NUCLEIC ACID STRUCTURE AND FUNCTION

Introduction

Although genes are composed of DNA, DNA is for the most part an information storage molecule. That information is released through the process of gene expression (namely transcription, RNA processing, and translation).

The process of converting the information contained in a DNA segment into proteins begins with the synthesis of mRNA molecules containing anywhere from several hundred to several thousand ribonucleotides, depending on the size of the protein to be made.

Genes are typically thought of as encoding RNAs that in turn produce proteins, but some RNAs are functional themselves (e.g. rRNA, tRNA, snRNAs, microRNA); thus some genes only encode RNAs, not proteins.

The transcribed strand of DNA is sometimes called the positive, plus, or sense strand. The template strand for the mRNA is sometimes called the negative, minus, or antisense strand.

Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) is the material of which genes are made. This had not been widely accepted until 1953 when J.D. Watson and F.H, Crick proposed a structure for DNA which accounted for its ability to self-replicate and to direct the synthesis of proteins. All living cells (both prokaryotic and eukaryotic) contain double stranded DNA as their genetic material.

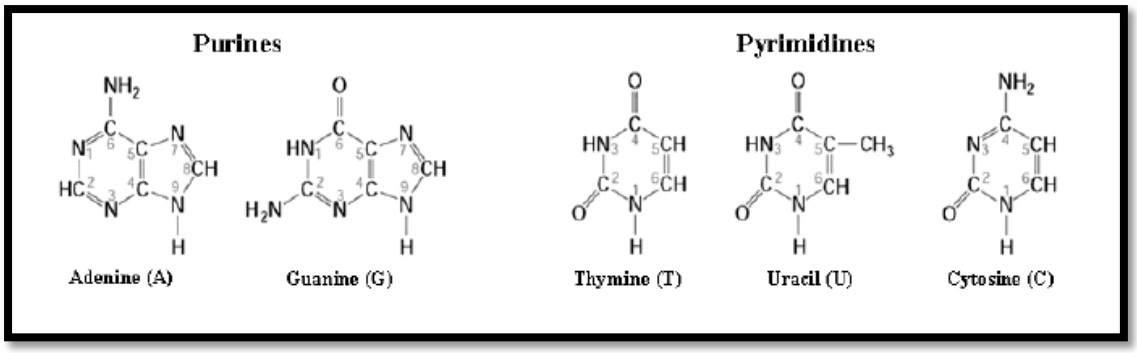
DNA is composed of a series of polymerized nucleotides, joined by phosphodiester bonds between the 5' and 3' carbons of deoxyribose units. DNA forms a double helix with these strands, running in opposite orientations with respect to the 3' and 5' hydrozxy groups.

The double helix structure is stabilized by base pairing between the nucleotides, with adenine and thymine forming two hydrogen bonds, and cytosine and guanine forming three.

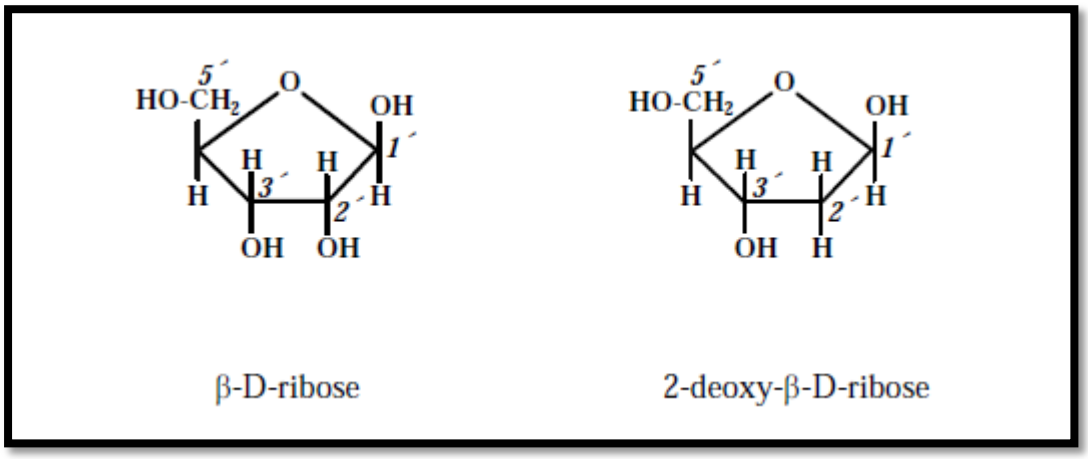
Base + Sugar = nucleoside

Base + Sugar + Phosphate Group = nucleotide

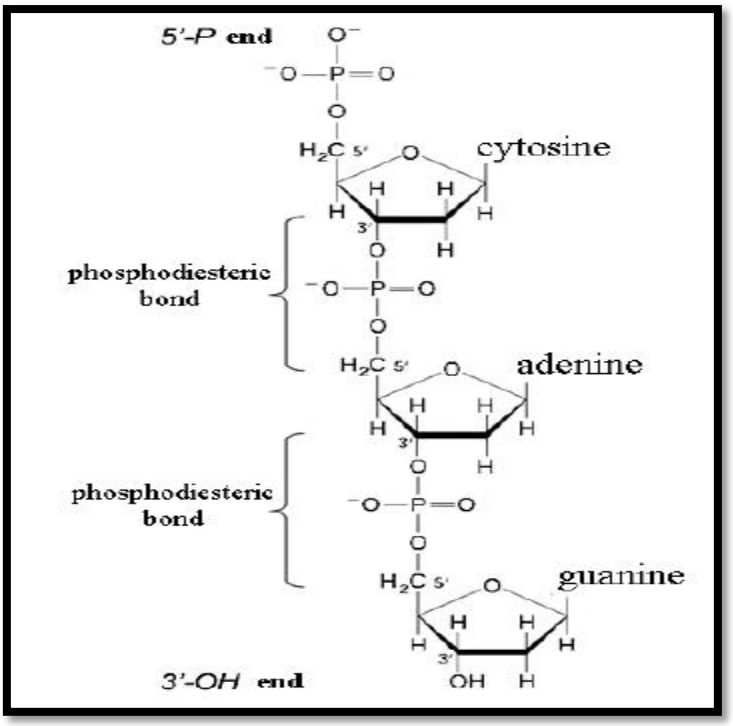
Attached to each sugar residue is one of the four essentially planar nitrogenic organic bases: Adenine A, Cytosine C, Guanine G, Thymine T, The plane of each base is essentially perpendicular to the helix axis. Encoded in the order of the bases along a strand is the hereditary information.



Linking any two sugar residues is an -O--P--O-, a phosphate bridge between the 3' carbon atom of one of the sugars and the 5' carbon atom of the other sugar. Note that in solution DNA is negatively charged due to the presence of the phosphate group.



Because deoxyribose has an asymmetric structure, the ends of each strand of a DNA fragment are different. At one end the terminal carbon atom in the backbone is the 5' carbon atom of the terminal sugar (the carbon atom that lies outside the planar portion of the sugar); and at the other end it is the 3' carbon atom (one that lies within the planar portion of the sugar).
DNA in a circular form is often supercoiled. Negatively supercoiled DNA is in a more compact shape than relaxed DNA and is partially unwound, facilitating interactions with enzymes such as polymerases.



Two enzymes work to maintain supercoiling in DNA:

1) Topoisomerases relax supercoiled DNA, and

2) DNA gyrase introduces supercoiling.

Topoisomerases

work by cleaving one or both strands of DNA, passing a segment of DNA through the break, and resealing the gap. The reaction to create supercoiled DNA requires an input in energy.

DNA gyrase

uses the hydrolysis of ATP as a source of free energy for the insertion of negative supercoils in DNA. DNA is wrapped around the enzyme, and both strands are cleaved when ATP binds to the complex. As with the topoisomerase depicted above, the 5' ends remains bound to specific tyrosine residues of the enzyme, important so that any supercoils which are already present won't be lost.

This activity is an important process; several antibiotics exert their effects on this system, inhibiting prokaryotic enzymes more than eukaryotic ones. Novobiocin blocks ATP binding to DNA gyrase, while nalidixic acid and ciproflaxin interfere with the cleavage and joining of the strands.

Ribonucleic acid

RNA is similar to DNA but differs in several respects.

1. It is shorter
2. It is single stranded (with few exception: few virus)

3. It is nuclear and cytoplasmic
4. It has ribose
5. It has uracil rather than thymine. The other bases are the same.

There are three basic types of RNA:

1. *Messenger RNA (mRNA):* relatively long strands that encode the information from a single gene (DNA). It is the template for protein synthesis**.** This is the product of transcription. An mRNA is an RNA that is translated into protein. mRNAs are very short-lived compared to DNA.

► In prokaryotic cells a primary transcript is used directly as an mRNA (often times before it is even completely transcribed).

► In eukaryotic cells a primary transcript is **processed** before being exported from the nucleus as an mRNA:

• A **5'CAP** of 7-methyl guanosine is added.

• A **poly (A) tail** is added to the 3' end of the transcript.

• **Introns** (intervening sequences) must be cut from the transcript by a process known as **RNA splicing**.

In prokaryotes

- They are only around for a few minutes.
- Continuous synthesis of protein requires a continuous synthesis of mRNA. This helps the prokaryotic cell respond quickly to a fluctuating environment and fluctuating needs.
- The mRNA of prokaryotic cells is **polycistronic** (one transcript can code for several different proteins).

In eukaryotic cells

- The mRNA are stable for 4-24 hrs.
- The mRNA of eukaryotic cells is **monocistronic** (each transcript only encodes a single protein)

2. *ribosomal RNA (rRNA)*: Ribosomes are composed of rRNA and protein. The rRNA forms base pairs with the nucleotides of mRNA during translation (protein synthesis).

3. *transfer RNA (tRNA):* short (90 nucleotides) RNA molecules responsible for translating nucleic acid language to protein language. In other words the "adapter" molecule that converts nucleic acid sequence to protein sequence.

Chemical differences between DNA & RNA

► Both RNA and DNA are composed of repeated units. The repeating units of RNA are ribonucleotide monophosphates and of DNA are 2'-deoxyribonucleotide monophosphates.

► Both RNA and DNA form long, unbranched polynucleotide chains in which different purine or pyrimidine bases are joined by N-glycosidic bonds to a repeating sugar-phosphate backbone.

► The chains have a polarity. The sequence of a nucleic acid is customarily read from 5' to 3'. For example the sequence of the RNA molecule is AUGC and of the DNA molecule is ATGC

► The base sequence carries the information, i.e. the sequence ATGC has different information that AGCT even though the same bases are involved.

Consequences of RNA/DNA chemistry

► The DNA backbone is more stable, especially to alkaline conditions. The 2' OH on the RNA forms 2'3'phosphodiester intermediates under basic conditions which breaks down to a mix of 2' and 3' nucleoside monophosphates. Therefore, the RNA polynucleotide is unstable.

► The 2' deoxyribose allows the sugar to assume a lower energy conformation in the backbone. This helps to increase the stability of DNA polynucleotides.

► Cytidine deamination to Uridine can be detected in DNA but not RNA because deamination of Cytidine in DNA leads to Uridine not Thymidine. Uridine bases in DNA are removed by a specific set of DNA repair enzymes and replaced with cytidine bases

► The role of DNA is long-term information storage. Thus DNA can be looked upon as a chemical information storage medium. All such media have certain common properties:

- The molecule must be able to carry information:
- The molecule must be able to hold information, without this property it is useless.
- The molecule must be readable:

- The information in the medium must be able to be used for some purpose. It is no use putting information into a storage medium if the information cannot be retrieved.
- The molecule must be stable and secure:
- The information storage medium must be passed from generation to generation. Thus the molecule must be able to remain essentially unchanged for many generations.

► The role of RNA is three-fold: as a structural molecule, as an information transfer molecule, as an information decoding molecule

► RNA molecules read and interpret the information in DNA. RNA molecules are key players in the reactions that turn information into useful work.

Plasmids

Besides the nucleoid (nuclear chromosome), the bacterial cells may contain in cytosol also other structures composed of DNA. They are called **plasmids**. They are extrachromosomal genetical elements (sc. episomes), which are made of a two-stranded circular DNA with a length of 2-15 kb. They replicate independently from the bacterial chromosome and often have ability for conjugation ("self" transport from one cell to another).

Each plasmid contains an origin of replication (ORI) and genes which ensure its own reproduction. They can also contain other sequences, as:

• genes for resistance against antibiotics and for synthesis of certain enzymes for example hyaluronidases etc., that give to the host bacteria selection advantages.

• restriction sites and selective markers, which allow the use of plasmids as vectors of genetic information.

Plasmids which contain the required genes, and are able to replicate in different host cells, are called „shuttle" vectors.

Plasmids were indentified also in some eukaryotic cells. A cryptic plasmid was found in yeast, which was made of a double stranded circular DNA, while in certain fungi and algae linear DNA plasmids were found.

CHROMOSOMES

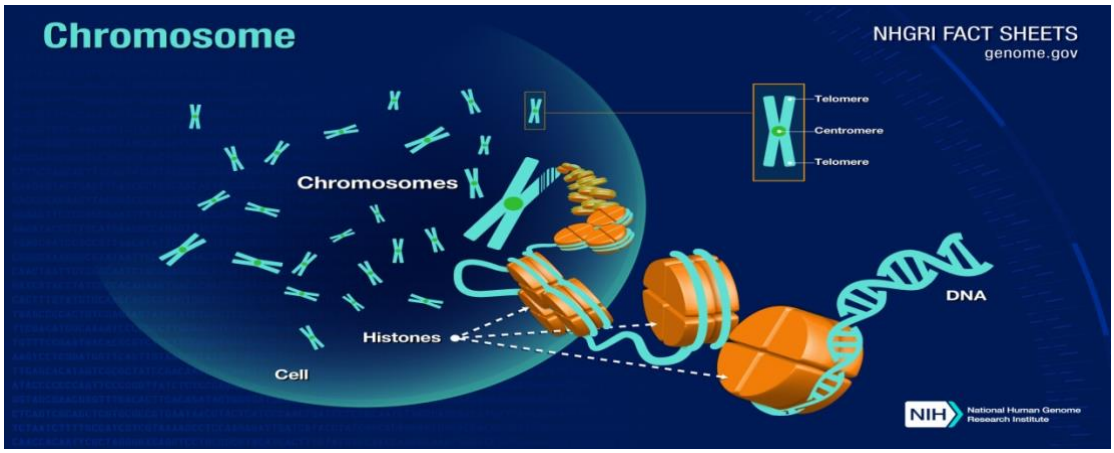
What is a Chromosome?

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)

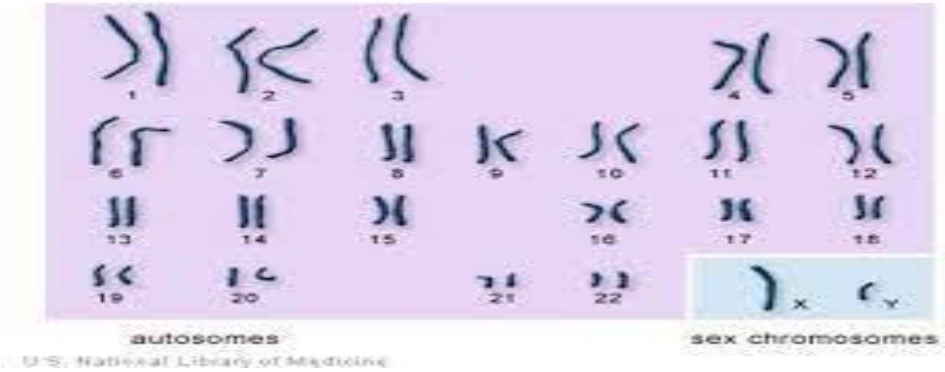


For an organism to grow and function properly, cells must constantly divide to produce new cells to replace old, worn-out cells. During cell division, it is essential that DNA remains intact and evenly distributed among cells. Chromosomes are a key part of the process that ensures DNA is accurately copied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

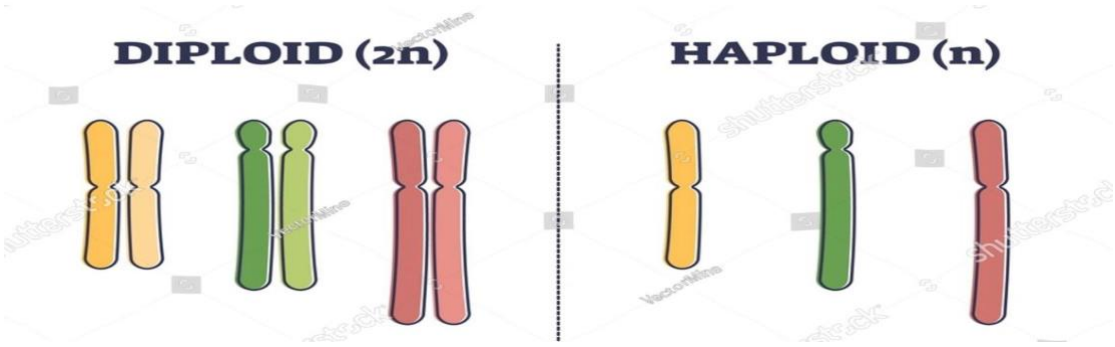


Besides the linear chromosomes found in the nucleus, the cells of humans and other complex organisms carry a much smaller type of chromosome similar to those seen in bacteria. This circular chromosome is found in mitochondria, which are structures located outside the nucleus that serve as the cell's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

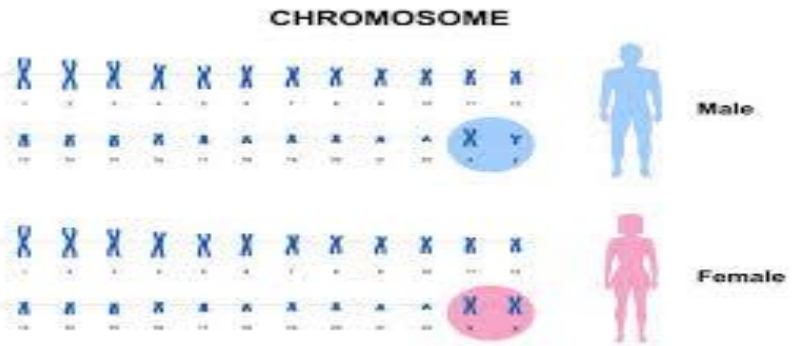
In humans and most other complex organisms, one copy of each chromosome is inherited from the female parent and the other from the male parent. This explains why children inherit some of their traits from their mother and others from their father.

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)

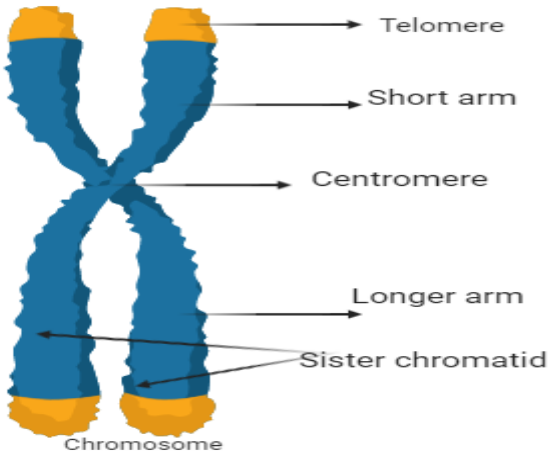


Shape of Chromosomes:

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)

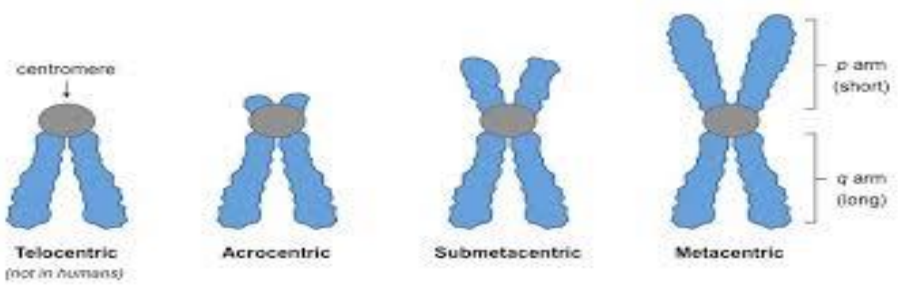


Centromeres help to keep chromosomes properly aligned during the complex process of cell division. As chromosomes are copied in preparation for production of a new cell, the centromere serves as an attachment site for the two halves of each replicated chromosome, known as sister chromatids.

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.

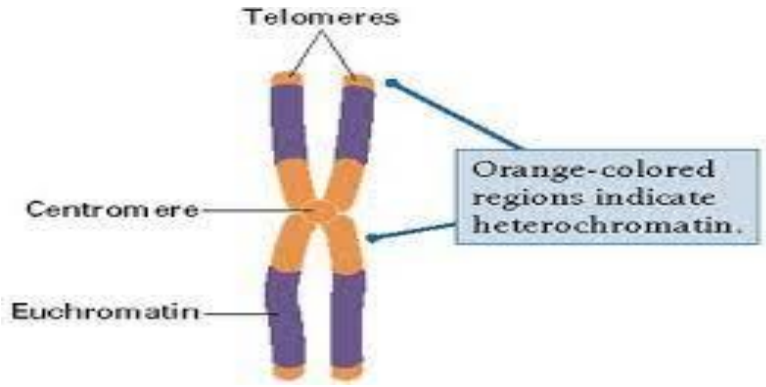
What are Telomere?

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.

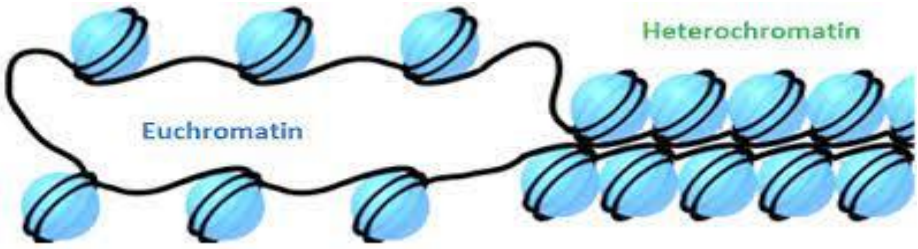
White blood cells and other cell types with the capacity to divide very frequently have a special enzyme that prevents their chromosomes from losing their telomeres. Because they retain their telomeres, such cells generally live longer than other cells.

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

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.

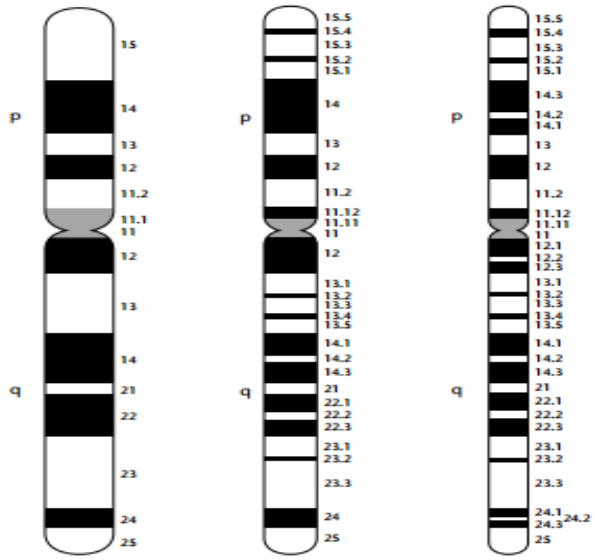


Fig (1): G-band ideograms of human chromosome 11 at (from left to right) 350, 550 and 850 band resolution.

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.

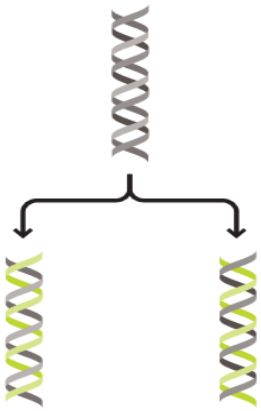
DNA REPLICATION

Introduction

Before cells divide, they must make a complete and faithful copy of the DNA in their chromosomes. This process of copying is called DNA replication. During replication, each strand of the DNA double helix is copied to make a new strand, thus producing two new daughter DNA double helices. Replication is a carefully process, requiring the activity of several important enzymes, each of whose role in DNA replication has been conserved across evolutionary time.

1- Each strand of the DNA double helix serves as a template for the synthesis of a complementary new strand.

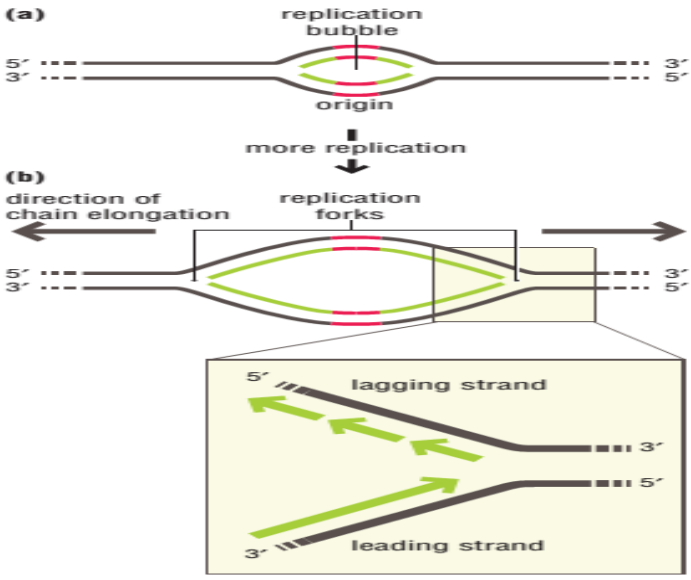
According to this basic mechanism of replication, both strands of the parental DNA molecule are copied during replication, each strand serving as a **template** for the synthesis of a new daughter strand. We call this process **semi-conservative**: when replication is complete, two daughter helices have been produced, each containing one old strand (which has been conserved from the parent) and one new strand.



The process of copying the template strand involves the recognition of a base in the template strand and the addition of the complementary base to the new daughter strand. This ensures that not only are the daughter molecules identical to the parent DNA, they are also identical to each other.

2- DNA synthesis occurs at replication forks that move outwards from origins of replication

In chromosomes, DNA replication starts at specialized sites called **origins** of replication and moves away from an origin in both directions, creating a structure known as a **replication bubble**. The DNA double helix is opened at the origins of replication and then unwound on both sides of the origin to form structures called **replication forks**.



The process of DNA replication can be divided into three consecutive phases: initiation, elongation, and termination.

3- Initiation of replication occurs at origins

During initiation, the first phase of DNA replication, the helix of the double stranded parent DNA is opened up in order to give replication enzymes and other proteins access to the single strands that will form the templates for the daughter strands that are to be synthesized. The origin is

recognized by a specific initiator protein that opens the double-stranded DNA and recruits a class of enzymes called helicases . **DNA helicases** act to unwind the double-stranded DNA to form the single-stranded templates required for DNA replication.

4- During elongation each base in the parent DNA strand is read by DNA polymerase to direct synthesis of a daughter strand in a 5 ! to 3 ! direction

After the replication machinery is in place, we enter the elongation phase of DNA replication. During this phase, the replication machinery moves along the parent DNA strands, copying the strands into daughter strands as it proceeds. The synthesis of a new DNA strand is catalyzed by an enzyme called **DNA polymerase III**. This has the very important consequence that DNA replication can only proceed in a **5 ′ to 3 ′** direction.

5- DNA replication starts with the synthesis of a short stretch of RNA

One unusual feature of DNA polymerase is that it cannot build a new DNA strand from the very start of the parent strand; it can only add nucleotides to the 3 ′ end of a nucleotide fragment that already exists. The nucleotide fragment on which the DNA polymerase builds its daughter strand is a short strand of RNA termed a primer, which provides the 3 ′ end the DNA polymerase requires.

The RNA primer is synthesized by a specialized polymerase called **primase**, To complete DNA replication, the RNA primer is degraded and replaced with DNA.

6- DNA synthesis is continuous on one strand and discontinuous on the other strand

The fact that DNA can only be synthesized in the 5 ′ to 3 ′ direction poses a problem at the replication fork: both strands of the DNA molecule must be copied at the same time but yet the two strands have opposite

orientations – one strand runs in the 5 ′ to 3 ′ direction called **Lading strand**, while its complementary strand runs in the 3 ′ to 5 ′ direction called **Lagging strand**. To solve this problem, DNA synthesis on the two strands is different. On one strand, synthesis is continuous: By contrast, on the other strand, synthesis occurs discontinuously: a series of short DNA fragments are synthesized from 5 ′ to 3 ′ called **Okazaki fragments**, which are then subsequently joined into a continuous strand by **Ligase**. Primer synthesis and polymerase loading occur once on the leading strand, but must occur repeatedly on the lagging strand.

7- Termination of replication occurs either when two forks meet or at the ends of linear chromosomes

Termination of DNA replication occurs when the two forks moving in opposite directions meet and the replication complexes are disassembled. After elongation by the replicative polymerases, the RNA primers are removed by **DNA Polymerase I** and the two adjacent newly synthesized DNA strands are connected by **DNA ligase** .

Replication in Eukaryotes

Eukaryotic DNA replication is clearly a much more complex process than bacterial DNA replication we discussed four aspects of eukaryotic DNA replication.

- In eukaryotes, the DNA molecules are larger than in prokaryotes and are not circular; there are also usually multiple sites for the initiation of replication.
- In E. coli chromosome forms only a single replication fork. In eukaryotes, these replicating forks, which are numerous all along the DNA, form "bubbles" in the DNA during replication.
- The replication fork forms at a specific point called autonomously replicating sequences (ARS). The ARS contains somewhat degenerate 11-bp sequences called the origin replication element

(ORE). The ORE is located adjacent to an 80-bp AT rich sequence that is easy to unwind

- Replication begins at some replication origins earlier in S phase than at others, but the process is completed for all by the end of S phase.

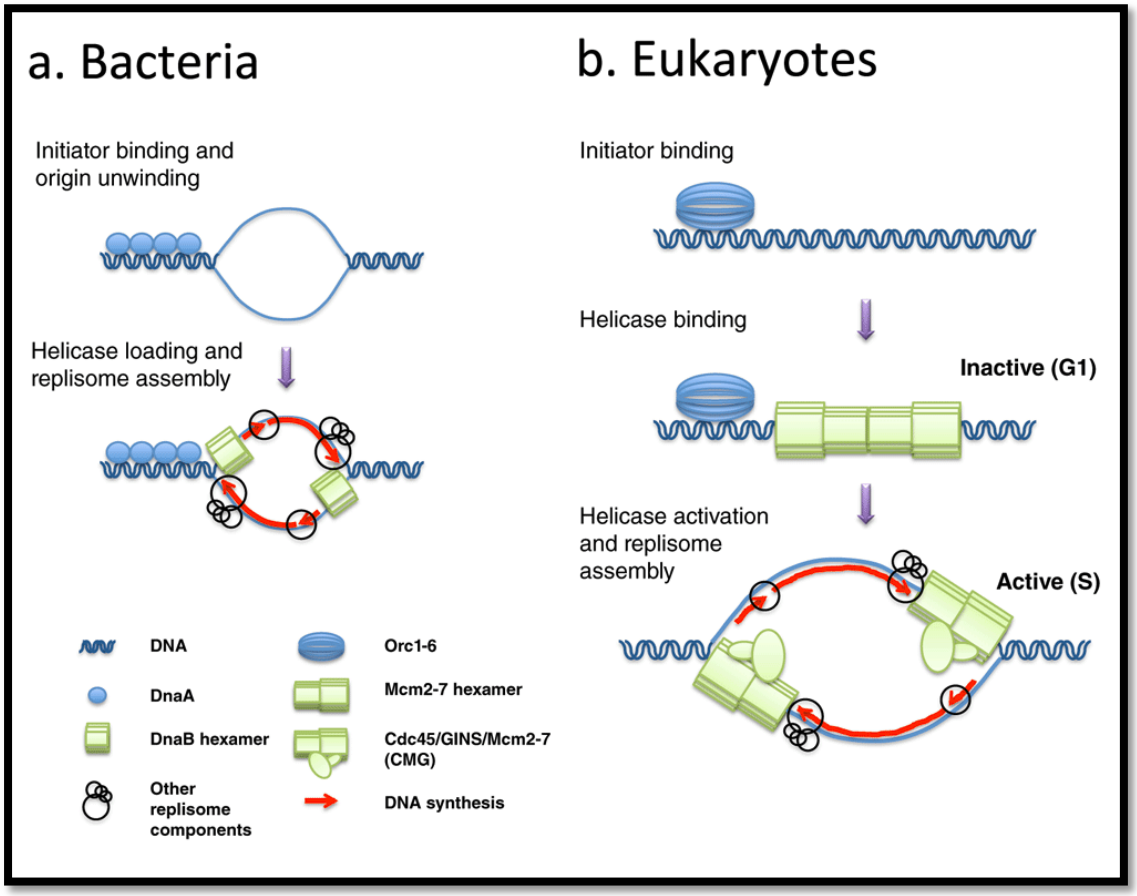
Regulating DNA Replication in Bacteria

The replication origin and the initiator protein DnaA are the main targets for regulation of chromosome replication in bacteria. The origin bears multiple DnaA binding sites, while DnaA contains ATP/ADP-binding and DNA-binding domains. When enough ATP-DnaA has accumulated in the cell, an active initiation complex can be formed at the origin resulting in strand opening and recruitment of the replicative helicase. DnaA activity is regulated by proteins that stimulate ATP-DnaA hydrolysis, yielding inactive ADP-DnaA in a replication-coupled negative-feedback manner, and by DnaA-binding DNA elements that control the subcellular localization of DnaA or stimulate the ADP-to-ATP exchange of the DnaA-bound nucleotide. Regulation of *dnaA* gene expression is also important for initiation.

Regulating DNA Replication in eukaryotic cell

To maintain genome integrity in eukaryotes, DNA must be duplicated precisely once before celldivision occurs. A process called replication licensing ensures that chromosomes are replicatedonly once per cell cycle. Its control has been uncovered by the discovery of the CDKs (cyclindependent kinases) as master regulators of the cell cycle and the initiator proteins of DNA replication, such as the Origin Recognition Complex (ORC), Cdc6/18, Cdt1 and the MCM complex. At the end of mitosis, the MCM complex is loaded on to chromatin with the aid of ORC, Cdc6/18and Cdt1, and chromatin becomes licensed for replication. CDKs, together with the Cdc7 kinase, trigger the initiation of

replication, recruiting the DNA replicating enzymes on sites of replication. The activated MCM complex appears to play a key role in the DNA unwinding step, acting as a replicating helicase and moves along with the replication fork, at the same time bringing the origins to the unlicensed state. The cycling of CDK activity in the cell cycle separates the two states of replication origins, the licensed state in G1-phase and the unlicensed state for the rest of the cell cycle. Only when CDK drops at the completion of mitosis, is the restriction on licensing relieved and a new round of replication is allowed.



Chromosomal Mutation

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.

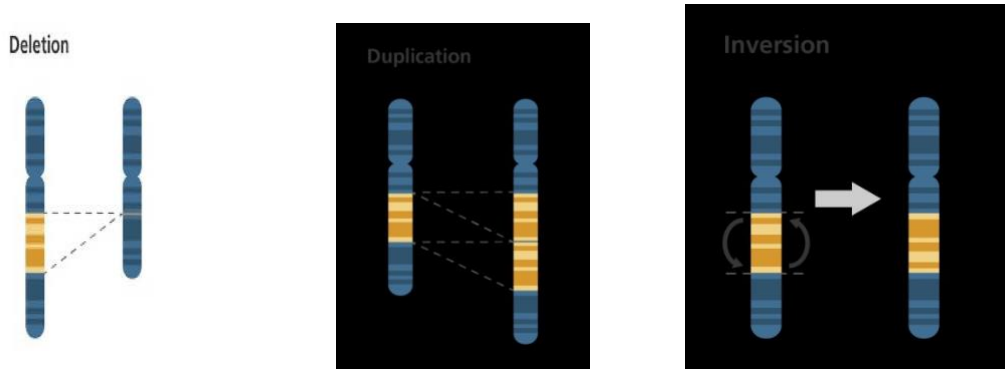
Both types of changes (structural and numerical) in chromosomes can be detected not only with a microscope (**cytologically**) but also by standard genetic analysis.

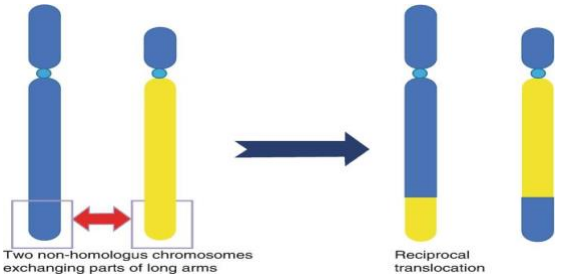
1- STRUCTURAL CHANGES IN CHROMOSOMES:

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.



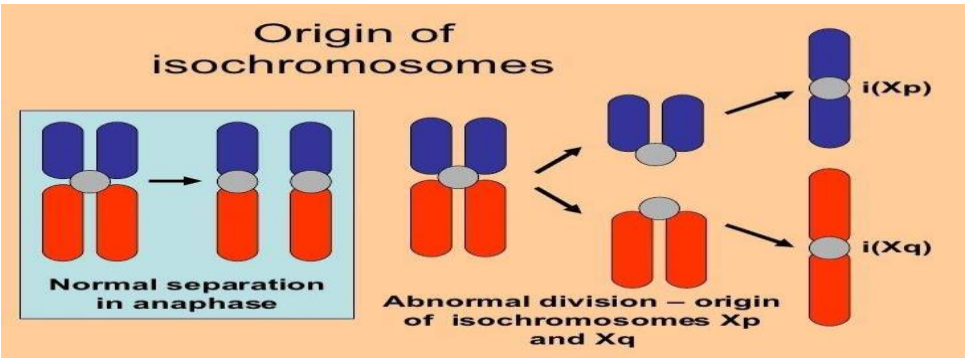


Variation in Chromosome Morphology:

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

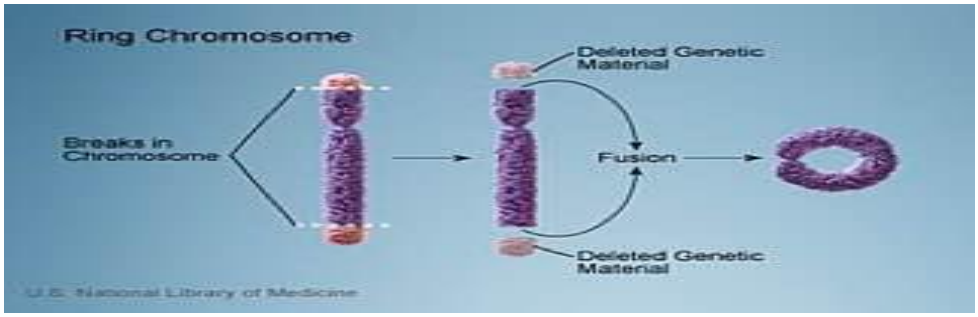
1. **Iso chromosomes.**

   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.



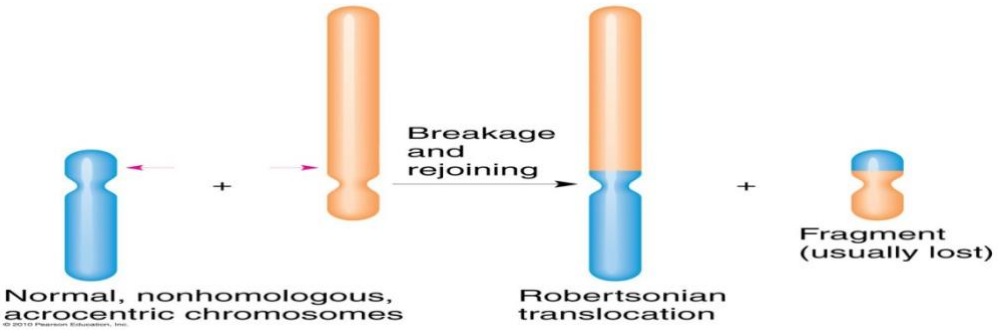
2. Ring chromosomes.

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.

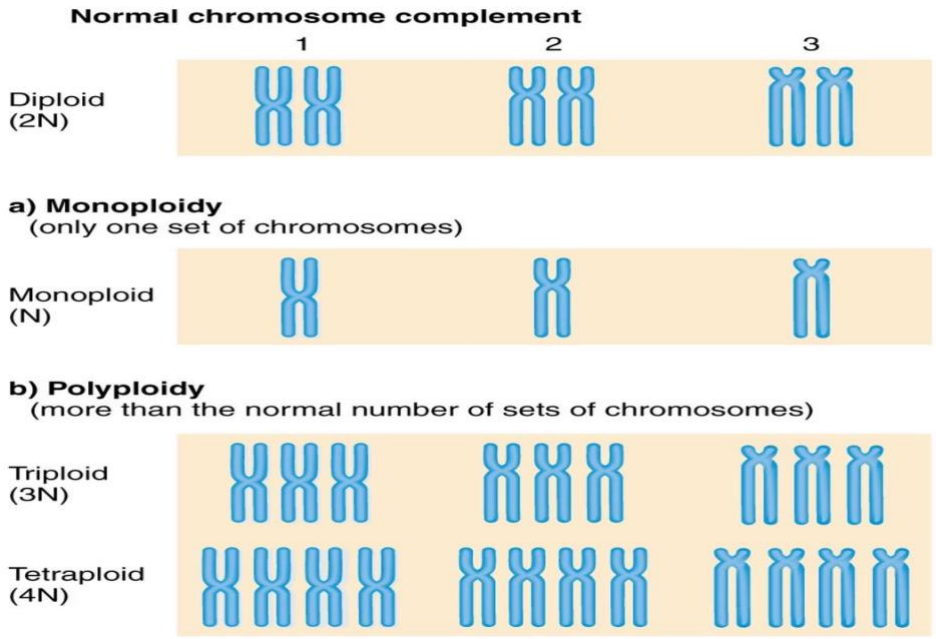
However, sometimes irregularities occur in nuclear division and causes Changes in number of whole chromosomes is called **heteroploidy** Heteroploidy may involve entire sets of chromosomes **(euploidy**), or loss or addition of single whole chromosomes (**aneuploidy**). Each may produce phenotypic changes.

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:

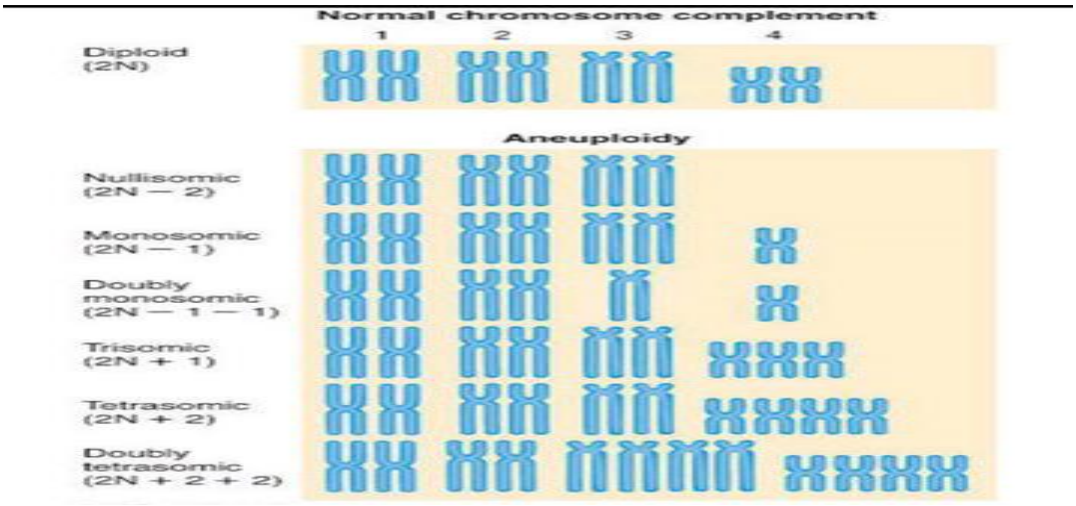
Variations in chromosome number may occur that do not involve whole sets of chromosomes, but only parts of a set.

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.



DNA Damage, Repair and Mutation

Lecturer: Dr. Owayes M. Hamed

Introduction

DNA in the living cell is subject to many chemical alterations (a fact often forgotten in the excitement of being able to do DNA sequencing on dried and/or frozen specimens. If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected. A failure to repair DNA produces a mutation. The recent publication of the human genome has already revealed 130 genes whose products participate in DNA repair. More will probably be identified soon.

Agents that Damage DNA

- Certain wavelengths of radiation: ionizing radiation such as gamma rays and x-rays and o Ultraviolet rays, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA.
- Highly-reactive oxygen radicals produced during normal cellular respiration as well as by other biochemical pathways.
- Chemicals in the environment: o many hydrocarbons, including some found in cigarette smoke and some plant and microbial products, e.g. the aflatoxins produced in moldy peanuts.
- Chemicals used in chemotherapy, especially chemotherapy of cancers.

Types of DNA Damage

- All four of the bases in DNA (A, T, C, G) can be covalently modified at various positions.
- Mismatches of the normal bases because of a failure of proofreading during DNA replication.
- Breaks in the backbone. Can be limited to one of the two strands (a single-stranded break SSB) or o on both strands (a double-stranded break DSB).
- Ionizing radiation is a frequent cause, but some chemicals produce breaks as well.

Repairing Damaged Bases

Damaged or inappropriate bases can be repaired by several mechanisms:

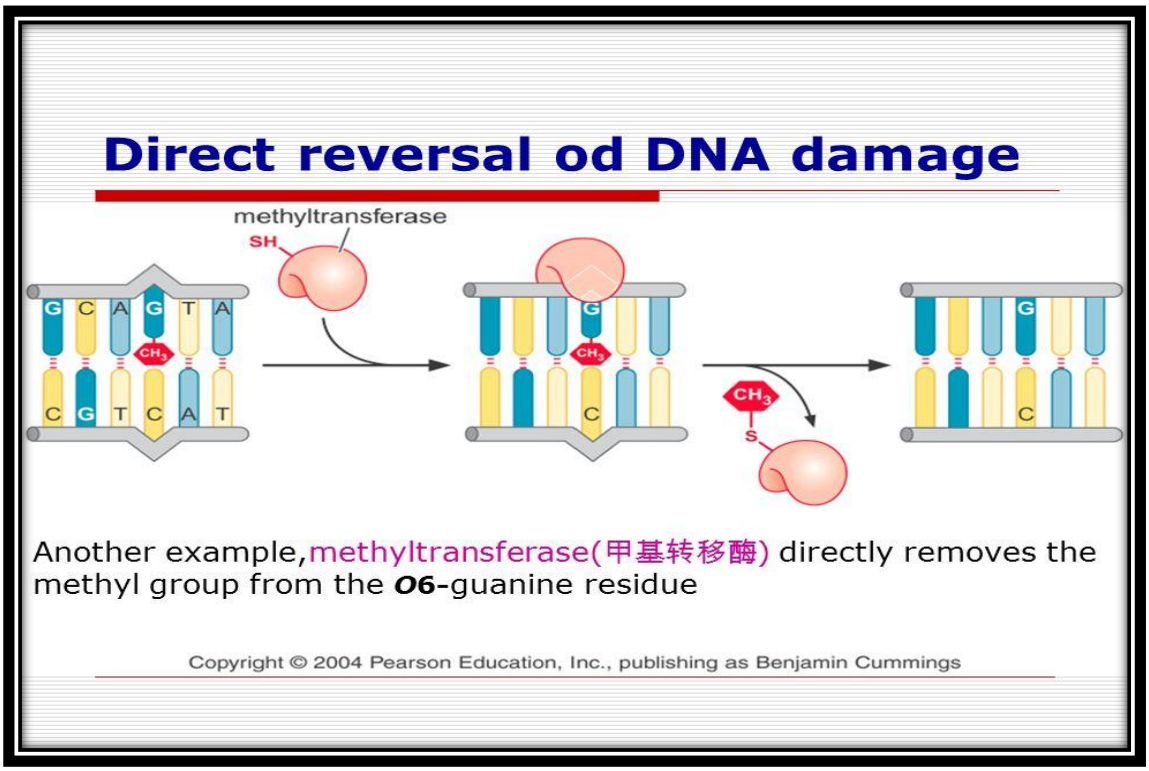
1- Direct Reversal of Base Damage

Perhaps the most frequent cause of point mutations in humans is the spontaneous addition of a methyl group (CH3-) (an example of alkylation) to Cs followed by deamination to a T. Fortunately, most of

these changes are repaired by enzymes, called glycosylases, that remove the mismatched T restoring the correct C. This is done without the need to break the DNA backbone (in contrast to the mechanisms of excision repair described below).

Some of the drugs used in cancer chemotherapy ("chemo") also damage DNA by alkylation. Some of the methyl groups can be removed by a protein encoded by our MGMT gene. However, the protein can only do it once, so the removal of each methyl group requires another molecule of protein.

This illustrates a problem with direct reversal mechanisms of DNA repair: they are quite wasteful. Each of the myriad types of chemical alterations to bases requires its own mechanism to correct. What the cell needs are more general mechanisms capable of correcting all sorts of chemical damage with a limited toolbox. This requirement is met by the mechanisms of excision repair.

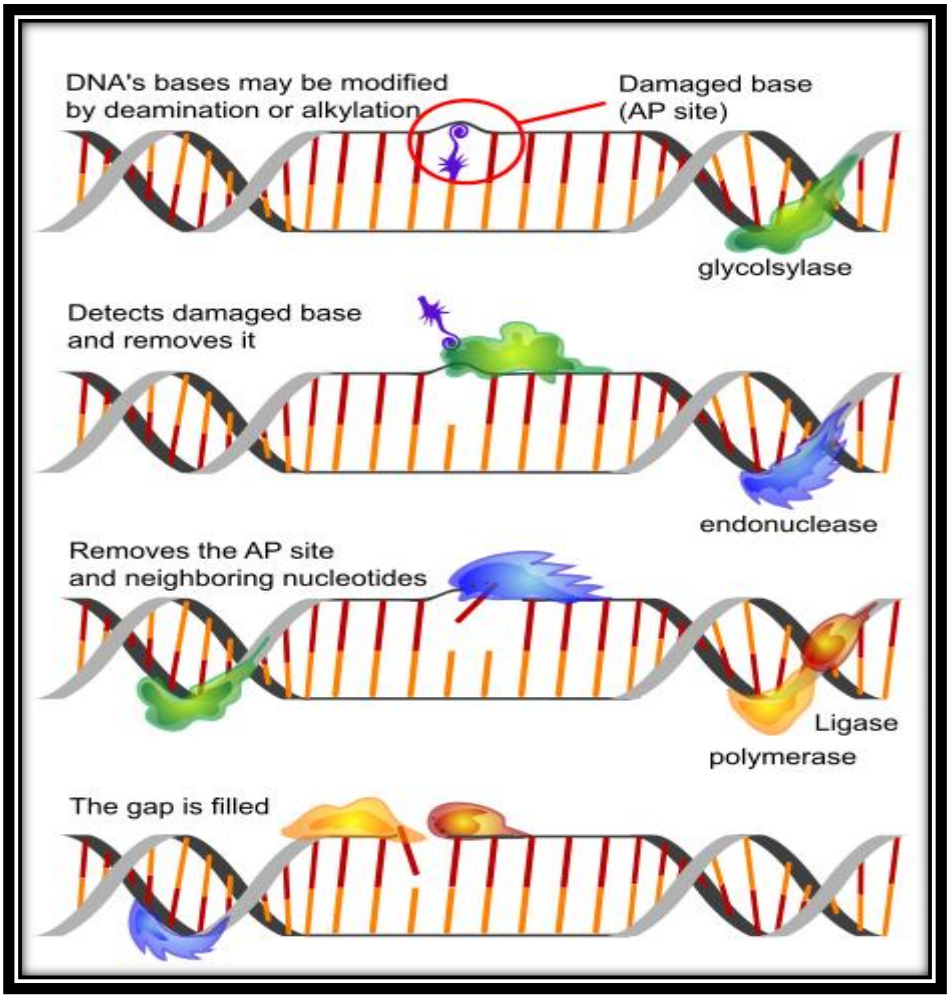


2- **Base Excision Repair (BER)**

The steps and some key players:

- Removal of the damaged base (estimated to occur some 20,000 times a day in each cell in our body) by a DNA glycosylase. We have at least 8 genes encoding different DNA glycosylases each enzyme responsible for identifying and removing a specific kind of base damage.

- Removal of its deoxyribose phosphate in the backbone, producing a gap. We have two genes encoding enzymes with this function.
- Replacement with the correct nucleotide. This relies on DNA polymerase beta, one of at least 11 DNA polymerases encoded by our genes.
- Ligation of the break in the strand. Two enzymes are known that can do this; both require ATP to provide the needed energy.



3- Nucleotide Excision Repair (NER)

NER differs from BER in several ways.

- It uses different enzymes.
- Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

The steps and some key players:

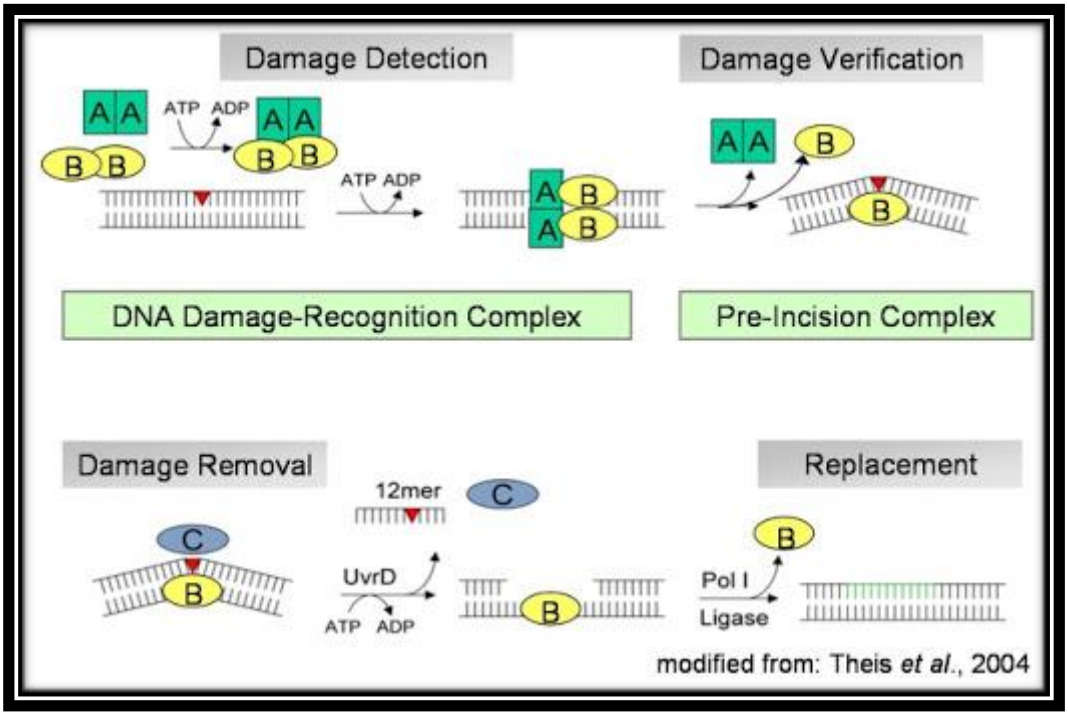
1- The damage is recognized by one or more protein factors that assemble at the location.

2- The DNA is unwound producing a "bubble". The enzyme system that does this is Transcription Factor IIH, TFIIH, (which also functions in normal transcription).

3- Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.

4- A fresh burst of DNA synthesis — using the intact (opposite) strand as a template — fills in the correct nucleotides. The DNA polymerases responsible are designated polymerase delta and epsilon.

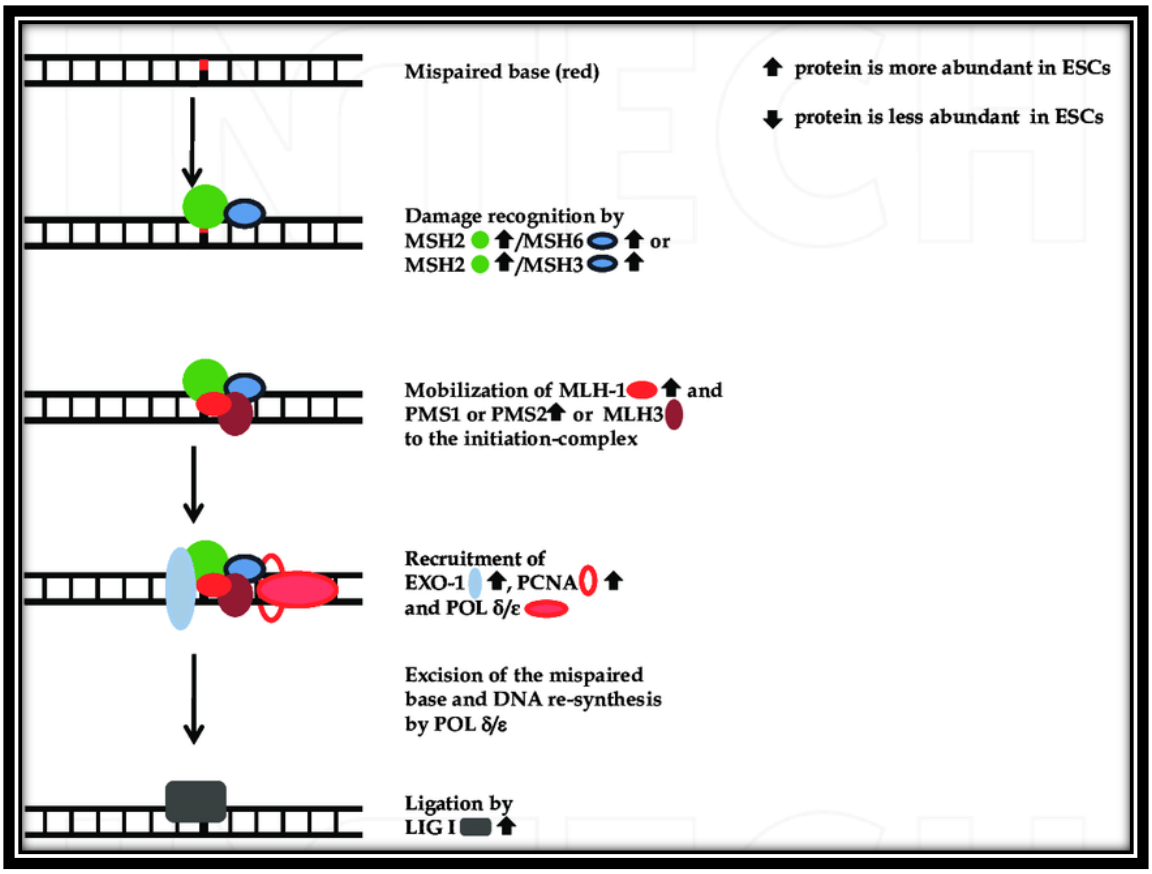
5- A DNA ligase covalent binds the fresh piece into the backbone.



4- Mismatch Repair (MMR)

Mismatch repair deals with correcting mismatches of the normal bases; that is, failures to maintain normal Watson-Crick base pairing (A•T, C•G). It can enlist the aid of enzymes involved in both base-excision repair (BER) and nucleotide-excision repair (NER) as well as using enzymes specialized for this function.

- Recognition of a mismatch requires several different proteins including one encoded by MSH2.
- Cutting the mismatch out also requires several proteins, including one encoded by MLH1.



Repairing Strand Breaks

Ionizing radiation and certain chemicals can produce both single-strand breaks (SSBs) and double-strand breaks (DSBs) in the DNA backbone.

Single-Strand Breaks (SSBs): Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).

Double-Strand Breaks (DSBs): There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:

- Direct joining of the broken ends. his requires proteins that recognize and bind to the exposed ends and bring them together for ligating. They would prefer to see some complementary nucleotides but can proceed without them so this type of joining is also called Non-homologous End-Joining (NHEJ).
- Errors in direct joining may be a cause of the various translocations that are associated with cancers.

Mutations

In the living cell, DNA undergoes frequent chemical change, especially when it is being replicated (in S phase of the eukaryotic cell cycle). Most of these changes are quickly repaired. Those that are not result in a mutation. Thus, mutation is a failure of DNA repair.

- **Single-base substitutions**

  A single base, say an A, becomes replaced by another. Single base substitutions are also called point mutations. (If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a transition. If a purine is replaced by a pyrimidine or viceversa, the substitution is called a transversion.)

- **Missense mutations**

  With a missense mutation, the new nucleotide alters the codon so as to produce an altered amino acid in the protein product.

- **Nonsense mutations**

  With a nonsense mutation, the new nucleotide changes a codon that specified an amino acid to one of the STOP codons (TAA, TAG, or TGA). Therefore, translation of the messenger RNA transcribed from this mutant gene will stop prematurely. The earlier in the gene that this occurs, the more truncated the protein Product and the more likely that it will be unable to function.

- **Silent mutations**

  Most amino acids are encoded by several different codons. For example, if the third base in the **TCT** codon for **serine** is changed to any one of the other three bases, serine will still be encoded. Such mutations are said to be silent because they cause no change in their product and cannot be detected without sequencing the gene (or its mRNA).

- **Splice-site mutations**

  The removal of intron sequences, as pre-mRNA is being processed to form mRNA, must be done with great precision. Nucleotide signals at the splice sites guide the enzymatic machinery. If a mutation alters one of these signals, then the intron is not removed and remains as part of the final RNA molecule. The translation of its sequence alters the sequence of the protein product.