of joined pieces of broken chromosomes.

Do all living things have the same types of Chromosomes?

Chromosomes vary in number and shape among living things. Most bacteria have one or two circular chromosomes. Humans, along with other animals and plants, have linear chromosomes that are arranged in pairs within the nucleus of the cell. (fig.2)

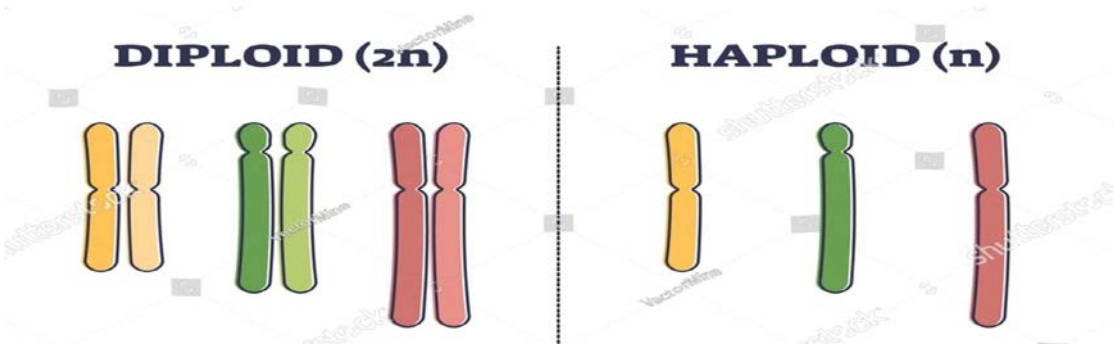


Besides the linear chromosomes found in the nucleus, the cells of humans and other complex organisms carry a much smaller type of chromosome similar to those seen in bacteria. This circular chromosome is found in mitochondria, which are structures located outside the nucleus that serve as the cell's powerhouses.

How many Chromosomes do humans have?

Humans have 23 pairs of chromosomes, for a total of 46 chromosomes. In fact, each species of plants and animals has a set number of chromosomes. A fruit fly, for example, has four pairs of chromosomes, while a rice plant has 12 and a dog, 39. The only human cells that do not contain pairs of chromosomes are reproductive cells, or gametes, which carry just one copy of each chromosome.

It is also crucial that reproductive cells, such as eggs and sperm, contain the right number of chromosomes and that those chromosomes have the correct structure. The number or set of the chromosomes of the gametic cells reduced or **Haploid** sets of chromosomes, and The somatic or body cells of most organisms contain two haploid set or genomes and genome.are knows as the **Diploid** cells.(fig.3)



How are Chromosomes inherited?

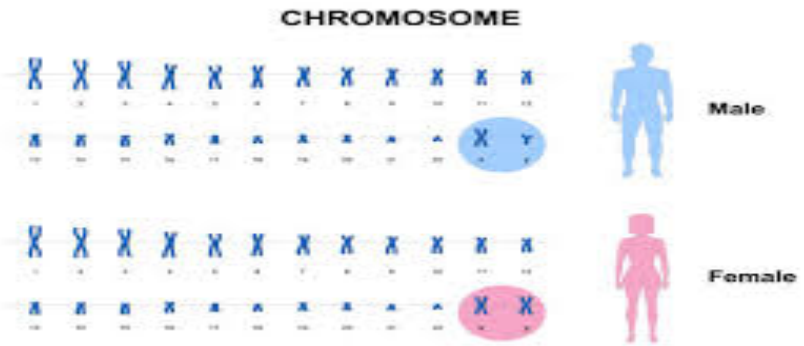
In humans and most other complex organisms, one copy of each chromosome is inherited from the female parent and the other from the male parent. This explains why children inherit some of their traits from their mother and others from their father.

The pattern of inheritance is different for the small circular chromosome found in mitochondria. Only egg cells - and not sperm cells - keep their mitochondria during fertilization. So, mitochondrial DNA is always inherited from the female parent. In humans, a few conditions, including some forms of hearing impairment and diabetes, have been associated with DNA found in the mitochondria.

Do males have different Chromosomes than females?

Yes, they differ in a pair of chromosomes known as the sex chromosomes. Females have two X chromosomes in their cells, while males have one X and one Y chromosome.

The XX-XY type of chromosomal sex determination is found in mammals, including human beings, many insects, and other animals, as well as in some flowering plants. The female is called the **Homogametic** sex because only one type of gamete (X-bearing) is produced, and the male is called the **Heterogametic** sex because two different types of gametes (X-bearing and Y-bearing) are produced. When the union of gametes in fertilization is random, a sex ratio at fertilization of 1:1 is expected because males produce equal numbers of X-bearing and Y-bearing sperm. (fig.4)

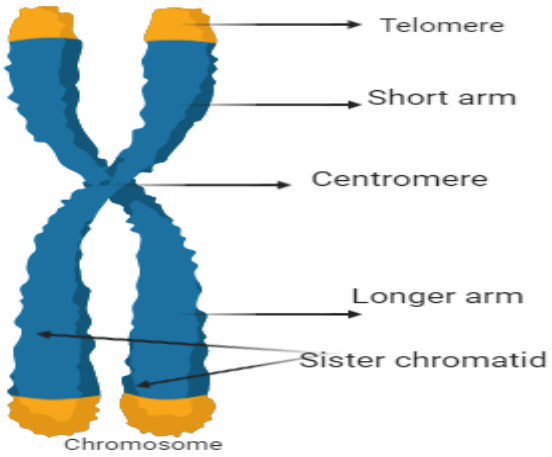


Shape of Chromosomes:

The shape of the chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division. In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread-like stainable structures.

What are Centromeres?

Each chromosome contains a high conserved sequences ,known as **centromere** or **kinetochore**, along their length. It usually not located exactly in the center of the chromosome and, in some cases, is located almost at the chromosome's end. The centromere divides the chromosomes into two parts, each part is called **chromosome arm,** P and **q** arms. (fig. 5)

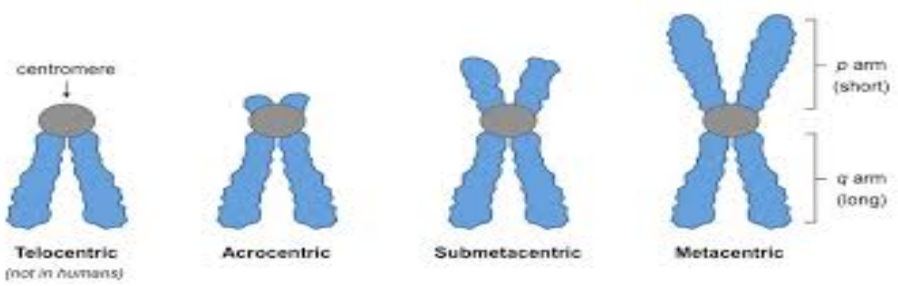


Centromeres help to keep chromosomes properly aligned during the complex process of cell division. As chromosomes are copied in preparation for production of a new cell, the centromere serves as an attachment site for the two halves of each replicated chromosome, known as sister chromatids.

The chromatid remains connected with the spherules of the centromere. Currently it is held that centromere is the region of the chromosome to which are attached the fibers of mitotic spindle. Centromeres are found to contain specific DNA sequences with special proteins bound to them, forming a disc-shaped structure, called **kinetochore**. During mitosis, 4 to 40 microtubules of mitotic spindle become attached to the kinetochore and provide the force for chromosomal movement during anaphase. The main function of the kinetochore is to provide a center of assembly for microtubules

The position of centromere varies from chromosome to chromosome and it provides different shapes to the chromosome which are following:

1. **Metacentric:** The metacentric chromosomes are X-shaped and in these chromosomes the centromere occurs in the center and forming two equal arms. The amphibians have metacentric chromosomes.
2. **Sub metacentric:** The sub metacentric chromosomes are L shaped. In these, the centromere occurs near the middle of chromosome.
3. **Acrocentric:** The acrocentric chromosomes are also rod-like in shape, the centromere is not central and is instead located near the end of the chromosome.
4. **Telocentric:** The centromere is located very close to the end of chromosome. (fig 6)



Depending upon size and centromere position, the 46 chromosomes have been divided into seven groups (A to G). For each chromosome in human karyotype, the chromosome are numbered for easy identification.

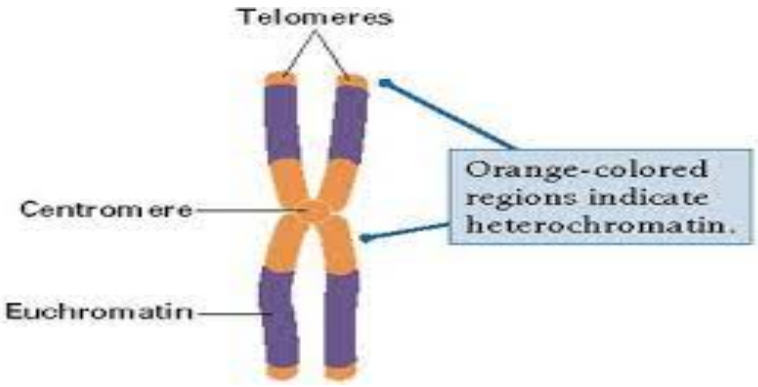
What are Telomere?

Telomere is a region of repetitive DNA sequences located at the ends of chromosomes. They protect the ends of chromosomes in a manner similar to the way the tips of shoelaces keep them from unraveling.

Each time a cell divides, the telomere become slightly shorter. Eventually, they become so short that the cell can no longer divide successfully, and the cell dies.

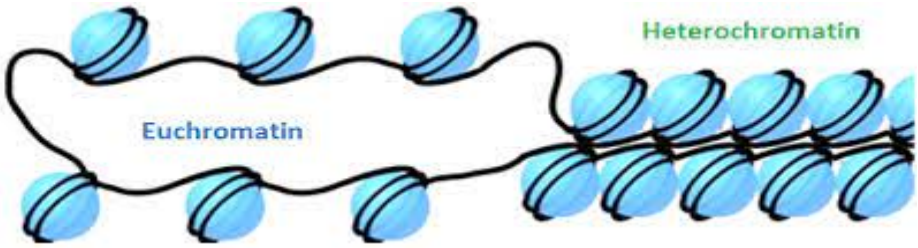
White blood cells and other cell types with the capacity to divide very frequently have a special enzyme that prevents their chromosomes from losing their telomeres. Because they retain their telomeres, such cells generally live longer than other cells.

Telomeres also play a role in cancer. The chromosomes of malignant cells usually do not lose their telomeres, helping to fuel the uncontrolled growth that makes cancer so devastating. (fig.7)



Material of Chromosome:

The material of the chromosomes is the chromatin, Depending on their staining properties, the following two types of chromatin may be distinguished in the interphase nucleus : (fig.8)



1. Euchromatin. Portions of chromosomes that stain lightly are only partially condensed; this chromatin is termed **euchromatin**. It represents most of the chromatin that disperse after mitosis has completed. Euchromatin contains structural genes which replicate and transcribe during G1 and S phase of interphase. The euchromatin is considered genetically active chromatin, since it has a role in the phenotype expression of the genes. In euchromatin, DNA is found packed in 3 to 8 nm fibre**.**

2. Heterochromatin. In the dark-staining regions, the chromatin remains in the condensed state and is called **heterochromatin**. In 1928, **Heitz** defined heterochromatin as those regions of the chromosome that remain condensed during interphase and early prophase and form the so-called **chromocentre**. Heterochromatin is characterized by its especially high content of repititive DNA sequences and contains very few, if any, structural genes**,** It is **late replicating** and is not transcribed.

Types of heterochromatin

Heterochromatin has been further classified into the following types :

1. Constitutive heterochromatin.

In such a heterochromatin the DNA is permanently inactive and remains in the condensed state throughout the cell cycle. This most common type of heterochromatin occurs around the centromere, in the telomeres and in the C-bands of the chromosomes.

2. Facultative heterochromatin.

Such type of heterochromatin is not permanently maintained in the condensed state ; instead it undergoes periodic dispersal and during these times is transcriptionally active. Frequently, in facultative heterochromatin one chromosome of the pair becomes either totally or partially heterochromatic. The best known case is that of the X-chromosomes in the mammalian female, one of which is active and remains euchromatic, whereas the other is inactive and forms at interphase, the **sex chromatin** or **Barr body.**

Karyotype Analysis and Chromosome Banding

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Chromosome analysis or karyotyping is a test that evaluate the number and structure of a person's chromosomes in order to detect abnormalities.

Chromosome banding is an essential technique used in chromosome karyotyping to identify normal and abnormal chromosomes for clinical and research purposes.

Methods of Chromosome Banding

Nearly all methods of chromosome banding rely on harvesting chromosomes in mitosis. This is usually achieved by treating cells with tubulin inhibitors, such as colchicine or colcemid, that depolymerize the mitotic spindle and so arrest the cell at this stage.

Chromosome banding methods are either based on staining chromosomes with a dye or on assaying for a particular function, there are available in various forms such as: G-band or (Giemsa), R-(reverse), C-(centromere) and Q-(quinacrine) banding. There are 2 types of bands observed:

1. Positive G band.
2. Negative G band.

Bands that show strong staining are referred to as positive bands; weakly staining bands are negative bands. G- positive bands likewise for R positive bands, C-bands contain constitutive heterochromatin. Q-bands are considered equivalent to G bands.

Uses of Chromosome Banding

G- and R –banding are the most commonly used techniques for chromosome identification (karyotyping) and for identifying abnormalities of chromosome number, translocations of material from one chromosome to another, deletions , inversions or amplifications of chromosome segments.

The detection of chromosome deletions associated with disorders, they may cause severe congenital anomalies and significant intellectual and physical disability. Similarly, translocations have been important in pinpointing the location of disease-associated genes and the characteristic translocations associated with some leukemia is important, not only for understanding the molecular basis of these cancers, but also for their diagnosis and prognosis.

Number and Size of Bands:

Idealized diagrams (ideograms) of G-banded chromosomes are published as standard reference points for chromosome banding. The G-bands are usually portrayed in black and the R-bands in white. Bands are numbered consecutively away from the centromere on both the short (p) and long (q) arms.

The total number of bands or 'resolution' in the human karyotype depends on how condensed the chromosomes are, and at what stage of mitosis they are in.

When a low-resolution band is subdivided, the number of each sub-band is placed behind a decimal point following the first band designation. For example the most distal low-resolution band on the short arm of human chromosome 11 (11p15) can be **subdivided** into bands 11p15.1, 11p15.2, 11p15.3, 11p15.4 and 11p15.5 at higher resolution.

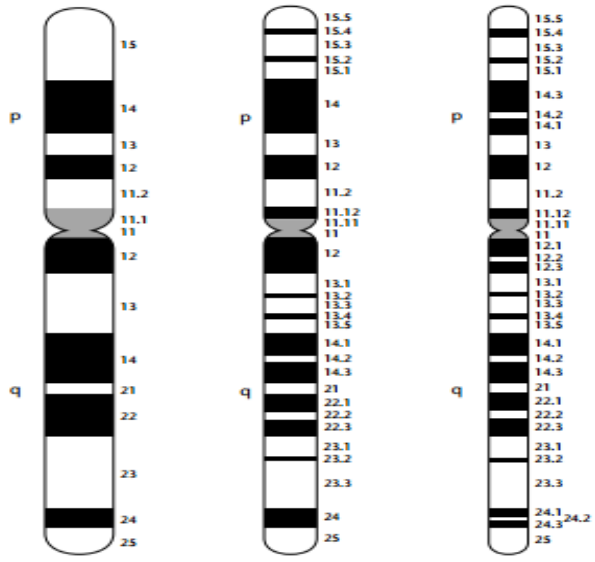

Fig (1): G-band ideograms of human chromosome 11 at (from left to right) 350, 550 and 850 band resolution.

Basis for G-/R-banding

G-banding involves staining protease-treated chromosomes with Giemsa dye and is thought to result from interactions of both DNA and protein with the thiazine and eosin components of the stain. The most common R-banding method involves heat denaturing chromosomes in hot acidic saline followed by Giemsa staining. This method is thought to preferentially denature AT-rich DNA and to stain the under-denatured GC-rich regions. T-banding identifies a subset of R-bands – the most intensely staining ones – by employing either a more severe heat treatment than R-banding. It is thought to identify the GC-richest R-bands, of which approximately half occur at telomeres in the human genome, hence the name.

Chromosomal Mutation

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The chromosomes of each species has a characteristic morphology (structure) and number. But, sometimes due to certain accidents or irregularities at the time of cell division, crossing over or fertilization, some alterations in the morphology and number of chromosomes take place. The changes in the genome involving chromosome parts, whole chromosomes, or whole chromosome sets are called **chromosome mutations**.

Chromosome mutation can occur in tow forms:

1- Structural change in chromosome.
2- Chang in number of chromosome.

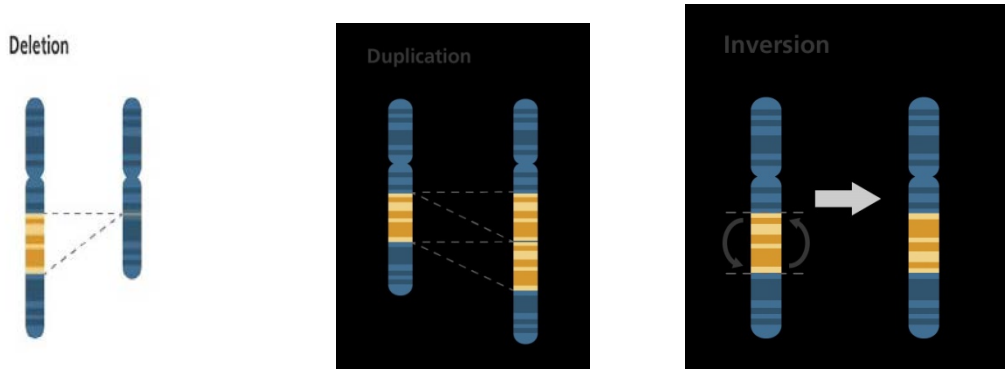
Both types of changes (structural and numerical) in chromosomes can be detected not only with a microscope (**cytologically**) but also by standard genetic analysis.

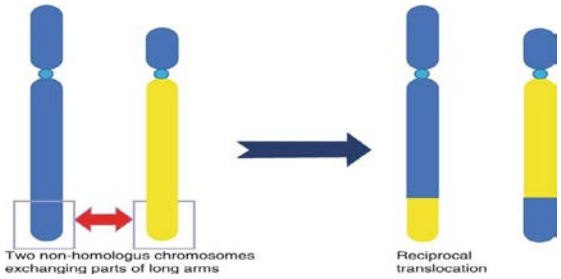
1- STRUCTURAL CHANGES IN CHROMOSOMES:

structural changes usually involve chromosome breakage; the broken chromosome ends are highly “reactive” or “sticky”, showing strong tendency to join with broken ends.

Structural changes in chromosome may be of the following types:

1- **deficiency** or **deletion** which involves loss of a broken part of a chromosome.
2- **duplication** involves addition of a part of chromosome (i.e., broken segment becomes attached to a homolog which, thus, bears one block of genes in duplicate).
3- **-inversion** in which broken segment reattached to original chromosome in reverse order, and
4- **translocation** in which the broken segment becomes attached to a non-homologous chromosome resulting in new linkage relations.



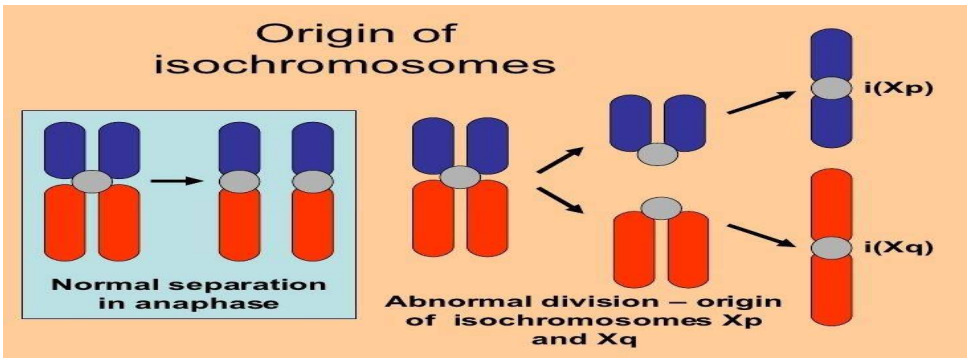


Variation in Chromosome Morphology:

Various changes in chromosome structure often produce variation in chromosome morphology such as: iso chromosomes, ring chromosomes and Robertsonian translocation.

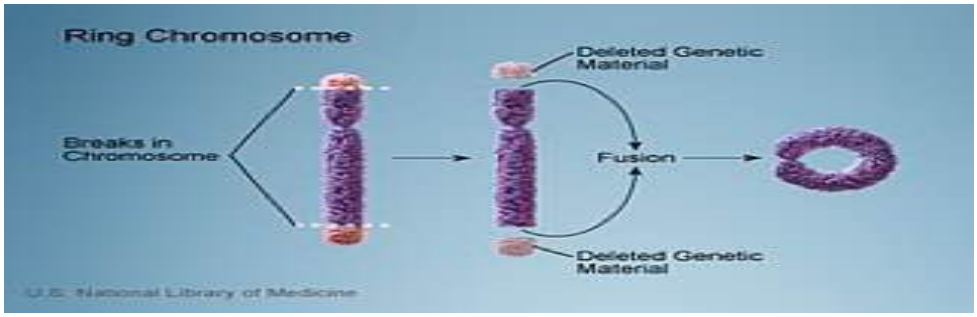
1. **Iso chromosomes.**

   An iso chromosome is a chromosome in which both arms are identical. It is thought to arise when a centromere divides in the wrong plane, yielding two daughter chromosomes, each of which carries the information of one arm only but present twice.



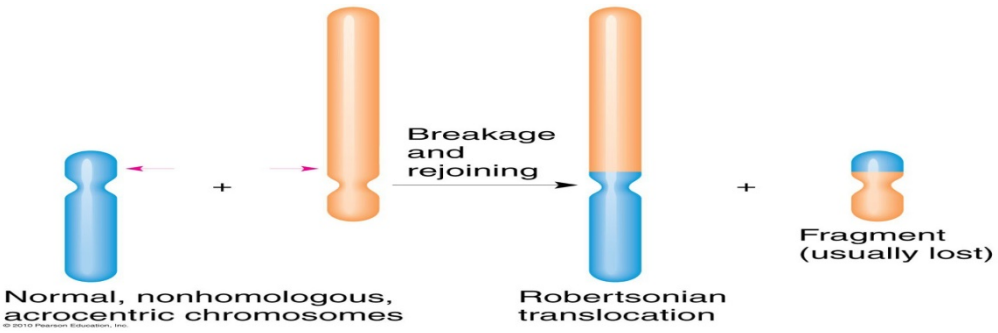
2. Ring chromosomes.

Chromosomes are not always rod-shaped. Occasionally ring chromosomes are encountered in higher organisms. Sometimes breaks occur at each end of the chromosome and broken ends are joined to form a ring chromosome. Crossing over between ring chromosomes can lead to bizarre anaphase.



3. Robertsonian translocation.

Thus, Robertsonian translocation is an eucentric reciprocal translocation where the break in one chromosome is near the front of the centromere and the break in the other chromosomes is immediately behind its centromere. The resultant smaller chromosome consists of largely inert heterochromatic material near the centromere; it normally contains no essential genes and tends to become lost. Thus, Robertsonian translocation results in a reduction of the chromosome number.



2- Changes in Chromosomes Number

Each species has a characteristic number of chromosomes in the nuclei of its gametes and somatic cells. The gametic chromosome number constitutes a basic set of chromosomes called **genome**. A gamete cell contains single genome and is called **haploid**. When haploid gametes of both sexes (male and female) unite in the process of fertilization a **diploid** zygote with two genomes is formed.

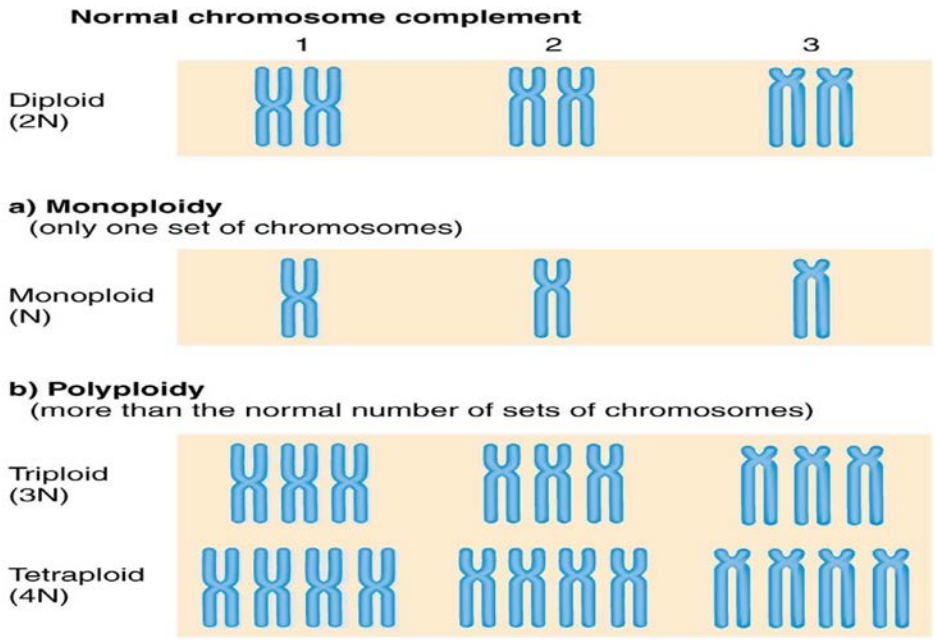
However, sometimes irregularities occur in nuclear division and causes Changes in number of whole chromosomes is called **heteroploidy** Heteroploidy may involve entire sets of chromosomes **(euploidy**), or loss or addition of single whole chromosomes (**aneuploidy**). Each may produce phenotypic changes.

A. EUPLOIDY: Changes in complete sets of chromosomes **:**

1- Monoploid: One set of chromosomes (1N) is characteristically found in the nuclei of some lower organisms such as fungi. Monoploids in higher organisms are usually smaller and less vigorous than the normal diploids. Few monoploid animals survive. A notable exception exists in male bees and wasps. Monoploidy is common in plant and rare in animals.

2- Triploid : Three sets of chromosomes (3N) can originate by the union of a monoploid gamete (1N) with a diploid gamete (2N). The extra set of chromosomes of the triploid is distributed in various combinations to the germ cells, resulting in genetically unbalanced gametes. Because of the sterility that characterizes triploids, they are not commonly found in natural populations.

3- Tetraploid : Four sets of chromosomes (4N) can arise in body cells by the somatic doubling of the chromosome number. Doubling is accomplished either spontaneously or it can be induced in high frequency by exposure to chemicals such as the alkaloid colchicine. Tetraploids are also produced by the union of unreduced diploid (2rt) gametes.



Phenotypic Effects of Polyploidy:

The increase in the genome's size beyond the diploid level is often caused following detectable phenotypic characteristics in a polyploid organism :

1- **Morphological effect of polyploidy.** The polyploidy is invariably related with **gigantism**.
2- **Physiological effect of polyploidy.** The ascorbic acid content has been reported to be higher in tetraploid cabbages and tomatoes than in corresponding diploids.
3- **Effect on fertility of polyploidy.** The most important effect of polyploidy is that it reduces the fertility of polyploid plants in variable degrees.
4- **Evolution through polyploidy.** Interspecific hybridization combined with polyploidy offers a mechanism whereby new species may arise suddenly in natural populations.

Polyploidy in humans have been found in liver cells and cancer cells. In them polyploidy is whether complete or as a mosaic, it leads to gross abnormalities and death.

B. ANEUPLOIDY:

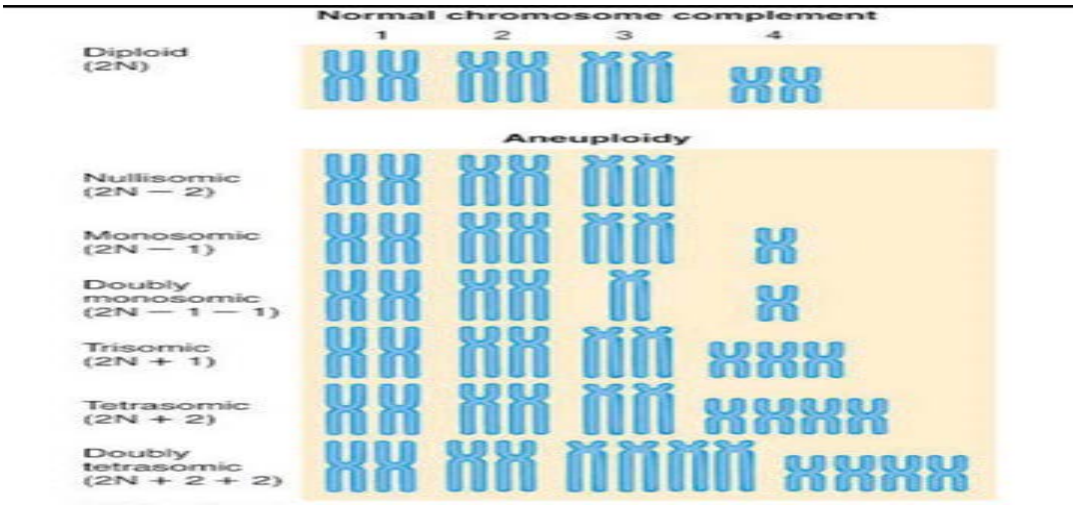
Variations in chromosome number may occur that do not involve whole sets of chromosomes, but only parts of a set.

1- Monosomic: Diploid organisms that are missing one chromosome of a single pair are monosomics with the genomic formula 2n - I. The single chromosome without a pairing partner may go to either pole during meiosis, but more frequently will lag at anaphase and fails to be included in either nucleus. In animals, loss of one whole chromosome often results in genetic unbalance. which is manifested by high mortality or reduced fertility.

2- Trisomic: Diploids which have one extra chromosome are represented by the chromosomal formula In N + 1 . One of the pairs of chromosomes has an extra member, so that a trivalent structure may be formed during meiotic prophase. If 2 chromosomes of the trivalent go to one pole and the third goes to the opposite pole, then gametes will be in + 1.

3- Tetrasotnic: When one chromosome of an otherwise diploid organism is present in quadruplicate, this is expressed as 2« + 2. A quadrivalent may form for this particular chromosome during meiosis which then has the same problem as that discussed for autotetraploids.

4- Nullisomy: An organism which has lost a chromosome pair is a nullosomic. The nullosomic organism has the genomic formula (2n–2). A nullosomic diploid often does not survive, however, a nullosomic polyploid (e.g., hexaploid wheat, 6x–2) may survive but exhibit reduced vigor and fertility.



Lecture 6

Mechanism of Prokaryotic Replication

DNA Replication

DNA replication in prokaryotes includes three stages :

- **initiation** – replication begins at an **origin of replication**
- **elongation** – new strands of DNA are synthesized by **DNA polymerase**
- **termination** – replication is terminated differently in prokaryotes and eukaryotes

Prokaryotic DNA Replication

The chromosome of most prokaryote is a circular molecule of DNA. Replication begins at a specific site in the DNA called the **origin of replication** (**ori**) there is one **origin of replication in prokaryotes** and proceeds in both directions around the chromosome.

- origins of replications usually contain 245 bp and are rich in Adenine and Thymine . DNA replication is **bidirectional** from the origin of replication .DNA replication occurs in both directions from the origin of replication in the circular DNA found in most bacteria.

DNA Replication in Bacteria

Initiation of replication :In *E.coli* replication initiated from the origin of replication which consists of two type of sequences ,three repeats of 13 bp called as a 13 mer and five repeats of 9bp called 9 mer. A Few proteins play important role in the DNA Replication:

DnaA- It recognizes oriC sequences for initiation of replication .

DnaB –Unwind DNA it is actually a helicase

DnaC- it helps helicase DnaB to recognize the site of its action

DNA Replication in Bacteria

DNA G- Its actually a primase ,it synthesizes anew RNA primer
.

DNA gyrase – it is DNA Topoisomerase II which helps in the unwinding of DNA . Higher concentration of DnaA signals to start cell division that bind to 9mer sequences of oriC with ATP ,This binding allows the opening of 13mer sequences of OriC by bending the DNA ,DnaB then bind to 13mer repeats which are recognize by DnaC and the activity of helicase (DnaB)generate tension on the remaining double strand of DNA then DNA gyrase helps in unwinding of DNA and releases the tension on the DNA strands by negative supercoiling the opening of DNA strands creates a replication fork that had Y shaped structure

DNA Replication in Bacteria

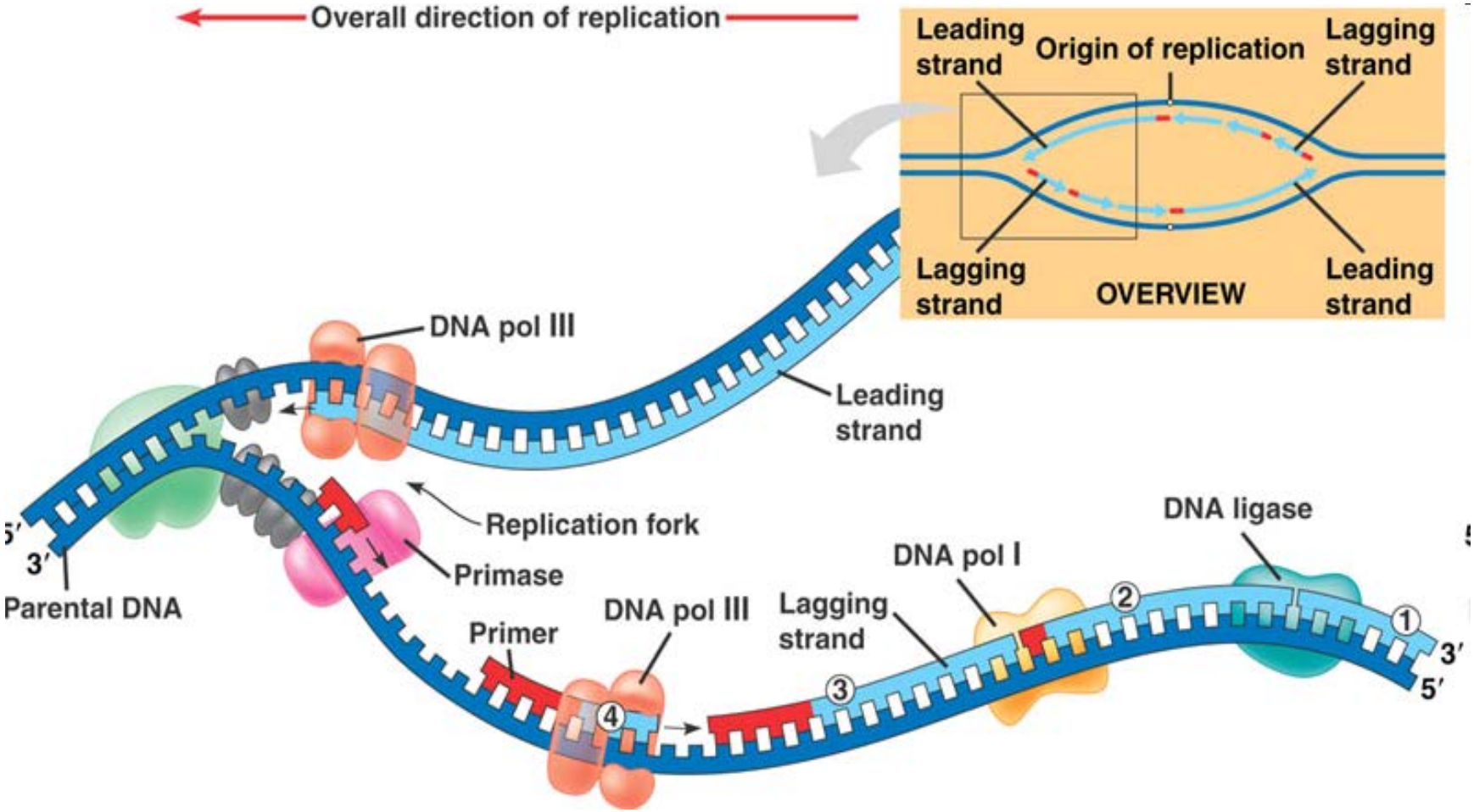
- When helicase unwinds DNA ,SSBP ,Single strand binding protein binding the single strands of DNA and prevent them from rejoining and from the attack of nucleases enzymes.

Elongation of Replication – Before of the strat of elongation , Primase synthesized a short 10bp long RNA Primer .the DNA Polymerase III can not able to add nucleotide without an RNA Primer because it provides a start site as free 3-OH group for the polymerase to work this enzyme add new nucleotides to the 3'-end of the primer by 5'- OH attachment to form 3'-5 phosphodiester bonds this enzyme can not work without the exist of free 3'-OH of the primer DNA polymerase III can't initiated new strand with out the existing of RNA primer but primase or RNA Polymerase can initiated a new strand.

DNA Replication in Bacteria

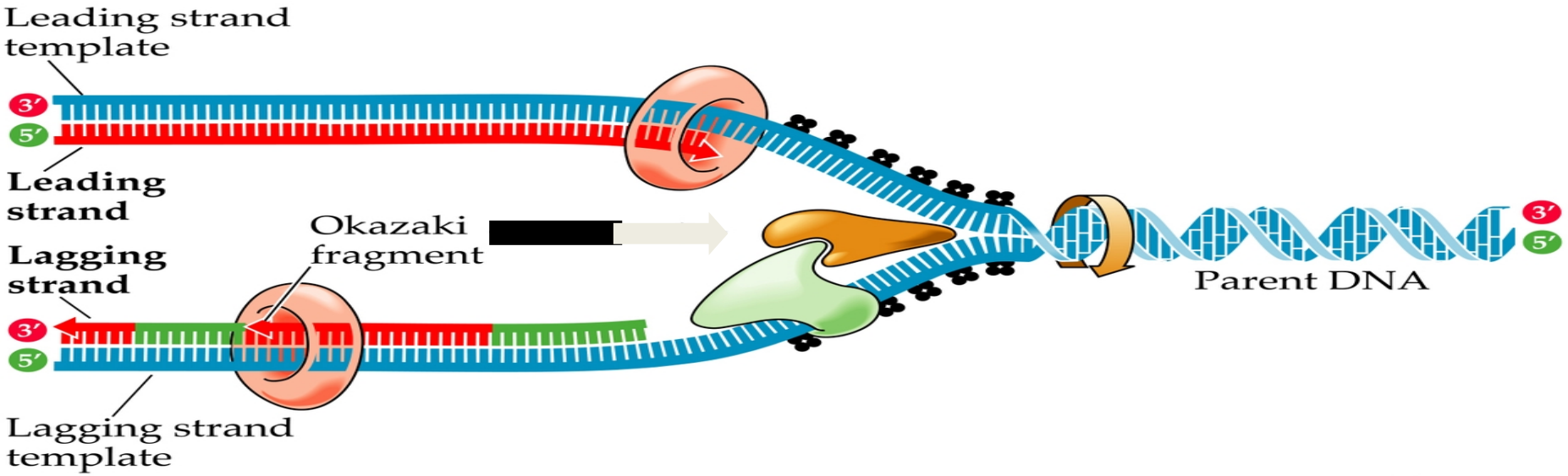
- Three different type of DNA polymerases had been exist .
- **DNA Polymerase I** –that remove RNA primer by exonucleases activity and it is very important enzyme in the proof reading in the DND Replication .
- **DNA Polymerase II-** It is very important enzyme in DNA repair.
- **DNA Polymerase III-** Add nucleotides from 5’-to 3’direction

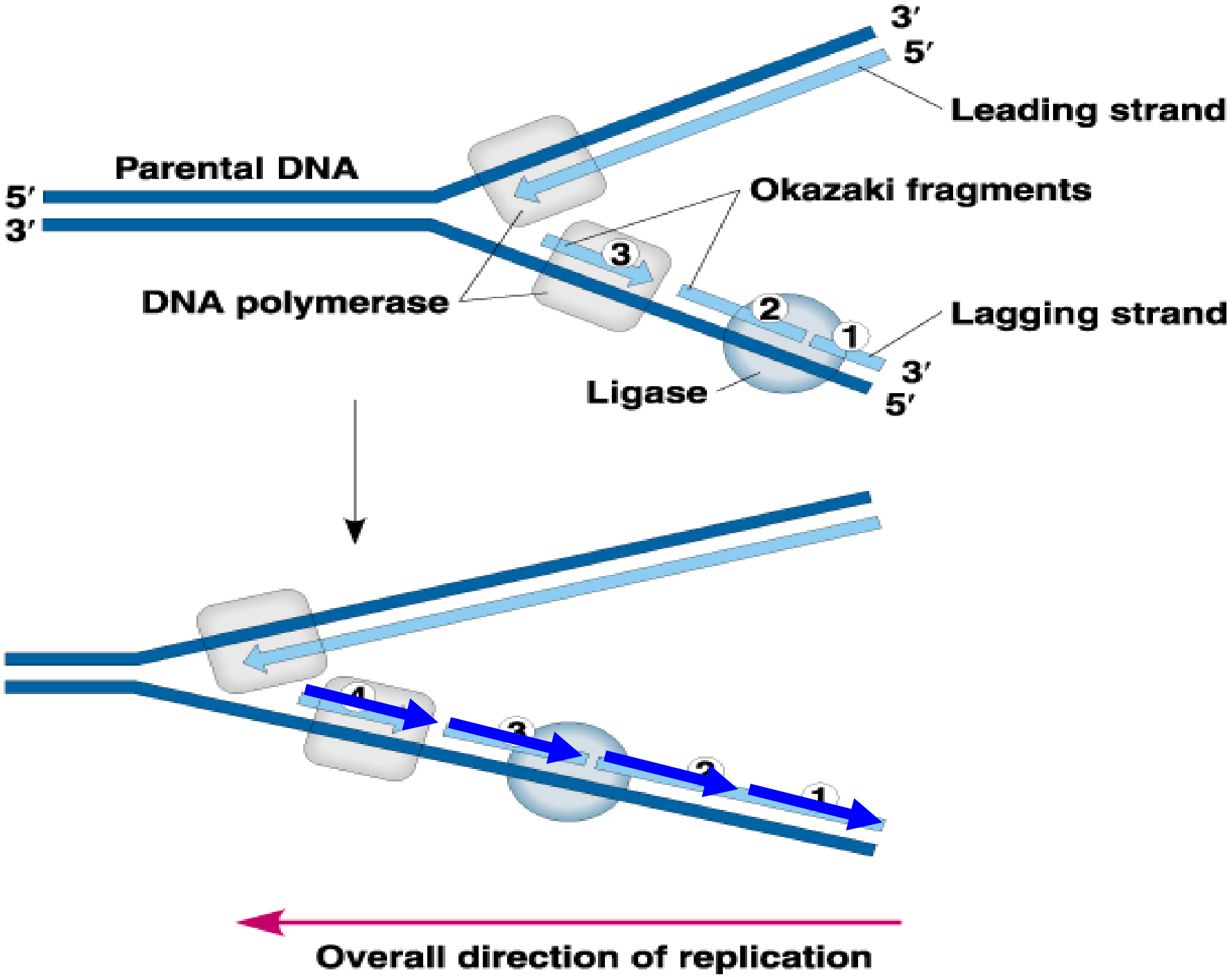
Replication Fork



The Mechanism of DNA Replication

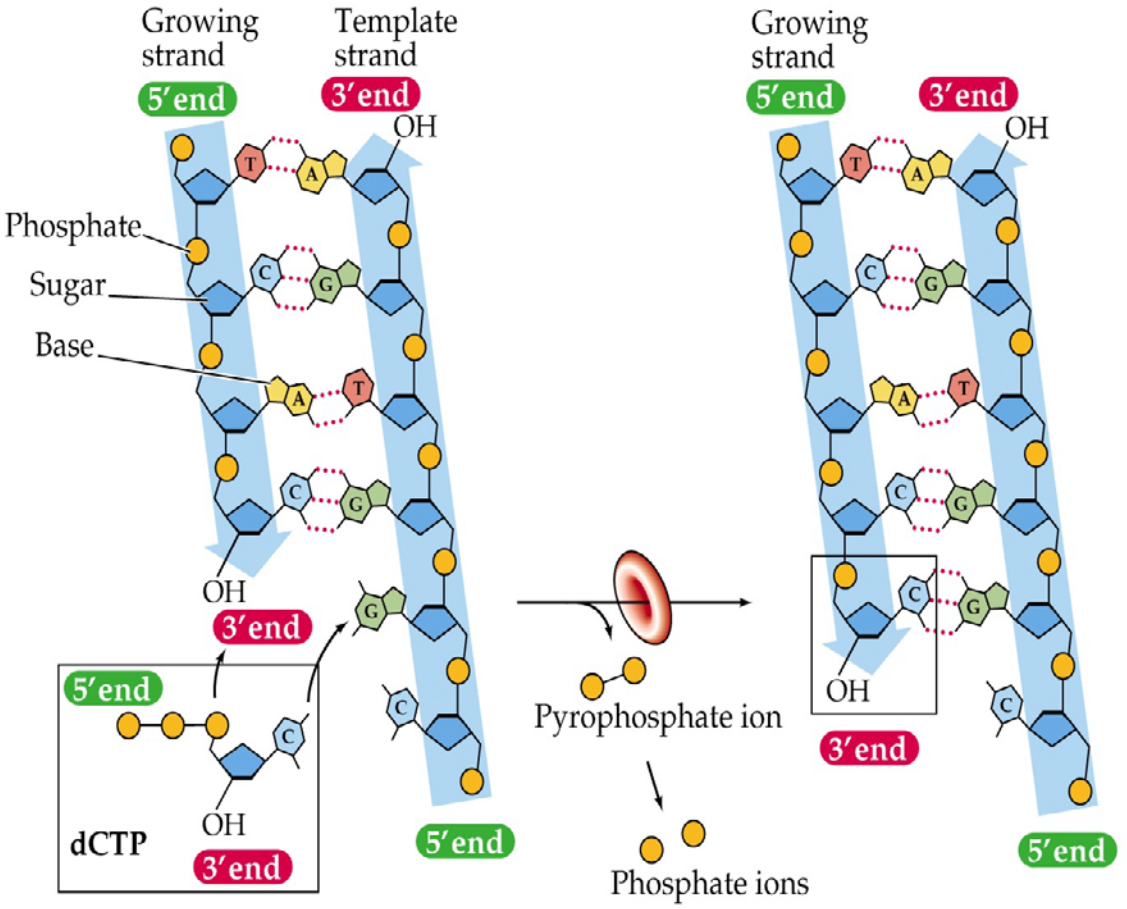
- DNA synthesis occurred in the 5’-to 3’ direction after the separation of the two DNA strands one strand in the direction of replication while the others in the opposite direction of replication. Replication of the strand that its direction in the opposite direction of replication 3’-----5’ occurred continuously and called leading strand while the other that its direction 5’------3’its replication in the opposite direction of replication then its replicated discontinuously and called lagging strand
- the leading strand is continuous
- The lagging strand grows the same *general* direction as the leading strand (in the same direction as the Replication Fork). However, DNA is made in the 5’-to-3’ direction




3′
5′
Leading strand
Parental DNA
5′
3′
Okazaki fragments
3
DNA polymerase
2
1
Lagging strand
3′
5′
Ligase
4
3
2
1
Overall direction of replication

Mechanism of DNA Replication

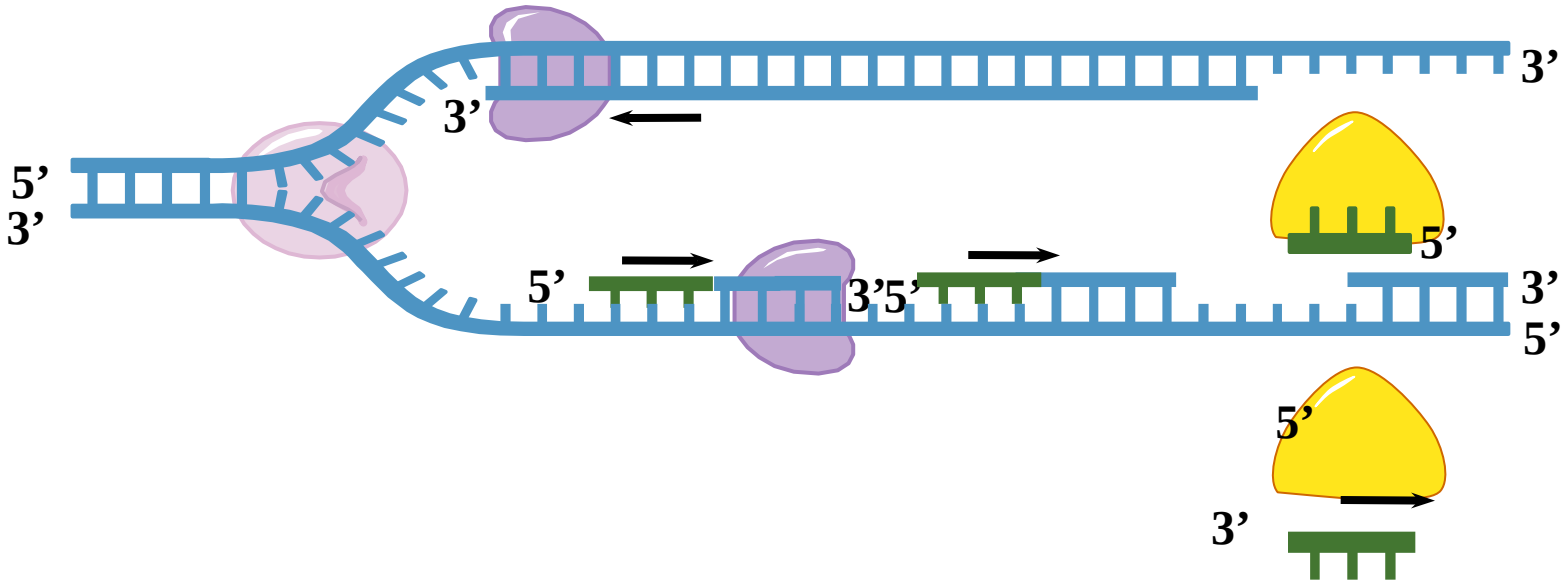
- Nucleotides are added by complementary base pairing with the template strand
- The substrates, deoxyribonucleoside triphosphates, are hydrolyzed as added, releasing energy for DNA synthesis.



DNA Replication in Bacteria

- The nucleotides lining up by complementary base pairing are deoxynucleosid triphosphates
- As the phospho-diester bond forms between the 5' phosphate group of the new nucleotide and the 3' OH of the last nucleotide in the DNA strand, two of the phosphates are removed providing energy for bonding .then nucleotides must be triphosphate but in the replication process it attached as nucleotide mono-phosphate the two other phosphates removed and the energy that released utilized to synthesize new bond 3-5phosphodiester bonds

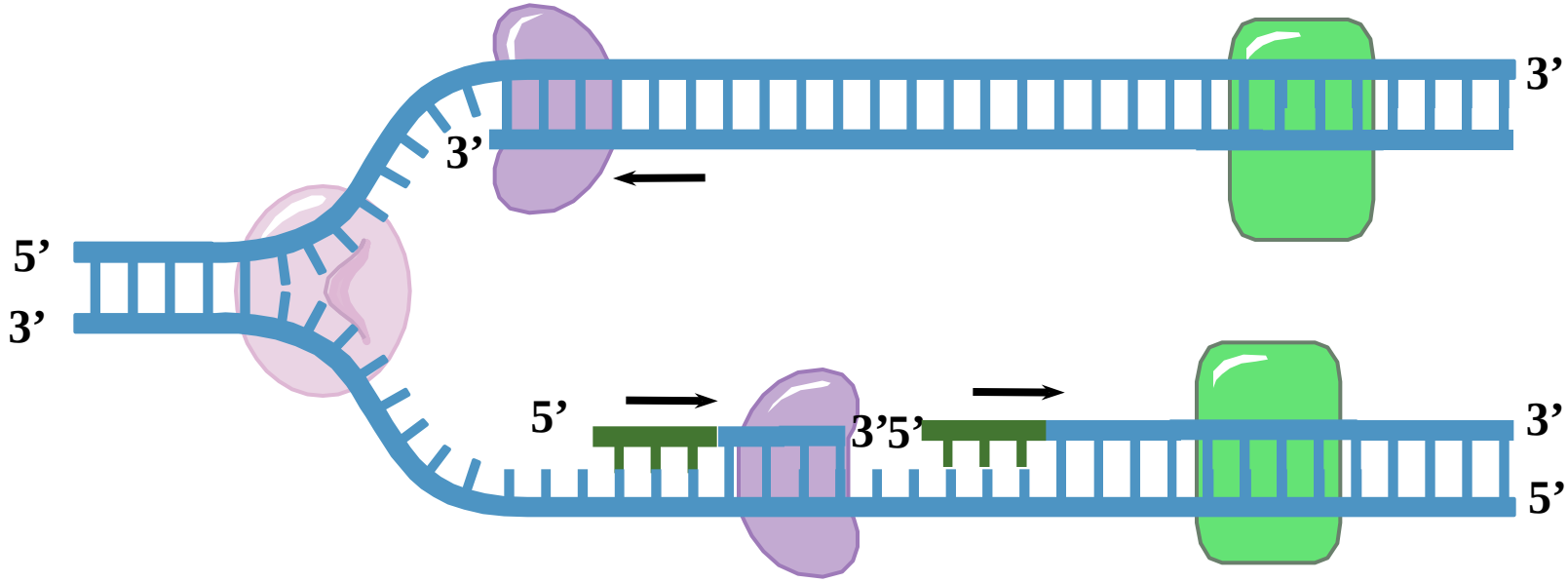
Replication



After the new DNA strand had been synthesized continuously in the leading strand and discontinuously in the lagging strand

Exonuclease activity of DNA polymerase I removes RNA primers by hydrolyzing one ribonucleotide and adding deoxy nucleotide instead until removal of the primer strand then the gap between the two okazaki fragment had been sealed of by the activity of ligase enzyme that is very important enzyme in the replication process

Replication



Polymerase activity of DNA polymerase I fills the gaps.
Ligase forms bonds between sugar-phosphate backbone.

Enzymes of Prokaryotic DNA Replication

The double helix is unwound by the enzymes

DNA gyrase- , DNA topoisomerase,removed the supercoiling of the double helix

helicase --,break the hydrogen bonds between the two strand of the DNA

SSBP (single stranded binding protein) helps keep strands separated

Primase ;Required for RNA primer synthesis which very important in the beginning of DNA replication

DNA polymerase III (pol III) is responsible for most of DNA synthesis

- adds nucleotides to the 3’ end of the daughter strand of DNA; DNA synthesis is from 5' to 3‘
- **Ligase** ; is very important in dna replication and is responsible for the attachment of Okasaki fragment after removing of the primer and filled the gap by DNA poly merase1 this enzyme is responsible for phosphodiester bond formation between nucleotides

DNA polymerase 1 (Exonuclease) removes RNA primer and inserts the correct **bases beside this enzyme**

- involved in proofreading and DNA repair

Proofreading

- DNA must be faithfully replicated…but mistakes occur
  - DNA polymerase111 (DNA pol) inserts the wrong nucleotide base in 1/10,000 bases
    - DNA pol1 has a proofreading capability and can correct errors
  - Mismatch repair: ‘wrong’ inserted base can be removed
  - Excision repair: DNA may be damaged by chemicals, radiation, etc. Mechanism to cut out and replace with correct bases

Termination of replication

During replication 1000 nucleotides are added per second ,the leading strand DNA synthesis is very striaght-forewored and beginning with the formation of one primer of RNA strand by RNA polymerase and other complex protein which called Primosome then DNA Polymerase III add nucleotides till the termination sequences . Termination sequences are unique conserved sequences which are recognized by polymerases enzymes at the end of replication ,while the lagging strand synthesis is little different from the leading strand here primase synthesized primers many time and DNA polymerase III added deoxy nucleotides to the 3’-OH end of the primers then DNA-polymerase I removed these primers and add deoxy nucleotides instead then ligase sealed the gaps between these okazaki fragments after the synthesis of leading and lagging strand the polymerase is detached from the site of replication

Termination of replication

At the last stage of termination two replication fork meet at terminator recognizing sequences called

Ter sequences , which is a specific sequences that attached to specific protein that called TUS protein that create a complex which arrests the replication process and prevents other cycle of replication to occurred at this complex the process of replication is completed and all other proteins and enzymes leave this site except DNA Polymerase II that remain in action .It cuts both strands dissociates Ter-TUS complex and two different circular DNA is generated