Ministry of Higher Education and Scientific Research

Medical Laboratory Techniques Department

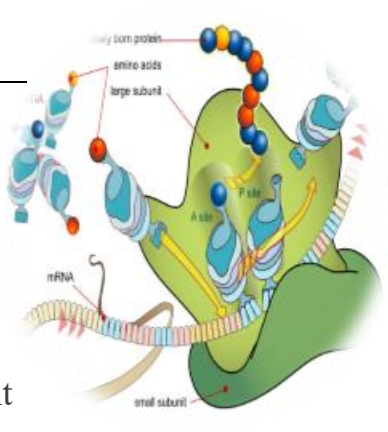
Training package In

Molecular Biology

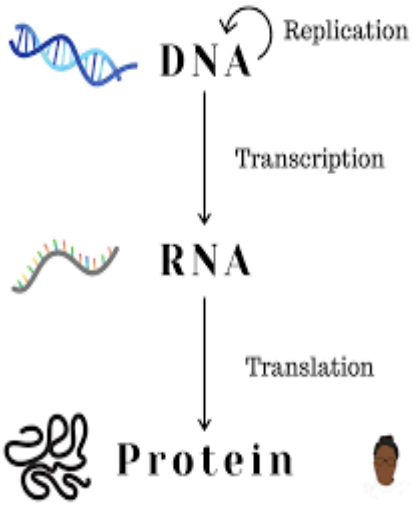
For 2nd Level Students

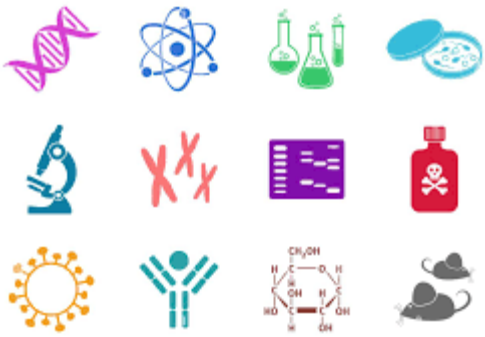
Objective:

MB



Molecular biology is a science that is concerned with the study of biology at the molecular level, so it overlaps with both microbiology and chemistry in several branches and intersects with biochemistry and genetics in several areas and disciplines. Molecular biology is concerned with the study of the various interrelationships between all Cellular systems, especially the relationships between deoxyribonucleic acid (DNA) and RNA (ribonucleic acid) and the process of protein synthesis. In addition to the mechanisms regulating this process and all vital processes in the body. At the end of this course, the student will be able to understand the three-dimensional structures and structural formations of nucleic acids in humans as well as understanding the molecular basis of the process of transcription, translation, DNA damage and mutations.





Lecture No.1:

Title of the lecture:

Introduction to Molecular Biology &Nucleic acids

-What is Molecular Biology?

Molecular biology, is the study of chemical and physical structure of biological macromolecules of the cell. It is a branch of biology that is focused especially on nucleic acids (e.g., DNA and RNA) and proteins—macromolecules that are essential to life processes—and how these molecules interact and behave within cells.

Molecular biology was first described as an approach focused on the foundations of biological phenomena—uncovering the structures of biological molecules as well as their interactions, and how these interactions explain observations of classical biology.

-What is molecular biology used for?

The term molecular biology was first used in 1945 by the physicist William Astbury where the field included techniques which enable scientists to learn about molecular processes and were used to efficiently target new drugs, diagnose disease, and better treatment choices.

Whereas, recently, molecular biology techniques play a vital role in advancing our understanding of genetics, cell biology, and biochemistry. Their research is used in a variety of fields, including: biotechnology, medicine, agriculture, and environmental science.

- Application of molecular biology in medical laboratory techniques:

Molecular biology has revolutionized medical laboratory science by shifting diagnostic focus from observing physical symptoms (phenotypes) to analyzing the genetic blueprint (genotypes). This allows for earlier, more precise detection of diseases and a shift toward personalized medicine.

These foundational methods are the "workhorses" of modern clinical diagnostics including:

- **Polymerase Chain Reaction (PCR):** Amplifies tiny amounts of DNA or RNA to detectable levels. It is the standard for detecting active infections (like COVID-19 or HIV) and identifying genetic mutations.

- **Next-Generation Sequencing (NGS):** Allows for rapid analysis of entire genomes, which is crucial for identifying rare genetic disorders, tracking pathogen mutations (surveillance), and precision oncology.
- **Gel Electrophoresis & Blotting:** Used to separate DNA, RNA, or proteins by size and charge to confirm the presence of specific molecules.
- **Fluorescence In Situ Hybridization (FISH):** Visualizes specific genes or chromosomal abnormalities directly within cells, often used in cancer diagnostics.

Molecular Biology in Medical Laboratory Applications

1. **Infectious Disease Diagnosis:**

1. **Rapid Pathogen Identification:** Detects slow-growing or unculturable organisms (e.g., *M. tuberculosis* or *T. pallidum*) much faster than traditional cultures.
2. **Viral Load Monitoring:** Quantifies the amount of virus in a patient (e.g., HIV or Hepatitis B) to monitor treatment effectiveness.
3. **Antimicrobial Resistance:** Identifies specific genes (like *mecA* for MRSA) to determine if an infection will respond to certain antibiotics.

2. **Oncology (Cancer Care):**

1. **Early Detection:** Identifies tumor-specific mutations even before symptoms appear.
2. **Targeted Therapy Selection:** Tests for specific markers (e.g., BRCA1/2, EGFR) to match patients with drugs that specifically attack their tumor's genetic profile.

3. **Genetic Testing & Hematology:**

1. **Inherited Disorders:** Diagnoses conditions like cystic fibrosis, sickle cell anemia, and hemophilia.
2. **Non-invasive Prenatal Testing (NIPT):** Analyzes fetal DNA in maternal blood to screen for chromosomal anomalies without invasive procedures.

4. **Pharmacogenomics:**

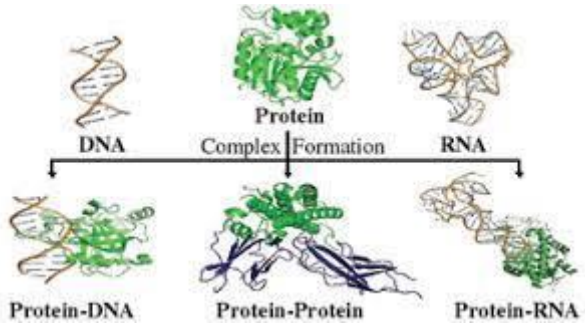
1. Studies how a patient's genetic makeup affects their response to drugs, allowing for "tailor-made" dosages to minimize side effects.

5. **Transfusion Medicine & HLA Typing:**

Improves blood group typing and matches organ donors with recipients more accurately through HLA-class II gene typing.

-Macromolecules examples studied in molecular biology:

There are two major classes of biological macromolecules (nucleic acids and proteins), and each is an important component of the cell and performs a wide array of functions.

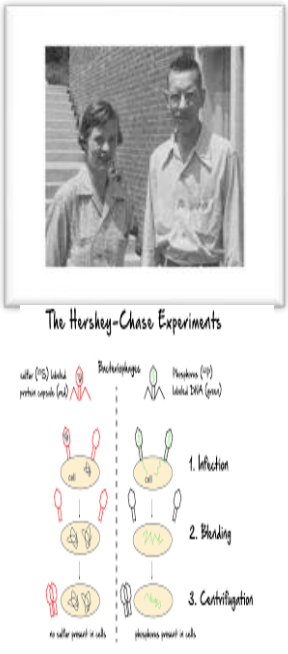


Nucleic acids

Nucleic acids and proteins are the basic macromolecules of living organisms. The linkage between nucleic acids and proteins is very close and each macromolecule has its unique function in the cell.

- The Function of the Nucleic Acids

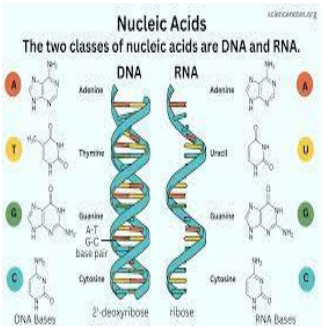
However, Since Gregor Mendel, the Austrian monk had discovered the basic principles of heredity through experiments in his garden where his observations became the foundation of modern genetics; scientists started to inquire about the "material" which was responsible about the transmission of the traits from one generation to the following. Three macromolecules were questioned as the carrier of the genetic traits they were namely (DNA, RNA and protein). However, after many experiments by many scientists all over the world, in 1952 an experiment was conducted by Hershey and Chase involving the bacteriophage T2 whose DNA was labeled by a radionuclide 32P, and the protein part by a 35S radionuclide. During the infection by a bacteriophage, only the DNA part of the virus entered the cell. This supported the evidence, that DNA was the carrier of the genetic information. However, certain viruses use the ribonucleic acid-RNA) as the carrier of the genetic material.



First and foremost, the genetic material must be capable of storing large amounts of information and instructions for all the traits and

functions of an organism. This information must have the capacity to vary, because different species and even individual members of a species differ in their genetic makeup. At the same time, the genetic material must be stable, because most alterations to the genetic instructions (mutations) are likely to be detrimental if turned around, errors in the genetic code lead to the synthesis of defective proteins, or to stop the synthesis of proteins overall.

-Primary structure of nucleic acid:



Nucleic acids were discovered as "nuclein" by a Swiss physician and biologist Miescher in the year 1869, and their name was created by Altmann (1889). For the synthesis of nucleic acids nucleosides are used, which are made of a nitrogenous base and pentose, but the structure of DNA differs than RNA.

2- Deoxyribonucleic acid (DNA) from the beginning

DNA was discovered as a major chemical of the nucleus able to transmit large amounts of hereditary information from generation to generation. Although DNA was known to be a very large molecule, it is relatively simple in structure, having an elegant and beautiful incomparable structure in comparison with the other large molecules in the body. It is useful to consider the structure of DNA at three levels of increasing complexity, known as the primary, secondary, and tertiary structures of DNA.

►Primary Structure of DNA

The primary structure of DNA consists of a string of nucleotides. (see figure 1.1).

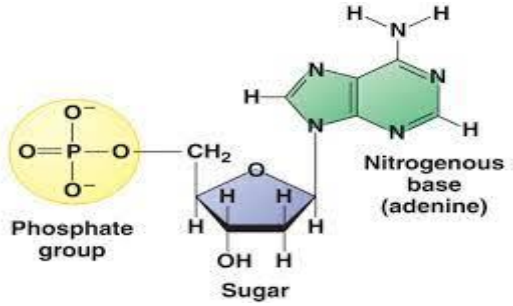
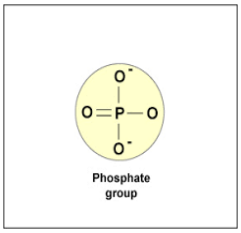


Figure (1.1):
The composition of a nucleotide in DNA.

Each nucleotide is composed of three parts:

1- Phosphate group
2- Sugar (pentose)
3- Nitrogenous base



The <u>first component of a nucleotide is the phosphate group</u>, which consists of a phosphorus atom bonded to four oxygen atoms. Phosphate groups are found in every nucleotide and frequently carry a negative charge, which makes DNA acidic. The phosphate is always bonded to the 5-carbon atom of the sugar in a nucleotide.

<u>The second component is the sugars of the nucleic acids</u> which is called "pentose sugar". This sugar consists of five carbon atoms, numbered 1′-, 2′-, 3′-, 4′- and 5′- see figure (1.2). In ribose sugar, five carbon atoms are joined by either a hydrogen (-H) or a hydroxyl (-OH) atoms to form a five-sided ring; the fifth (5′-) carbon atom projects upward from the ring. While, in the 2-Deoxyribose sugar, the carbon atom number two is attached to hydrogen atom (-H) instead of a hydroxyl groups (-OH) found attached to the carbon atom number two in ribose sugar making it deficient in an oxygen atom and gaining the name 2′-Deoxyribose.

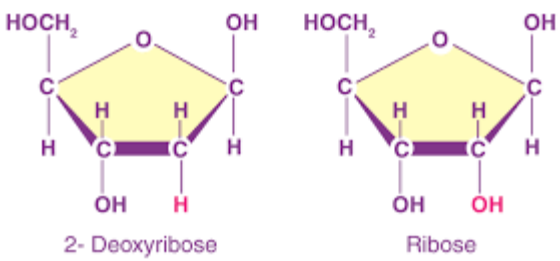
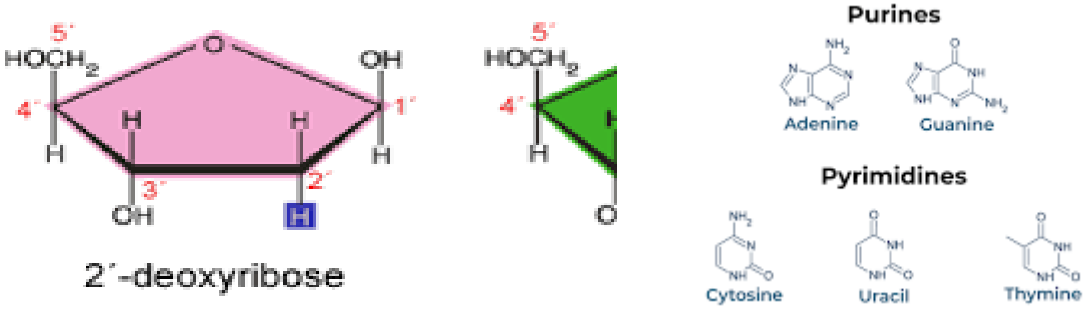


Figure (1.2):
The composition of pentose sugars in the nucleic acids.

This minor chemical difference in the ribose sugar between DNA and RNA is recognized by all the cellular enzymes that interact with DNA or RNA, thus yielding specific functions for each nucleic acid. Furthermore, the additional oxygen atom in the RNA nucleotide makes it more reactive and less chemically stable than DNA. For this reason, DNA is better suited to serve as the long-term storehouse of genetic information.

Structures of deoxyribose and ribose, the pentose sugars of DNA and RNA, respectively.



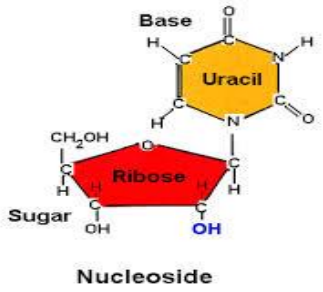
The third component of a nucleotide is its nitrogenous base, which may be of two types; a purine or a pyrimidine. See figure (1.3).

Figure (1.3):
The chemical structure of nitrogenous bases in the nucleic acids.

Purines are the larger of the two types of nitrogen bases found in DNA and RNA. They are composed of nine atoms that make up the fused rings (5 carbon and 4 nitrogen atoms) that are numbered from one to nine. All ring atoms lie in the same plane. Adenine (A) and Guanine (G) are purines they occur in both DNA and RNA. On the other hand, **pyrimidines** are composed of six atoms (4 carbon and 2 nitrogen atoms) they are numbered from one to six. Like purines, all pyrimidine ring atoms lie in the same plane and Cytosine (C), Uracil (U) and Thymine (T) are pyrimidines. Each purine consists of a six-sided ring attached to a five-sided ring, whereas each pyrimidine consists of a six-sided ring only.

DNA and RNA both contain two purines, adenine and guanine (A and G). There are three pyrimidines found in nucleic acids: cytosine (C), thymine (T), and uracil (U). Cytosine is present in both DNA and RNA; however, thymine is restricted to DNA, and uracil is found only in RNA.

In a nucleotide, the nitrogenous base always forms a covalent bond (glycosidic bond/ N-glycosidic linkage) with the carbon number one atom of the sugar. However, a deoxyribose (or ribose) sugar and a base together are referred to as a nucleoside.



Nucleoside

Figure (1.4):
The chemical structure of a nucleoside in the nucleic acid (RNA).

On the other hand, a phosphodiester bond is formed between the sugar and the phosphate group in the nucleotide. However, the "backbone" of a DNA molecule in each cell consists of polynucleotide chains composed of many nucleotides connected by covalent bonds, which join the 5′- phosphate group of one nucleotide to the 3′-carbon atom of the ribose sugar in the next nucleotide as shown below in figure (1.5). where those nucleotides were joined together by phosphodiester linkages.

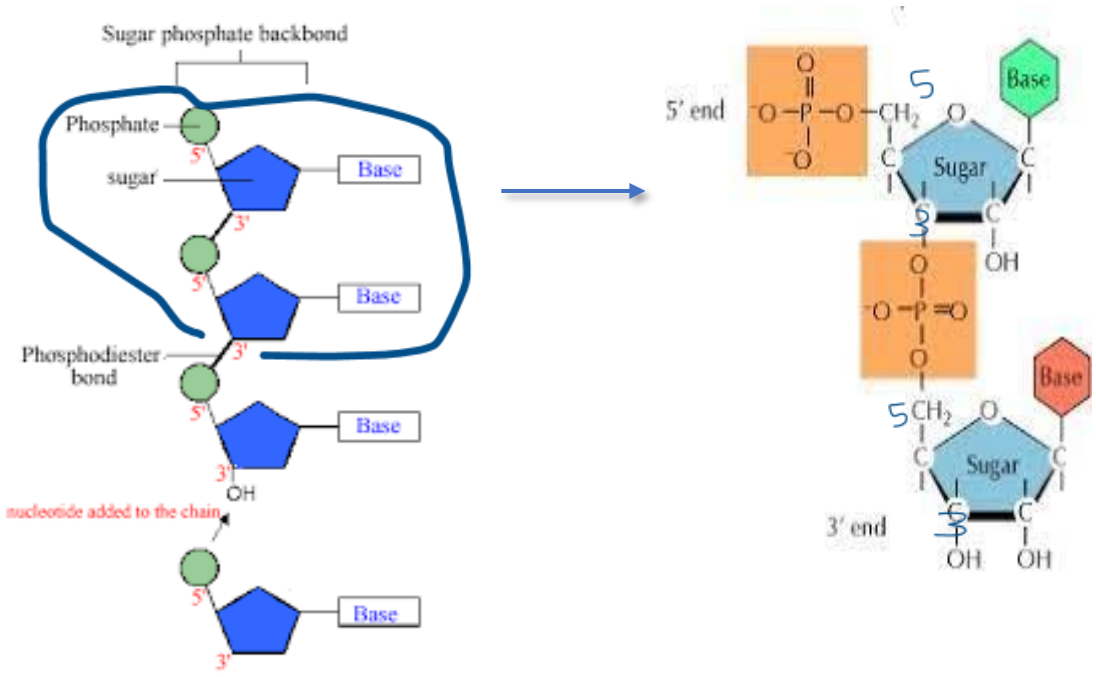
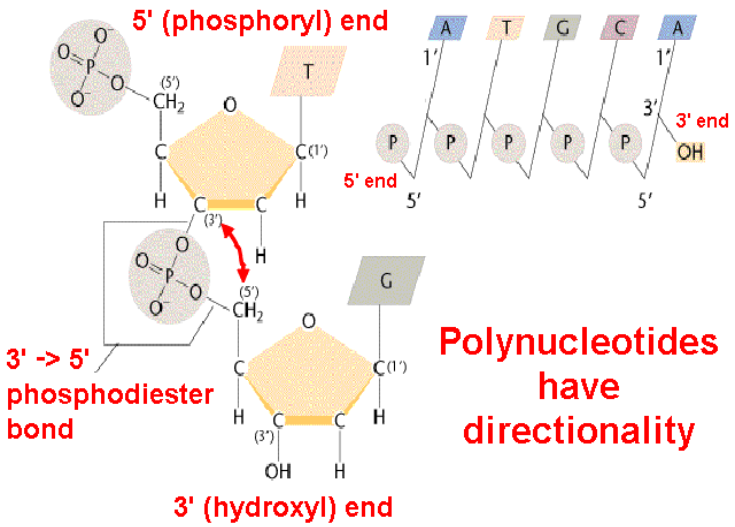


Figure (1.5): Polynucleotide chain structure in the nucleic acids.

The phosphodiester linkages, are relatively strong covalent bonds; a series of nucleotides linked in this way constitutes a polynucleotide strand. The backbone of the polynucleotide strand is composed of alternating sugars and phosphates; the bases project away from the long axis of the strand and the negative charges of the phosphate groups are frequently neutralized by the association of positive charges on proteins, metals, or other molecules. Thus, the DNA is typically a very long molecule and is therefore, termed a macromolecule, if stretched out straight, would be several centimeters in length.

Direction of DNA polynucleotide

An important characteristic of the polynucleotide strand is its direction, or polarity. At one end of the strand a phosphate group is attached to the 5′-carbon atom of the sugar in the nucleotide which is therefore, referred to as the 5′- end. On the other hand, a 3′-carbon atom of another sugar molecule is attached to the same phosphate group and referred as the 3′-end. Thus, the polynucleotide chains have a 5'-to-3' directionality. A fundamental feature of the polynucleotide chain is that its ends are dissimilar. Thus, the 3'- hydroxyl is displayed at one terminus, (or so called the 3'- end), and the 5'- phosphoryl at the other terminus, (or called the 5'- end).



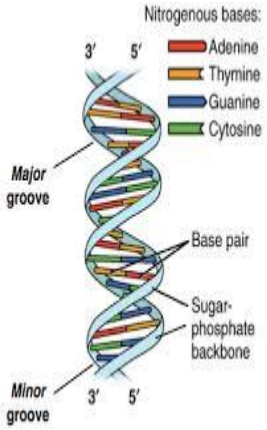
The directionality of the polynucleotide chain is very important feature of the DNA since the **two ends of the molecule have a very different biochemical properties, and** behave very differently in molecular processes. DNA directionality is also important in governing various cellular events.

Furthermore, the nitrogenous bases of each polynucleotide chain is bond to each other by hydrogen bonds and align in an antiparallel orientation in the double helix forming the secondary structure of DNA.

Lecture No.2:

Title of the lecture

Secondary Structure of DNA



The model of the secondary structure of DNA consists of two polynucleotide strands twisted around each other forming a unique structure called (a double helix). This double helix is composed after the linkage of sugar–phosphate on the outside of the helix, and the staking of the purine and pyrimidine bases bonded by hydrogen bonds in the interior of the molecule forming a complex of two polynucleotide chains twisted around a mutual axis so that, the carbohydrate chain protrude outside the chain and the nucleic acid bases are directed inwards the chain. See figure (2.1).

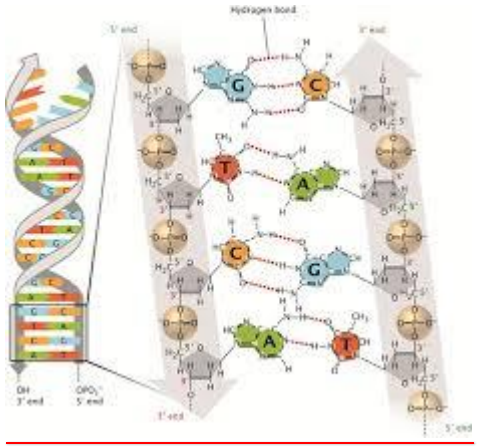
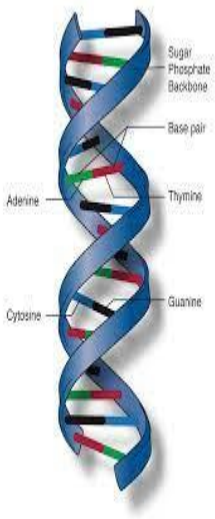


Figure (2.6): Secondary structure of DNA.

Still, the basic structure of DNA can be divided into two portions: the external sugar-phosphate backbone, and the internal bases. The sugar phosphate backbone, as its name implies, is the major structural component of the DNA molecule. The backbone is constructed from alternating ribose sugar and phosphate molecules which are highly polar. Because the backbone is polar, it is hydrophilic which means that it likes to be immersed in water. While, the interior portion of a DNA molecule is composed of a series of four

nitrogenous bases: adenine (A), guanine (G), thymine (T), and cytosine (C). These bases are non-polar therefore they are hydrophobic (they don't like water). Inside a DNA molecule these bases pair up, A to T and C to G, forming hydrogen bonds that stabilize the DNA molecule. Because the interior bases pair up in this manner, we say that; the DNA double helix is complimentary. It is this sequence of bases inside the DNA double helix that we refer to as the genetic code. However, all of the DNA molecules are built in 5′ to 3′ direction while the other strand lane in the opposite direction making the two strands "antiparallel" showing an opposite chemical polarity.

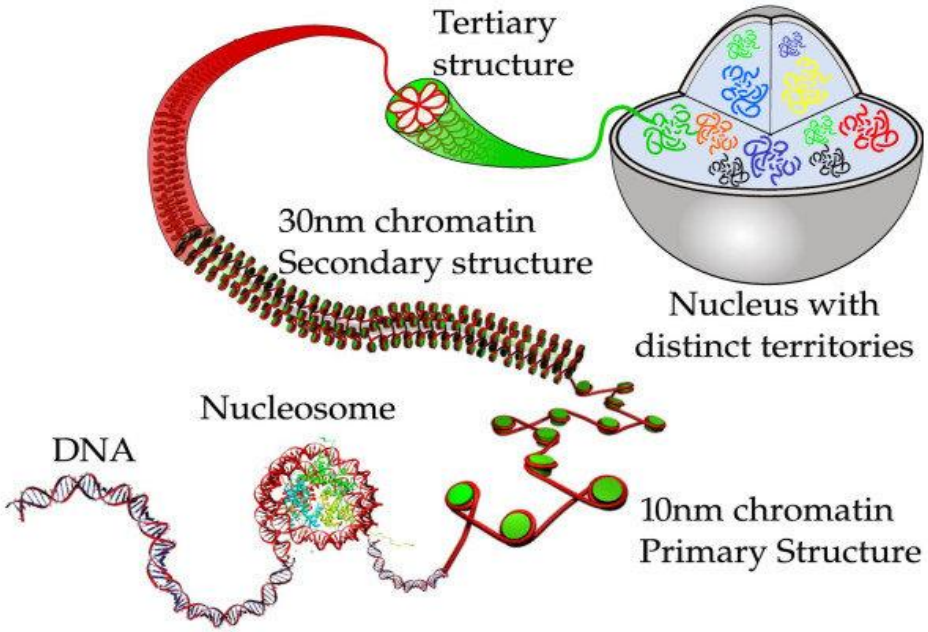


What is the double helix of a DNA?

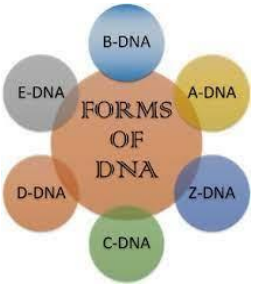
Double helix, as related to genomics, is a term used to describe the physical structure of DNA. A DNA molecule is made up of two linked strands that wind around each other to resemble a twisted ladder in a helix-like shape.

Why is DNA double helical?

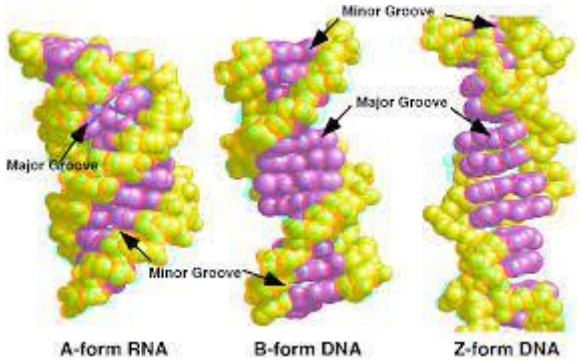
To maximize the efficiency of base-pair packing, the two sugar-phosphate backbones wind around each other to form a double helix, with one complete turn every ten base pairs



►Tertiary Structure of DNA



Nucleic acid tertiary structure is the three-dimensional shape of a nucleic acid polymer. RNA and DNA molecules are capable of diverse functions ranging from molecular recognition to catalysis. Such functions require a precise three-dimensional structure. Thus, the DNA tertiary structure is the three-dimensional geometrical formation of the nucleotides and can include B-DNA, A-DNA, and Z-DNA which is determined by the base pair geometry.



Property	A-DNA	B-DNA	Z-DNA
Helix Handedness	Right	Right	Left
Base Pairs per turn	11	10.4	12
Rise per base pair along axis	0.23nm	0.34nm	0.38nm
Pitch	2.46nm	3.4nm	4.56nm
Diameter	2.55nm	2.37nm	1.84nm
Major Groove	Present	Present	Absent
Minor Groove	Present	Present	Deep Cleft

The most popular forms of DNA

- A-DNA: It is a right-handed double helix, short and fat compared to B-DNA, occur only in dehydrated cells
- B-DNA: This is the most common DNA conformation and is a right-handed helix, described by Watson and Crick. ...
- Z-DNA: Z-DNA is a left-handed DNA where the double helix winds to the left in a zig-zag pattern. Long and thin in comparison to B-DNA

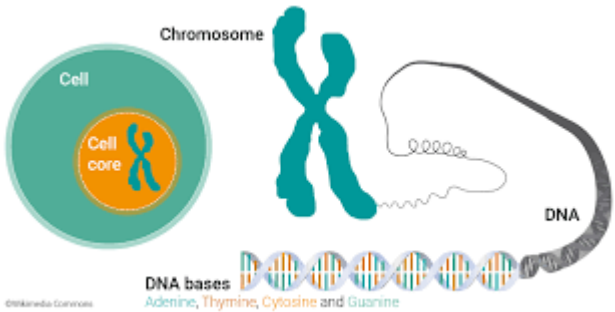
Types of DNA according to cell location

According to its location inside the cell we can divide DNA into more types. The most important is the chromosomal DNA. The mitochondrial DNA and plasmid DNA.

1-Chromosomal DNA

Chromosomal DNA can be divided into the certain groups according to function, coding and non-coding regions. The coding DNA determines the sequence of amino in the polypeptide chain (structural genes), or nucleotides order in the RNA types

There are more types of non-coding DNA – for example the DNA which has a control and regulatory function (for instance promoters). Some types of DNA have a specific function inside the chromosomes, for instance the repetitive sequences in the region of the centromeres or telomeres.

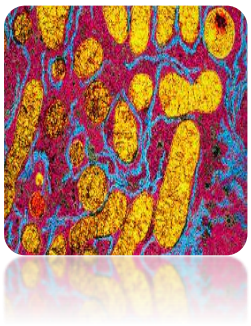


A chromosome is formed from a single, enormously long DNA molecule that contains a linear array of many genes. The human genome contains 3.2 × 109DNA nucleotide pairs, divided between 22 different autosomes and 2 sex chromosomes. Only a small percentage of this DNA codes for proteins or structural and catalytic RNAs.

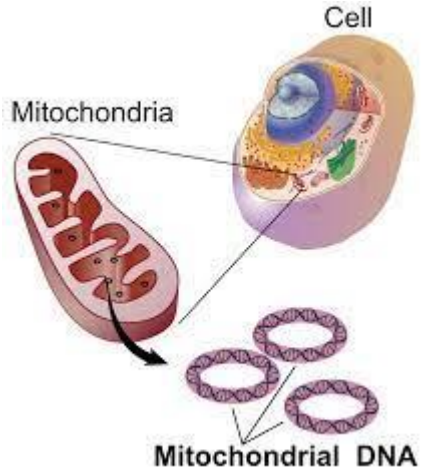
What is the difference between DNA and chromosomal DNA?

A chromosome is a long chain of DNA molecules that contains part of all of the genetic material of an organism. DNA is a fundamental molecule that carries the genetic instruction of all living organisms. DNA is packed into chromosomes with the help of special proteins called histones.

2-Mitochondrial DNA (mtDNA)



In humans, the non-chromosomal DNA is located in the mitochondria. Mitochondrial DNA is the circular chromosome found inside the cellular organelles called mitochondria. Located in the cytoplasm, mitochondria are the site of the cell's energy production and other metabolic functions. Offspring inherit mitochondria — and as a result mitochondrial DNA — from their mother.



Mitochondria therefore have their own DNA (mtDNA), circular and double-stranded, closer to a prokaryotic genome than nuclear DNA, with a genetic code slightly different from the universal genetic code found in the nucleus of eukaryotic cells.

Mitochondrial DNA (mtDNA) has many special features such as a high copy number in cell, maternal inheritance, and a high mutation rate which have made it attractive to scientists from many fields.

Is mitochondrial DNA only found in females?

Mitochondrial DNA (mtDNA) is passed from mother to child. Both sons and daughters receive mtDNA, but only daughters pass the mtDNA on to their own children. Since both sons and daughters receive their mother's mtDNA, both men and women can take mtDNA tests.

What are the differences between mitochondrial and nuclear DNA?

Nuclear DNA is located within the nucleus of eukaryote cells and usually has two copies per cell while mitochondrial DNA is located in the mitochondria and contains 100–1,000 copies per cell.

Is mitochondrial DNA single or double-stranded?

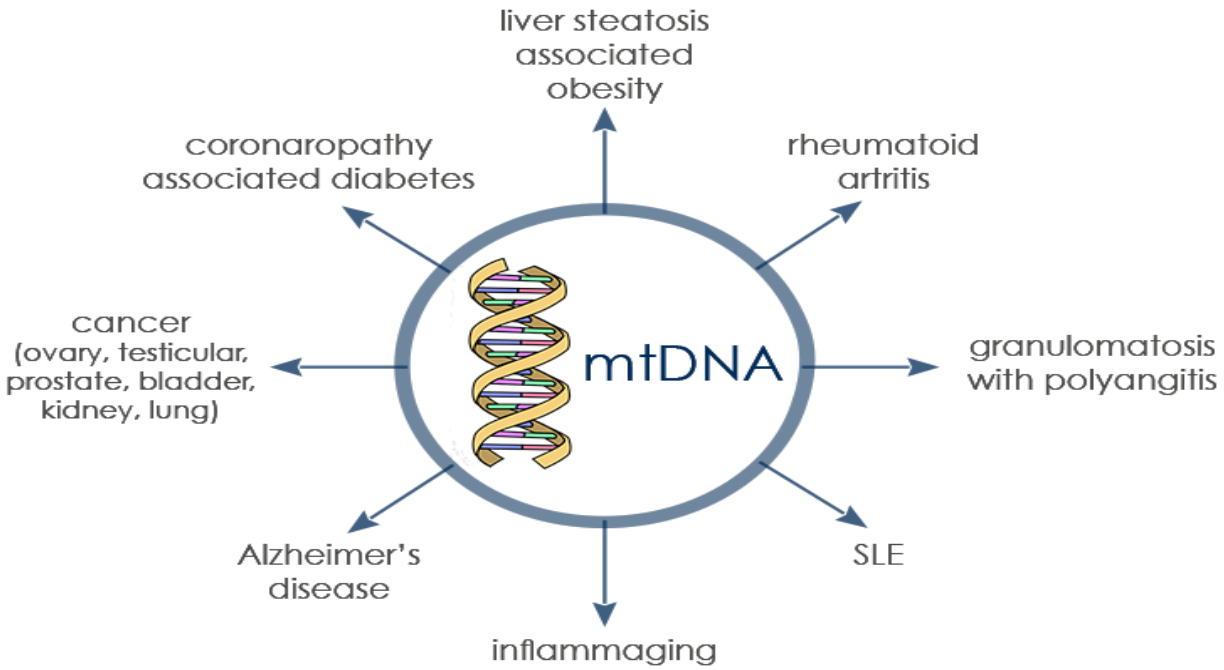
Mitochondrial DNA (mtDNA) is a double-stranded molecule of 16.6 kb

How many genes are in mitochondrial DNA? 37 genes

This genetic material is known as mitochondrial DNA or mtDNA. In humans, mitochondrial DNA spans about 16,500 DNA building blocks (base pairs), representing a small fraction of the total DNA in cells. Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function

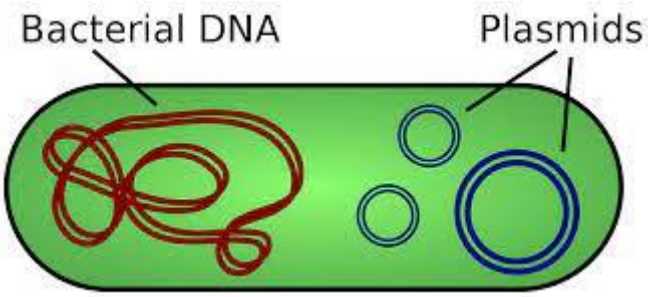
Does mitochondrial DNA have RNA?

Thanks to its mtDNA mitochondria possess their own set of tRNAs, rRNAs and mRNAs that encode a subset of the protein subunits of the electron transport chain complexes.



3-Plasmids

A plasmid is a small circular DNA molecule found in bacteria and some other microscopic organisms. Plasmids are physically separate from chromosomal DNA and replicate independently.



Plasmids naturally exist in bacterial cells but, they also occur in some eukaryotes. With no mechanism to penetrate most eukaryotic cells. However, viruses can and are used to transport DNA into eukaryotic cells. Still, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance.

How plasmids are formed?

Plasmids found in nature often give their hosts beneficial traits that allow them to survive in competitive environments. Plasmids derived directly from the environment are sometimes called 'natural' plasmids, to distinguish them from the modified versions we usually work with in the lab.

The construction of artificial plasmids is crucial in modern molecular biology. In many cases, plasmids are constructed *in vitro* by digesting (cutting) DNA fragments with restriction enzymes at specific sites (restriction sites) and then ligating (joining) the resulting fragments.

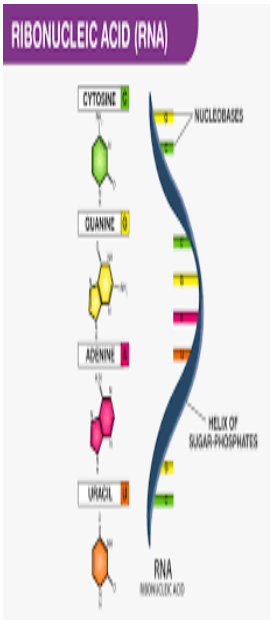
Are plasmids found in eukaryotic mitochondria?

Mitochondrial genomes exhibit diverse features among eukaryotes in the aspect of gene content, genome structure, and the mobile genetic elements such as integrons and plasmids.

Lecture No.3:

Title of the lecture:

Molecular Structure of RNA



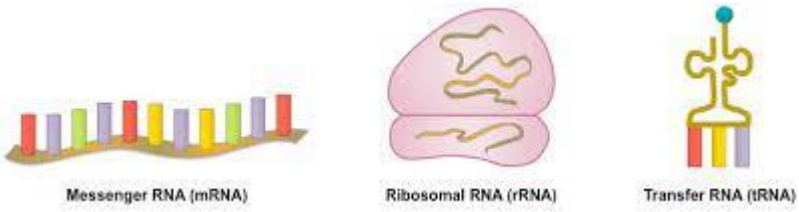
RNA, abbreviation of ribonucleic acid, a complex compound of a high molecular weight that functions in cellular protein synthesis and replaces DNA (deoxyribonucleic acid) as a carrier of genetic codes in some viruses. RNA consists of ribose nucleotides (nitrogenous bases appended to a ribose sugar) attached by phosphodiester bonds, forming strands of varying lengths. The nitrogenous bases in RNA are adenine, guanine, cytosine, and uracil, which replaces thymine in DNA. The ribose sugar of RNA is a cyclical structure consisting of five carbons and one oxygen. The presence of a chemically reactive hydroxyl (−OH) group attached to the second carbon group in the ribose sugar molecule makes RNA prone to hydrolysis. This chemical lability of RNA, compared with DNA, which does not have a reactive −OH group in the same position on the sugar moiety (deoxyribose), is thought to be one reason why DNA evolved to be the preferred carrier of genetic information in most organisms. The structure of the RNA molecule was described by R.W. Holley in 1965.

RNA structure

RNA typically is a single-stranded biopolymer. However, the presence of self-complementary sequences in the RNA strand leads to intrachain base-pairing and folding of the ribonucleotide chain into complex structural forms. The three-dimensional structure of RNA is critical to its stability and function, allowing the ribose sugar and the nitrogenous bases to be modified in numerous different ways by cellular enzymes that attach chemical groups (e.g., methyl groups) to the chain. Such modifications enable the formation of chemical bonds between distant regions in the RNA strand, leading to complex contortions in the RNA chain, which further stabilizes the RNA structure.

Types and functions of RNA

Of the many types of RNA, the three most well-known and most commonly studied are messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA), which are present in all organisms.



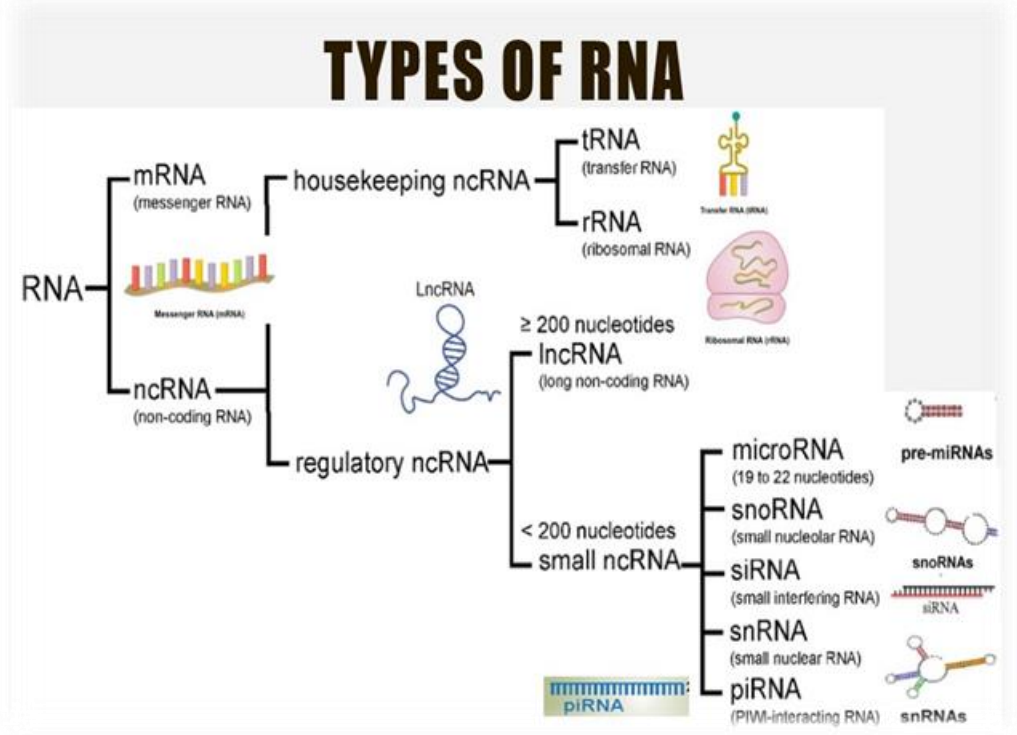
They primarily carry out biochemical reactions, similar to enzymes. Some, however, also have complex regulatory functions in cells. Owing to their involvement in many regulatory processes, to their abundance, and to their diverse functions, RNAs play important roles in both normal cellular processes and diseases. In protein synthesis, mRNA carries genetic codes from the DNA in the nucleus to ribosomes, the sites of protein translation in the cytoplasm.

Ribosomes are composed of rRNA and protein. The ribosome protein subunits are encoded by rRNA and are synthesized in the nucleolus. Once fully assembled, they move to the cytoplasm, where, as key regulators of translation, they "read" the code carried by mRNA.

A sequence of three nitrogenous bases in mRNA specifies incorporation of a specific amino acid in the sequence that makes up the protein.

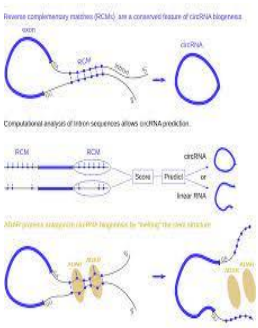
Molecules of tRNA (sometimes also called soluble, or activator, RNA), which contain fewer than 100 nucleotides, bring the specified amino acids to the ribosomes, where they are linked to form proteins. In addition to mRNA, tRNA, and rRNA, RNAs can be broadly divided into coding (cRNA) and noncoding RNA (ncRNA).

There are two types of ncRNAs, housekeeping ncRNAs (tRNA and rRNA) and regulatory ncRNAs, which are further classified according to their size. Long ncRNAs (lncRNA) have at least 200 nucleotides, while small ncRNAs have fewer than 200 nucleotides. Small ncRNAs are subdivided into micro RNA (miRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), small-interfering RNA (siRNA), and pi-interacting RNA.



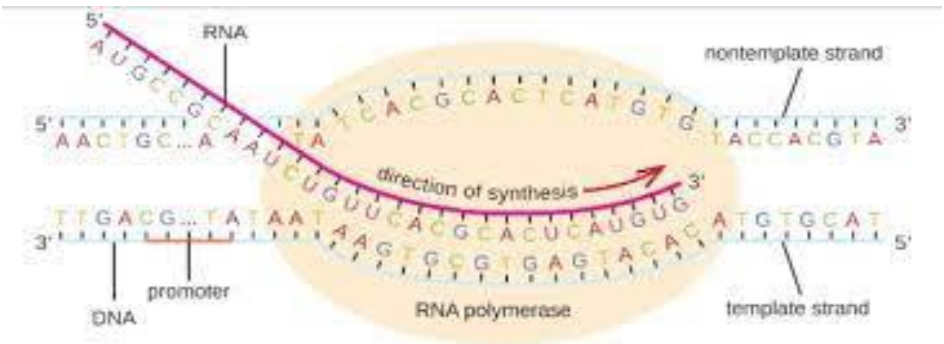
The miRNAs are of particular importance. They are about 22 nucleotides long and function in gene regulation in most eukaryotes. They can inhibit (silence) gene expression by binding to target mRNA and inhibiting translation, thereby preventing functional proteins from being produced. Many miRNAs play significant roles in cancer and other diseases. For example, tumor suppressor and oncogenic (cancer-initiating) miRNAs can regulate unique target genes, leading to tumorigenesis and tumor progression. Also, of functional significance are the piRNAs, which are about 26 to 31 nucleotides long and exist in most animals. They regulate the expression of transposons (jumping genes) by keeping the genes from being transcribed in the germ cells (sperm and eggs). Most piRNA are complementary to different transposons and can

specifically target those transposons. Circular RNA (circRNA) is unique from other RNA types because its 5′ and 3′ ends are bonded together, creating a loop. The circRNAs are generated from many protein-encoding genes, and some can serve as templates for protein synthesis, similar to mRNA. They can also bind miRNA, acting as "sponges" that prevent miRNA molecules from binding to their targets. In addition, circRNAs play an important role in regulating the transcription and alternative splicing of the genes from which circRNAs were derived.

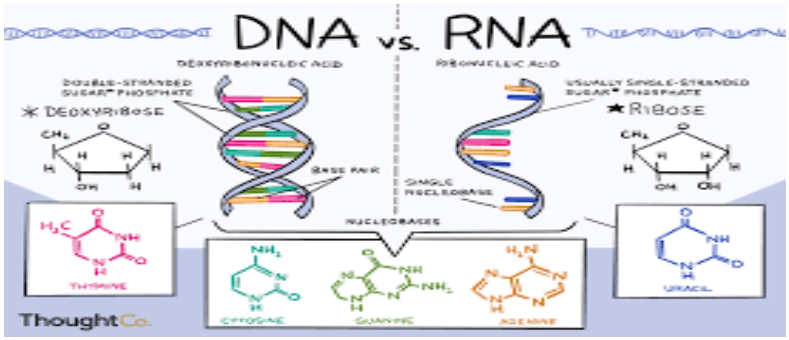


Synthesis of RNA

Synthesis of RNA is usually catalyzed by an enzyme—RNA polymerase—using DNA as a template, a process known as transcription. Initiation of transcription begins with the binding of the enzyme to a promoter sequence in the DNA (usually found "upstream" of a gene). The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3' to 5' direction, synthesizing a complementary RNA molecule with elongation occurring in the 5' to 3' direction. The DNA sequence also dictates where termination of RNA synthesis will occur. Primary transcript RNAs are often modified by enzymes after transcription. For example, a poly(A) tail and a 5' cap are added to eukaryotic pre-mRNA and introns are removed by the spliceosome. There are also a number of RNA-dependent RNA polymerases that use RNA as their template for synthesis of a new strand of RNA. For instance, a number of RNA viruses (such as poliovirus) use this type of enzyme to replicate their genetic material.



The Differences between DNA and RNA



There are a number of differences that distinguish DNA from RNA: (a) RNA contains the sugar ribose, while DNA contains the slightly different sugar deoxyribose (a type of ribose that lacks one oxygen atom),

(b) RNA has the nucleobase uracil while DNA contains thymine.

(c) DNA is a double-stranded molecule that has a long chain of nucleotides. RNA is a single-stranded molecule which has a shorter chain of nucleotides.

(d) DNA replicates on its own, it is self-replicating. RNA does not replicate on its own.

(e) DNA and RNA molecules both contain four nitrogenous bases. Three of these (adenine, cytosine, and guanine) are found in both types of nucleic acid.

(f) DNA molecules are self-replicating, whereas RNA molecules are synthesized by a process called transcription.

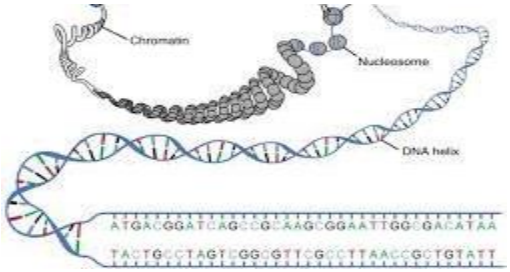
(g) While DNA contains deoxyribose, RNA contains ribose, characterized by the presence of the 2′-hydroxyl group on the pentose ring (Figure 5). This hydroxyl group make RNA less stable than DNA because it is more susceptible to hydrolysis.

(h) DNA and RNA molecules have different functions. DNA stores genetic information for the cell, whereas RNA codes for amino acids and acts as a messenger between DNA molecules and the ribosomes.

Lecture No.4:

Title of the lecture:

DNA Organization in prokaryotic and Eukaryotic cells



DNA ORGANIZATION IN PROKARYOTIC CELLS

A cell's DNA, packaged as a double-stranded DNA molecule, is called its **genome.** In prokaryotes, the genome is composed of a single, double-stranded DNA molecule in the form of a loop or circle (Figure 4.1). The region in the cell containing this genetic material is called a **nucleoid** (remember that prokaryotes do not have a separate membrane-bound nucleus). Some prokaryotes also have smaller loops of DNA called **plasmids** that are not essential for normal growth. Bacteria can exchange these plasmids with other bacteria, sometimes receiving beneficial new genes that the recipient can add to their chromosomal DNA. Antibiotic resistance is one trait that often spreads through a bacterial colony through plasmid exchange.

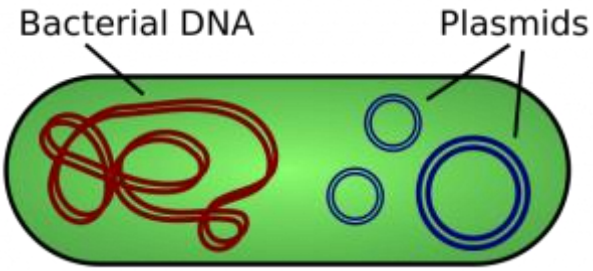


Figure (4.1): Bacterial DNA and plasmids are both circular.

The size of the genome in one of the most well-studied prokaryotes, *E. coli,* is 4.6 million base pairs (which would be approximately 1.1 mm in length, if cut and stretched out). So how does this fit inside a small bacterial cell?

The DNA is twisted by what is known as supercoiling. Supercoiled DNA is coiled more tightly than would be typically be found in a cell (more than 10 nucleotides per twist of the helix). If you visualize twisting a rope until it twists back on itself, you have a pretty

good visual of supercoiled DNA. This process allows the DNA to be compacted into the small space inside a bacterium.

DNA ORGANIZATION IN EUKARYOTIC CELLS

Eukaryotes have much more DNA than prokaryotes. For example, an *E. coli* bacterium contains roughly 3 million base pairs of DNA, while a human contains roughly 3 *billion*. In eukaryotes such as humans and other animals, the genome consists of several double-stranded linear DNA molecules (Figure 4.2), which are located inside a membrane-bound nucleus. Each species of eukaryotes has a characteristic number of chromosomes in the nuclei (plural of nucleus) of its cells. A normal human **gamete** (sperm or egg) contains 23 chromosomes. A normal human body cell, or **somatic** cell, contains 46 chromosomes (one set of 23 from the egg and one set of 23 from the sperm). The letter *n* is used to represent a single set of chromosomes; therefore, a gamete (sperm or egg) is designated *1n*, and is called a **haploid** cell. Somatic cells (body cells) are designated 2*n* and are called **diploid** cells.

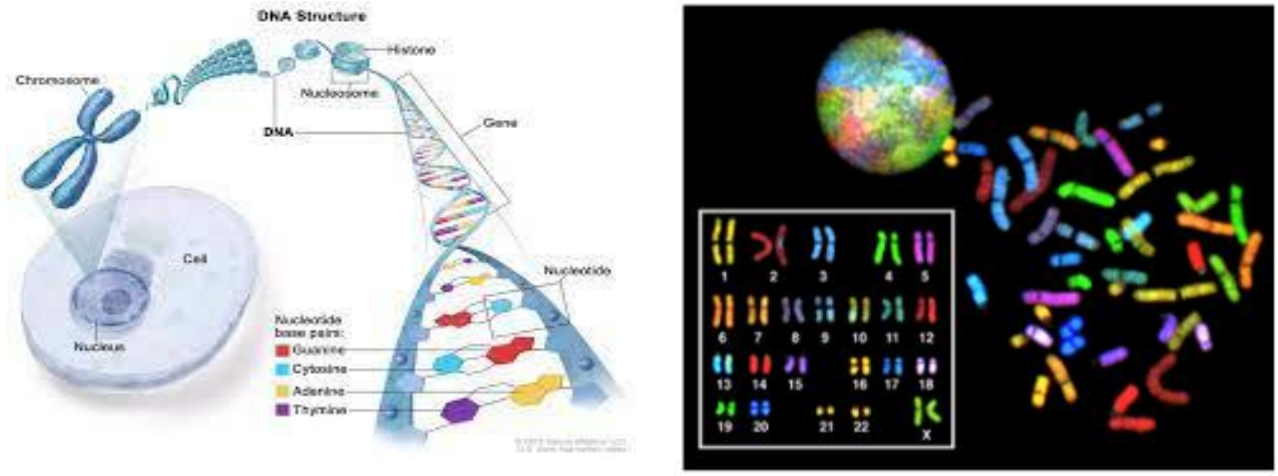
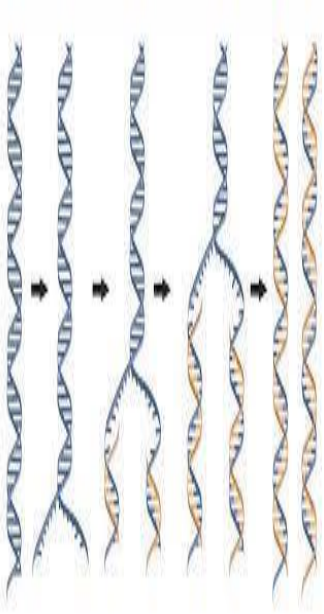


Figure (4.2): There are 23 pairs of homologous chromosomes in a female human somatic cell. The condensed chromosomes are viewed within the nucleus (top), removed from a cell in mitosis and spread out on a slide (right), and artificially arranged according to length (left); an arrangement like this is called a karyotype. In this image, the chromosomes were exposed to fluorescent stains for differentiation of the different chromosomes. A method of staining called "chromosome painting" employs fluorescent dyes that highlight chromosomes in different colors.

Lecture No.5:

The title of the Lecture: DNA Replication

DNA replication



The replication of the genome is essential for the continuity of life. The molecular mechanism is very similar in all groups of organisms. Although the basics of replication are already well understood, researchers are still focusing on questions relating to DNA replication. These questions not only deal with the understanding of a basic biological process, but also with related medical aspects. One attribute of living things is their ability to reproduce. The information required to pass on traits to the next generation is mainly stored in cellular DNA. Daughter and parent cells need to be equipped with an identical copy of DNA during cell division. "The molecular basis of this process is the replication of DNA". However, in 1958, Matthew Meselson and Franklin Stahl showed that newly replicated bacterial DNA consists of a new and an old DNA strand. In the 1970s and 1980s, further evidence was found relating to the mechanism of 'semiconservative' replication. Replication consists of three phases: initiation, elongation and termination. The basis of replication is the pairing of the four bases found in DNA: adenine pairs with thymine, cytosine with guanine. The process, which results in two DNA helices instead of one, is mediated by several dozen proteins.

However, the process is not the same at the end of the chromosomes, where a replication fork can no longer progress due to the lack of bases. At the site of the lagging strand where the DNA primase places the last RNA primer, the DNA polymerase is no longer able to continue replication. The terminal DNA segment cannot be replicated. The DNA strand gets shorter and shorter as the number of cell division rounds increases.

So, to protect themselves against the rapid shortening of the DNA, eukaryotic chromosomes possess sequence repeats (telomeres) at their extremities, which do not code for proteins. Since the

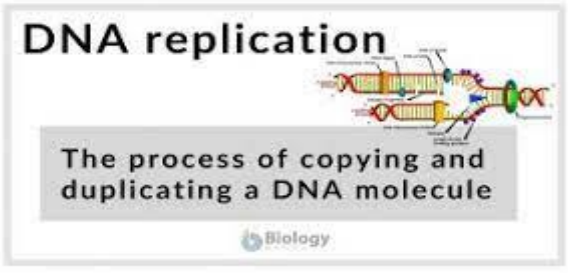
telomeres do not contain any important information, the key parts of the DNA are protected. The telomeres get shorter each time a cell divide. The length of these so-called telomere caps defines the number of possible divisions and hence the lifespan of a cell. Some cell types (for example maturing sex cells or certain tumor cells) contain the enzyme telomerase, which prevents telomere shortening and thus protects the cell from cell ageing and programmed cell death.

Order in the cells

A cell must at all costs prevent errors from occurring during the replication of DNA. The strict order of the copying process is therefore essential. In eukaryotic cells, the DNA is kept as clearly arranged as possible: genome regions that are not undergoing replication are densely packed in chromosomes. Scientists also assume that the chromosomes in eukaryotic cells are spanned over a cytoskeleton consisting of protein tubes and wires. The replication enzymes are bound to this so-called nuclear matrix and motor proteins pull the genome past them. It appears that bacterial cells have similar mechanisms.

All in a good time

Many bacteria divide once every thirty minutes, others replicate even faster. Eukaryotic cells only replicate their genome when new cells have to be created. This happens as a result of external signals, for example tissue loss or inflammation. The life cycle of eukaryotic cells underlies an accurately defined sequence of activities (cell cycle). However, any errors occur during the control of the cell cycle will coast cells to divide more quickly and more frequently as is the case with many cancer cells



The process of DNA replication

Replication is a complex process in which dozens of proteins, enzymes, and DNA structures take part; a single defective component can disrupt the whole process. The solution to this problem is central to replication. A huge amount of genetic information and an enormous number of cell divisions are required to produce a multicellular adult organism; even a low rate of error during copying would be fatal.

How is this extraordinarily accurate and rapid process accomplished?

1- The complementary nature of the two nucleotide strands in a DNA molecule suggested that, during replication, each strand can serve as a template for the synthesis of a new strand.
2- 2- The specificity of base pairing (adenine with thymine; guanine with cytosine) implied that only one sequence of bases can be specified by each template, and so two DNA molecules built on the pair of templates will be identical with the original.
3- Eukaryotic DNA is replicated in more than replication foci.

(a) DNA Synthesis Begins at Replication Origins

The process of DNA replication begun by initiator proteins that bind to the DNA and separate the two strands apart, breaking the hydrogen bonds between the bases. The positions at which the DNA is first opened are called **replication origin**. They are usually marked by a particular sequence of nucleotides figure (4.1).

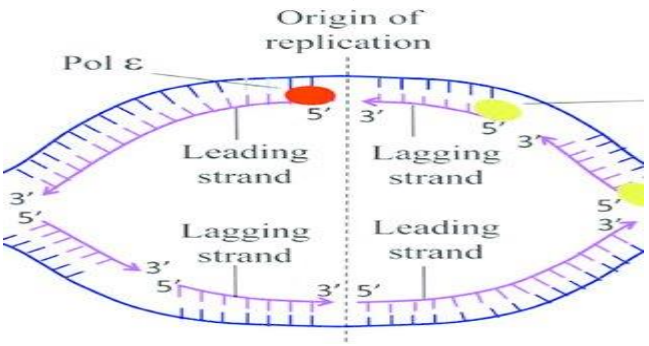


Figure (4.1):
Schematic diagram of origin of replication in human.

(b) DNA Synthesis Occurs at Replication Forks

During the DNA replication it is possible to see Y-shaped junctions in the DNA, called "the replication forks". See figure (4.2) At these forks, the replication machine is moving along the DNA, opening up the two strands of double helix and using each strand as a template to make new daughter strand. Two replication forks are formed starting from each replication origin, and they move away from the origin in both directions, unzipping DNA as they go.

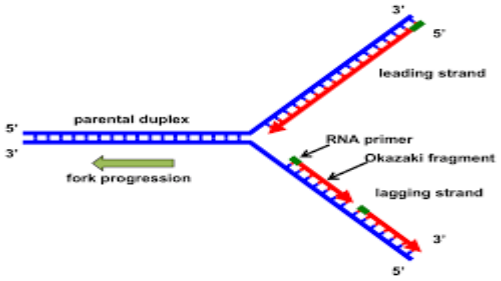


Figure (4.2):
Simple replication fork

(c) Elongation of a new strand

Elongation of new DNA at replication fork is catalyzed by enzymes called DNA polymerase. As nucleotides align with complementary bases along "old" template strand of DNA, they are added by polymerase, one by one, to the growing end of the new DNA strand. The rate of elongation is about 50 nucleotides per second in human cells. As each monomer joins the growing end of DNA strand, it losses two phosphate groups. Hydrolysis of phosphate is the exergonic reaction that drives polymerization of nucleotides to from DNA.

Replication Requirements

Although the process of replication includes many components, there are four components very essential for the process, they include: -

1. A template consisting of single-stranded DNA;(in eukaryotes, the replication fork forms at the replication initiation point (RIP) where the helicase enzymes unwinds the DNA double helix. The enzyme DNA polymerase, situated at each replication fork, builds a new DNA strand (template) by adding nucleotides in the 5' to 3'

direction. Also performs proof-reading and error correction. DNA polymerase replicates a DNA template with remarkable fidelity.

2. Substrates to be assembled into a new nucleotide strand; the four types of the deoxyribonucleotide – triphosphate (dCTP, dTTP, dGTP, dATP). These substrates are provided from two pathways for nucleotide biosynthesis (the de novo pathway: nucleotides are constructed from simple precursors and the salvage pathways: recovery and recycling of nucleotides obtained in the diet).

*In humans, dietary nucleotide bases are rarely incorporated into nucleotides. As a result, humans must synthesize their own nucleotide bases. (With the exception of a few parasitic prokaryotes, all organisms can synthesize nucleotides.) Although all nucleated eukaryotic cells can synthesize nucleotides, most human synthesis occurs in the liver. Nucleotide synthesis is tightly regulated. Nucleotide synthesis is somewhat expensive in that the pathways use several molecules with other uses. In addition, although pyrimidines can be degraded into standard metabolic intermediates, purine catabolism does not alter the basic purine structure, and excessive levels of purines can be toxic.

3. Enzymes including (DNA helicases- unwinds the double helix, DNA polymerase- build a new DNA and performs proof- reading error correction, primase- provide a primer and DNA ligase that joins the Okazaki fragments together) and other proteins that "read" the template and assemble the substrates into a DNA molecule.

4. Primer (short sequence of DNA or RNA) in the template with a free –OH end. This short sequence is provided by the primase enzyme to provide a starting point for the DNA polymerase to begin synthesis of the new DNA strand.

The Mechanism of Replication

DNA replication takes place in four stages: initiation, unwinding, elongation, and termination, illustrated in (Figure 4.3).

1- Initiation of replication Eukaryotic cells utilize thousands of replication origins due to the large size of their genome, and so the entire genome can be replicated in a timely manner. The use

of multiple origins, however, creates a special problem in the timing of replication: the entire genome must be precisely replicated once and only once in each cell cycle so that no genes are left unreplicated and no genes are replicated more than once. How does a cell ensure that replication is initiated at thousands of origins only once per cell cycle? The precise replication of DNA is accomplished by the separation of the initiation of replication into two distinct steps: -

◄ -In the first step, the origins are licensed, meaning that they are approved for replication. This step is early in the cell cycle when a replication licensing factor attaches to an origin.

◄In the second step, initiator proteins cause the separation of DNA strands and the initiation of replication at each licensed origin. The key is that initiator proteins function only at licensed origins. As the replication forks move away from the origin, the licensing factor is removed, leaving the origin in an unlicensed state, where replication cannot be initiated again until the license is renewed. To ensure that replication takes place only once each cell cycle, the licensing factor is active only after the cell has completed mitosis and before the initiator proteins become active.

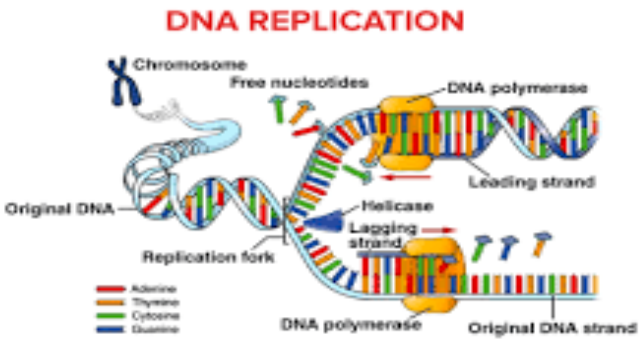
2. Unwinding Several helicases that separate double-stranded DNA have been isolated from eukaryotic cells, as have single strand- binding proteins and topoisomerases. These enzymes and proteins are assumed to function in unwinding eukaryotic DNA.

3. Elongation Eukaryotic cells contain a number of different DNA polymerases that function in replication, recombination, and DNA repair. However, through the elongation process, the DNA polymerase α _, which contains primase activity, initiates nuclear DNA synthesis by synthesizing RNA primer, followed by a short string of DNA nucleotides. After DNA polymerase _ has laid down from 30 to 40 nucleotides, DNA polymerase δ _ completes replication on the leading and lagging strands. DNA polymerase β _ does not participate in replication but is associated with the repair and recombination of nuclear DNA.

However, the following concepts review the mechanism of eukaryotic replication:

1. Replication is always semiconservative.
2. Replication begins at sequences called origins.
3. DNA synthesis is initiated by short segments of RNA called primers synthesized by primase enzyme to make a template with free 3- OH end to be used by the DNA polymerase that add nucleotides in the new strands.
4. The elongation of DNA strands is always in the (5′→3′) direction.
5. New DNA is synthesized from dNTPs; in the polymerization of DNA, two phosphates are cleaved from a dNTP and the resulting nucleotide is added to the 3-OH group of the growing nucleotide strand.
6. Replication is continuous on the leading strand and discontinuous on the Lagging strand to create Okazaki fragments, these fragments are made of 1000 nucleotides, after replication stops, these fragments undergo excision process where DNA polymerase removes the primers from these fragments and are ligated by DNA ligase together to form an elongated strand complementary to their template.
7. New nucleotide strands are made complementary and antiparallel to their template strands.
8. Replication takes place at very high rates and is accurate, due to Precise nucleotide selection, proofreading, and repair mechanisms.

4. Termination In some DNA molecules, replication is terminated whenever two replication forks meet. In others, specific termination sequences block further replication.

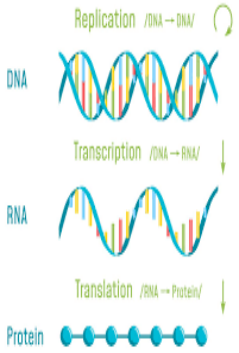


Lecture No.6:

Title of the lecture:

DNA Transcription & Post transcriptional Modification

DNA Transcription



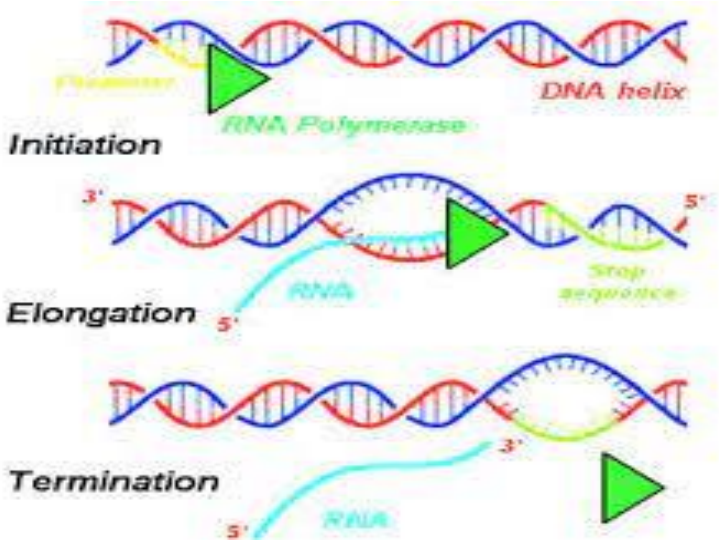
Since the food that enters our bodies cannot be used as it is. Thus, it should go through a number of chemical processes to be digested. Thereby, the chemical processes in our stomach uses different proteins and enzymes to break down the food particles into usable nutrients our cells can absorb. All of the instructions needed for these proteins to be manufactured are stored in our DNA. The DNA contain genes which are a string of nucleotides that encode for the information required for protein in a process called (Gene expression). The process of gene expression is divided into two processes (transcription and translation). In eukaryotic cell the process is carried out in the nucleus. DNA transcription requires that sequences on DNA are accessible to RNA polymerase and other proteins. However, to achieve this; the chromatin structure is modified before transcription so that; the DNA is in a more open configuration and is more accessible to the transcription machinery.

Thus, in simple terms, **DNA transcription** is the process of "copying" a segment of DNA into RNA. It is the first step of **gene expression**, where the instructions stored in our genome are read to eventually create proteins.

However, Transcription occurs in three distinct steps, governed primarily by an enzyme called **RNA polymerase**.

1. **Initiation:** RNA polymerase binds to a specific DNA sequence called a **promoter**. This signals the DNA to unwind so the enzyme can "read" the bases.
2. **Elongation:** The enzyme moves along the DNA strand. It creates a matching RNA strand by pairing complementary bases.
    - *Note:* In RNA, Uracil (U) replaces Thymine (T).

3. **Termination:** Once the enzyme reaches a "stop" signal (terminator), the process ends, and the newly formed RNA strand is released



During initiation, the promoter region of the gene acts as a recognition site for RNA polymerase to bind. Figure 5.1 ((this is where the majority of gene expression is controlled by either permitting or blocking the access of RNA polymerase to this site. Moreover, another type of controlling elements also plays an important role in the transcription initiation; they are called ((the enhancers)).

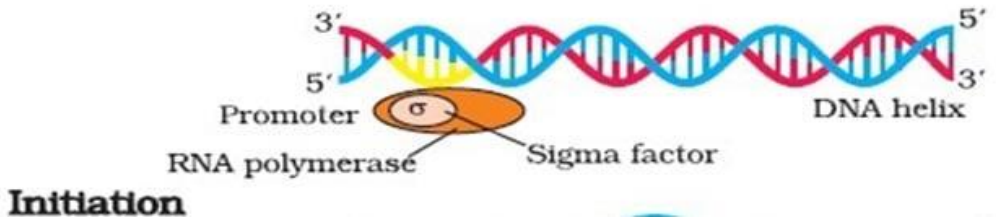


Figure (5.1): Initiation in DNA Transcription

An enhancer, it can affect the transcription of genes that are thousands of nucleotides away, and their positions relative to start sites can vary. See figure 5.2.

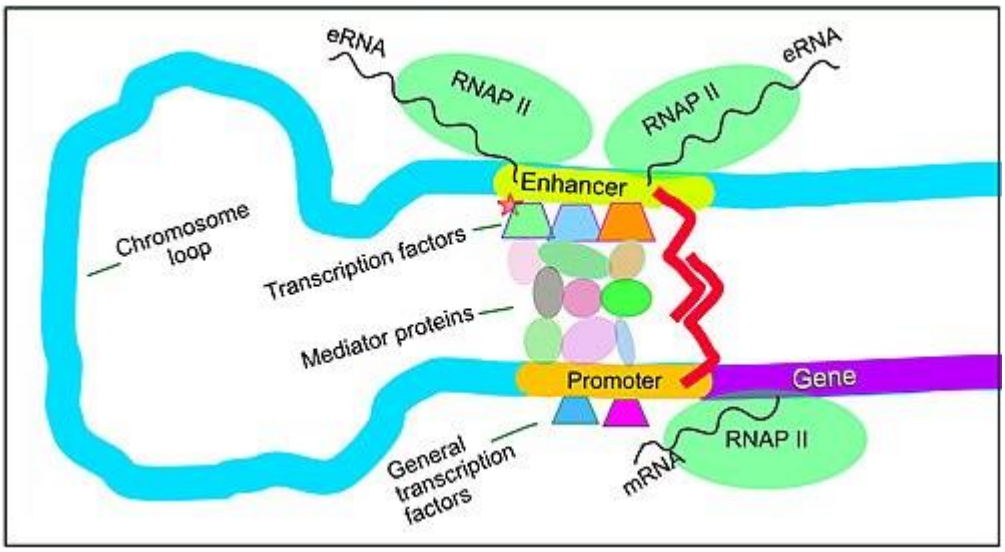


Figure (5.2): Enhancer driven initiation of Transcription.

Binding of RNA polymerase to this site cause the DNA double helix to unwind and open. Thereby, during elongation step.

In the elongation step, the RNA polymerase slides along the template DNA strand. As the complementary bases pair up in the 5 to 3 direction, the DNA transcription elongation is in the direction of 5′ → 3 ′ and Transcription begins at the start site, which is determined, the consensus sequences. A short stretch of DNA is unwound near the start site and used as a template this is called "the sense strand", it determines the amino acid sequence in the produced protein. On the other hand, the other strand is called "the antisense strand" that is not transcribed into mRNA but it is used as a template in the DNA replication for the synthesis of the sense strand. figure 5.3.

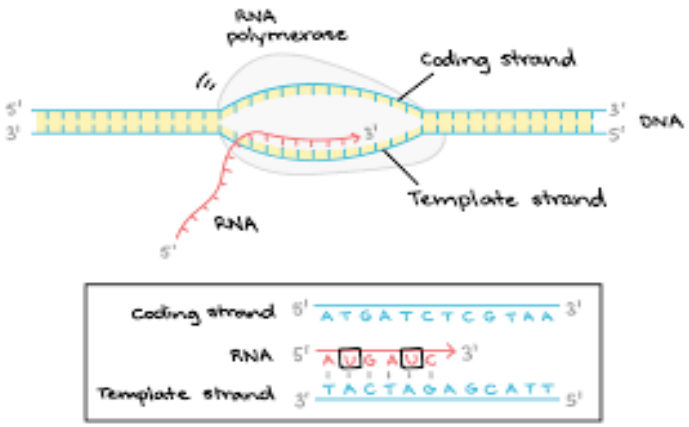


Figure (5.3): Elongation in DNA Transcription

Once RNA polymerase reaches to the termination site of the gene. Then, RNA polymerase, the DNA molecule and the messenger RNA dissociate from each other pronouncing the termination of the transcription see figure 5.4.

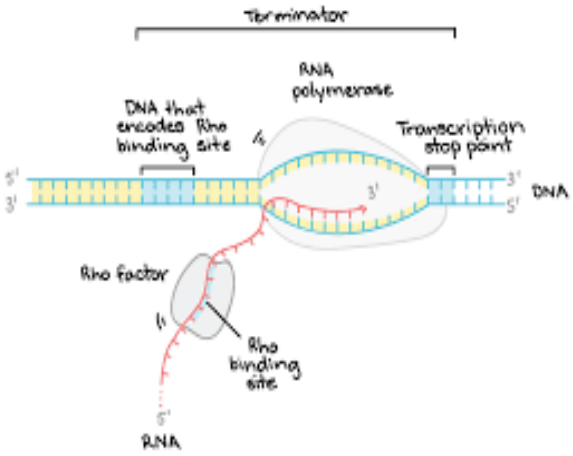


Figure (5.4): Elongation in DNA Transcription

However, three types of RNA polymerase transcribe different RNA molecules.

1- RNA polymerase I = rRNA
2- RNA polymerase II = mRNA
3- RNA polymerase III = tRNA + rRNA

consequently, RNA molecule will result from the transcription process that differs between prokaryotes, figure 5.6 and eukaryotes as figure 5.7 showed.

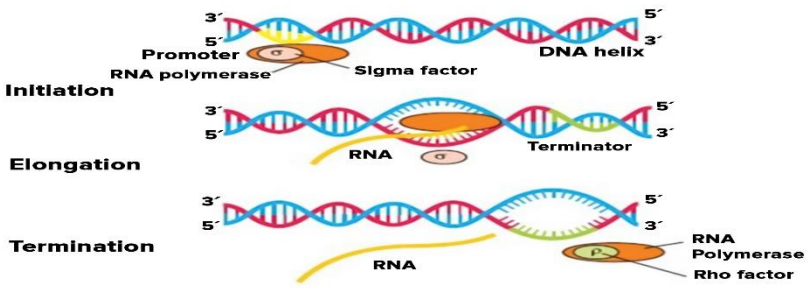


Figure (5.): DNA Transcription in prokaryotes

The resulting mRNA in eukaryotes is called ((pre-mRNA)) because it contains ((Exons – the protein coding regions)) and ((Introns- the non-coding regions)). This primary transcript needs some processing to become mature mRNA. See figure 5.7

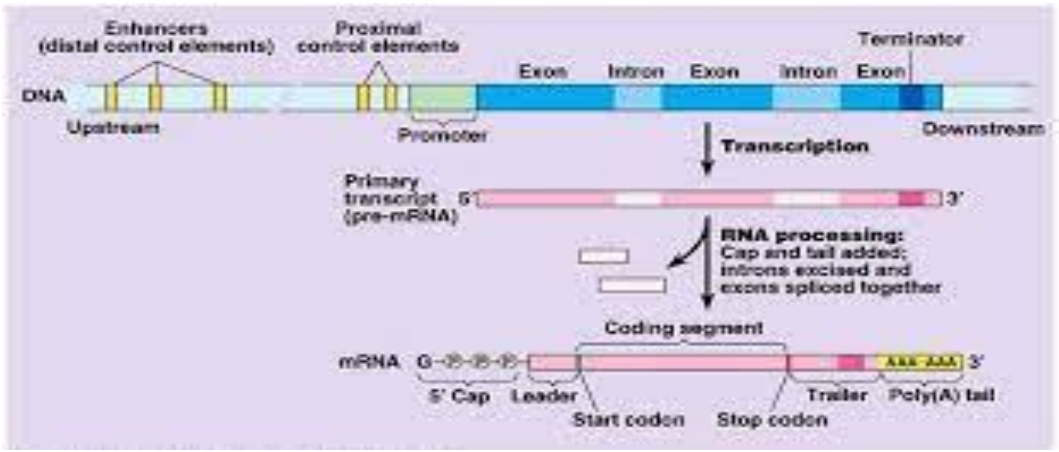
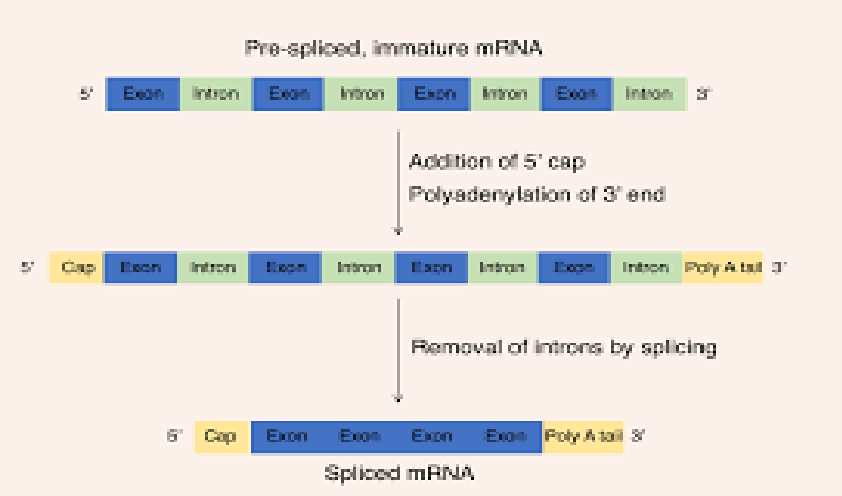


Figure (5.7): DNA Transcription in eukaryotes

Post transcriptional modification

Moreover, the produced primary transcript (pre-mRNA) is not ready to be transformed to the ribosomes for translation. Yet, it should be subjected to the "Post – transcriptional modifications". ((Post Transcriptional Modification)) Which comprises: -



1. The addition of "Cap" This process, known as mRNA capping, is highly regulated and vital in the creation of stable and mature messenger RNA able to undergo translation during protein synthesis.

Capping is accomplished through the unusual 5′ to 5′ triphosphate linkage of a guanine nucleotide to mRNA. This guanine is methylated on the 7′ position directly after capping by a methyl transferase. It is referred to as a 7-methylguanylate cap. The 5′ cap has four main functions:

a. Regulation of nuclear export
b. Prevention of degradation by exonucleases
c. Promotion of translation
d. Promotion of intron excision

2. Polyadenylation is the addition of a poly (A) tail to a messenger RNA. It consists of multiple adenosine monophosphates. In eukaryotes, this process protects the mRNA molecule from enzymatic degradation in the cytoplasm and aids in transcription termination, export of the mRNA from the nucleus, and translation. See (figure 5.8).

3. The excision of Introns This process is called (Intron splicing) and is performed by a complex made of proteins and RNA called (a spliceosome). This complex removes the intron segments and join the adjacent exons to produce mature mRNA strand that can leave the nucleus through a nuclear pore and enter the cytoplasm to begin translation.

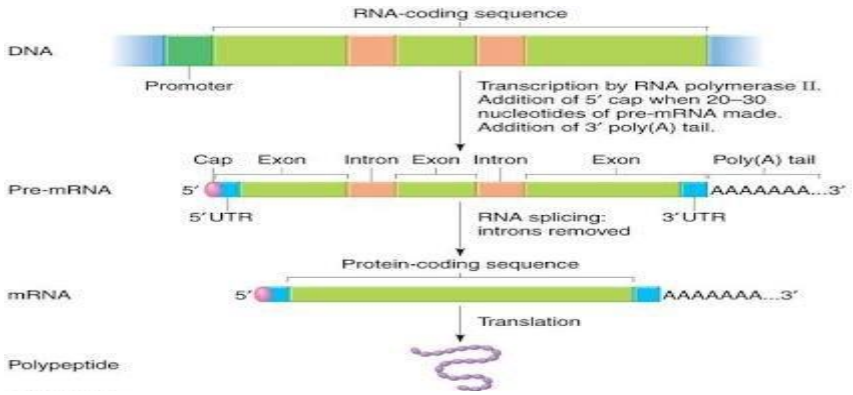
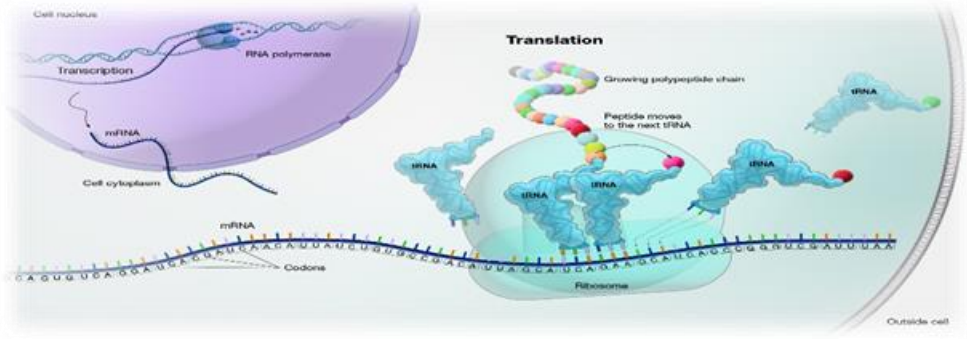


Figure (5.8): Summary of Eukaryotic DNA Transcriptional modification and mRNA processing.

Lecture No7:

Title of the lecture: DNA Translation



How does the cell convert DNA into working proteins?

As you Knew before, the genes in our DNA encode for protein molecules, which are the "work horses" of the cell, carrying out all the functions necessary for life. For example, enzymes, including those that metabolize nutrients and synthesize new cellular constituents, as well as DNA polymerases and other enzymes that make copies of DNA during cell division, are all proteins.

During translation, which is the second major step in gene expression, the mRNA is "read" according to the genetic code which relates the DNA sequence to the amino acid sequence in proteins. Each group of three bases in mRNA constitutes a codon (every three bases specify a codon) and each codon specifies a particular amino acid where the genetic code includes ((64 codons)). The mRNA sequence is thereby used as a template to assemble—in order—the chain of amino acids that form a protein where there are four special codons in the genetic code; one codes for Start (AUG) and three code for Stop (UGA, UAG, UAA).

Where Translation Occurs?

Within all cells, the translation machinery resides within a specialized organelle called the ribosome.

In eukaryotes, mature mRNA molecules must leave the nucleus and travel to the cytoplasm, where the ribosomes are located. Eukaryotic ribosomes have two unequal subunits, designated small subunit (40S) and large subunit (60S) according to their sedimentation coefficients. Both subunits contain dozens of ribosomal proteins arranged on a scaffold composed of ribosomal RNA (rRNA). See (figure 6.1).

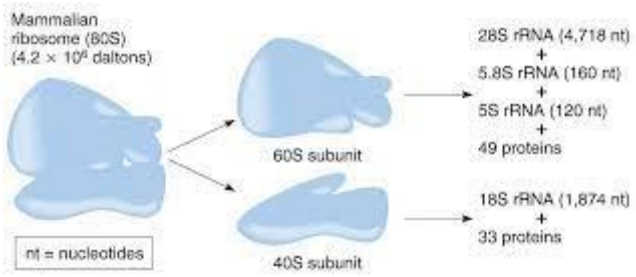


Figure (6.1): Eukaryotic 80S ribosome

How many ribosomes are in eukaryotes?

A single actively replicating eukaryotic cell, for example, may contain as many as 10 million ribosomes. In the bacterium Escherichia coli (a prokaryote), ribosomes may number as many as 15,000, constituting as much as one-quarter of the cell's total mass

Why does 60S and 40S make 80S?

The 80S ribosome is identified according to its sedimentation coefficients in Svedberg units. Since it is a measure of sedimentation time, it is not simply a sum of subunits. Svedberg units are a nonlinear function, and 60S and 40S are two separate subunits that together sediment at 80S

On the other hand, Bacteria and archaebacteria have smaller ribosomes, termed 70S ribosomes in their cytoplasm, which are composed of a small 30S subunit and large 50S subunit. The "S" stands for Svedberg's, a unit used to measure how fast molecules move in a centrifuge.

What is difference between 70S and 80S?

The 70S ribosomes are smaller and have a simpler structure than the 80S ribosomes. The 70S ribosomes are composed of a small number of proteins and RNA molecules, while the 80S ribosomes are composed of a larger number of proteins and RNA molecules. The 80s ribosomes are also more stable than the 70S ribosomes. Figure (6.2).

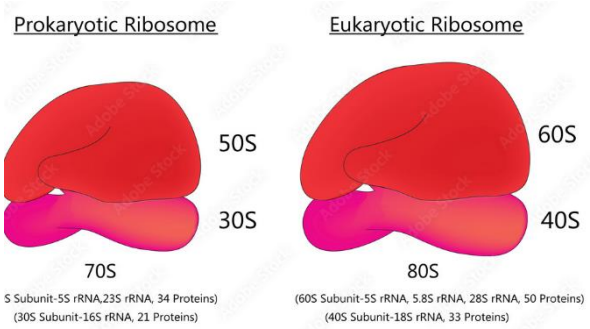


Figure (6.2): Eukaryotic 80S ribosome VS Prokaryotic 70S ribosome

Still, each subunit of the ribosomes exist separately in the cytoplasm, but the two join together on the newly transcribed mRNA molecule in order to start translation.
Translation consists of three steps:
1- Initiation
2- Elongation
3- Termination

1- Initiation

Translation begins when the mRNA strand binds to the small ribosomal subunit upstream the start codon, "AUG" start codon. See figure below.

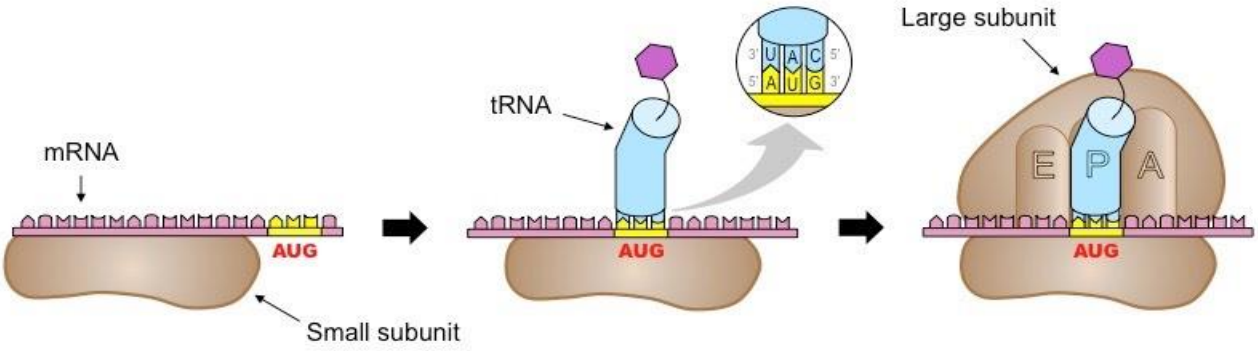


Figure (6.3): Initiation of translation

Interestingly, the "start codon- AUG" alone is not enough to initiate translation, it needs other factors to initiate the translation chain whether in eukaryotes or in prokaryotic cells.

In eukaryotes, there is an area near the 5' end of the molecule that is known as the untranslated region (UTR) or leader sequence or more specifically called "Kozak box".

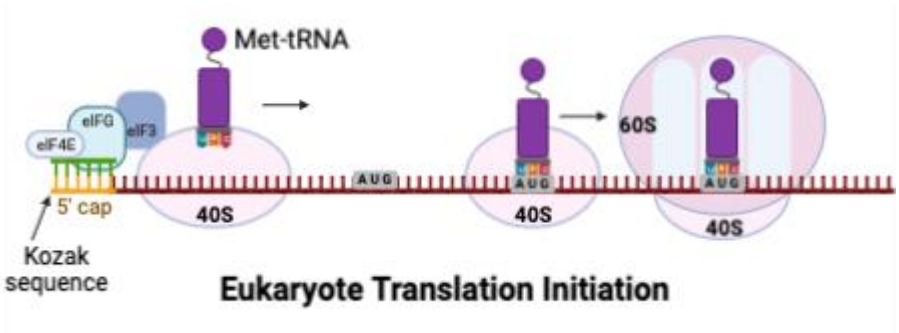


Figure (6.4): Kozak sequence

The Kozak sequence is named after the scientist Marilyn Kozak who discovered it. It is a nucleic acid motif that initiates protein translation in eukaryotic mRNA. A start codon is located within the Kozak sequences where the assembly of ribosomes begins.

What is the Kozak sequence in human cells?

The Kozak sequence directs the pre-initiation complex (PIC) and ribosome to the translation initiation site (start codon) and mediates ribosome assembly ensuring the correct protein sequence is translated. The consensus Kozak sequence is generally considered as GCCGCCACCATGG, where ATG is the start codon.

Is Kozak sequence only in eukaryotes?

The scanning mechanism of initiation, which utilizes the Kozak sequence, is found only in eukaryotes and has significant differences from the way bacteria initiate translation. This portion of mRNA is located between the start codon (AUG) and the first nucleotide that is transcribed in the coding region, and it does not affect the sequence of amino acids in a protein.

So, what is the purpose of the UTR? It turns out that, the leader sequence is important because it is a protein translation initiation site found on the mRNA of eukaryotes.

On the other hand, in bacteria, this site is known as the Shine-Dalgarno box (AGGAGG), after scientists John Shine and Lynn Dalgarno who first characterized it.

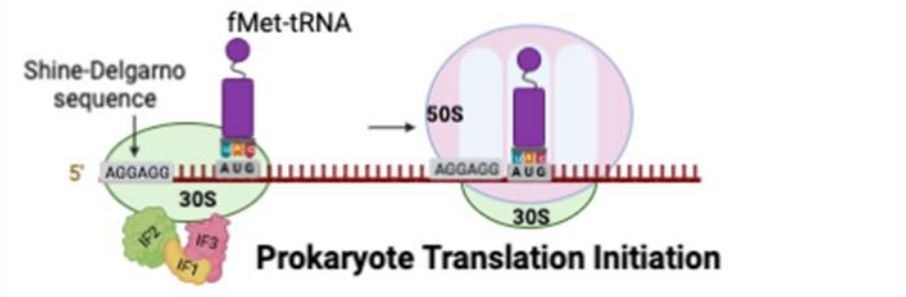


Figure (6.5): Shine-Dalgarno box

The Shine–Dalgarno (SD) sequence motif facilitates translation initiation and is frequently found upstream of bacterial start codons.

The translation of mRNA begins with the formation of a complex on the mRNA where, three initiation factor proteins (known as IF1, IF2, and IF3) bind to the small subunit of the ribosome (40S) Then, a methionine-carrying tRNA will bind to the mRNA, near the AUG start codon, forming the initiation complex.

► Although methionine (Met) is the first amino acid incorporated into any new protein; it is not always the first amino acid in mature proteins. In many proteins, methionine is removed after translation. In fact, if a large number of proteins are sequenced and compared with their known gene sequences.

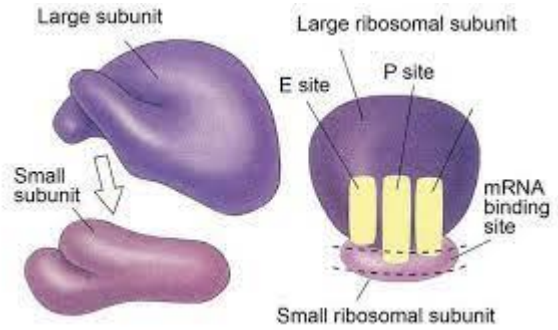
However, each amino acid is brought to the ribosome by a specific tRNA molecule and the type of amino acid depends on the anticodon sequence of the tRNA molecule where a complementary occurs between the codon of the mRNA and the anticodon of the tRNA.

After the initiation tRNA binds to the mRNA strand, the large rRNA subunit (60S) binds to the mRNA to form the translation complex and the ignition then is completed.

2- Elongation

In the large ribosomal subunit, there are three distinct regions; called (E, P, A) sites. See figure (6.6).

Figure (6.6): Ribosome structure



Where,
E= Exit site
P= Peptide bond- tRNA binding site
A= Amino acid tRNA binding site

During elongation, individual amino acids are brought to the mRNA strand by tRNA molecule through complementary base pairing between the codons (in mRNA) and the anticodons (in tRNA) where it corresponds to a particular amino acid.

Elongation occurs in these steps: Figure (6.7)

1- A charged tRNA molecule binds to the A site and a peptide bond forms between its amino acid and the one of the initiators tRNA at the P site.

2- The complex slides one codon to the right where the uncharged tRNA exits from the E site.

4- The A site is open now to accept a new charged tRNA molecule according to the codon of the mRNA and Elongation continues until a stop codon is reached.

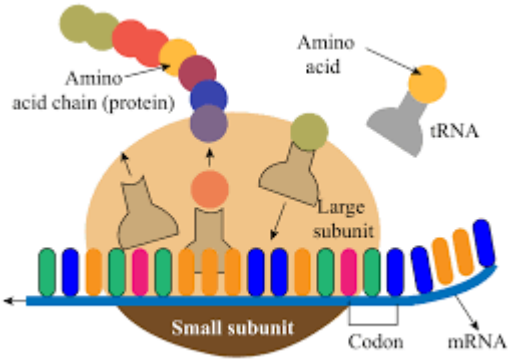


Figure (6.7): Elongation in translation

3- Termination

A release factor binds to the A site at a stop codon and the polypeptide is released from the tRNA in the P site and the entire complex dissociates and can reassemble to begin the process again where the purpose of translation is to produce polypeptides quickly and accurately.

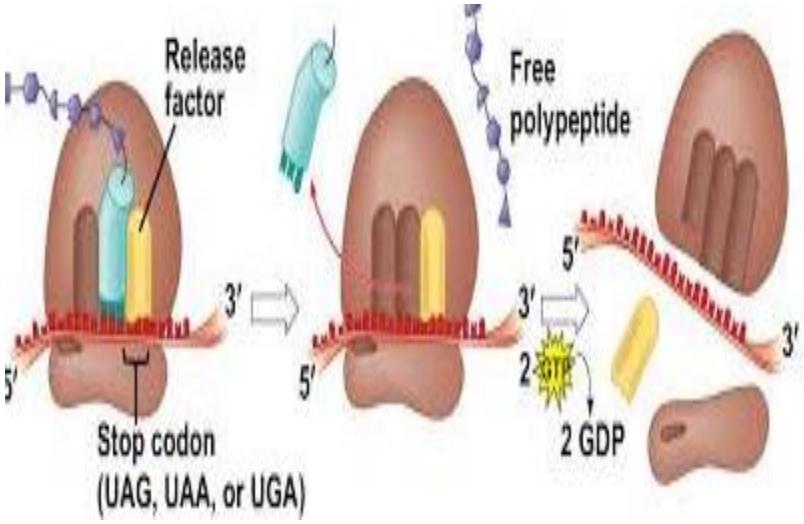


Figure (6.8): Termination of translation

The difference between prokaryotes and eukaryotes in translation

Diff.	Prokaryotes	Eukaryotes
1	It is a continuous process as both transcription and translation processes occur in the cytoplasm	It is a discontinuous process as transcription occurs in the nucleus and translation occurs in the cytoplasm
2	Little mRNA processing	Extensive mRNA processing of three steps
3	Polycistronic mRNA (synthesis information of many proteins under a single control)	**Monocistronic mRNA** (synthesis information of only one protein under a single control)
4	It occurs on 70S ribosomes	It occurs on 80S ribosomes
5	Ribosome small subunit binds to Shine Dalgarno sequence	Translation initiation occurs at Kozak box
6	Initiator tRNA is (fMet/tRNA) which codes for formyl methionine	Initiator tRNA is (Met/tRNA) which codes for methionine
7	Simple initiation of translation	Complex initiation process involves many factors
8	Termination involves the release of many factors	Only one release factor recognizes all three stop codons
9	Low number of post transcriptional modifications occur in the cytoplasm	Extensive post translational modifications occur in the Endoplasmic reticulum and Golgi apparatus before becoming a fully functional protein
10	mRNA half life is short (few seconds) unstable	mRNA half life is longer (few hours to few days) quite stable
11	It is a faster process Add 17-21 amino acids /sec.	Comparatively slower Add 6-9 amino acids/sec.

Protein Synthesis

Protein synthesis is the process whereby biological cells generate new proteins; and the process is balanced by the loss of cellular proteins via degradation or export and the need to a new protein. This process is accomplished through two processes (DNA transcription and Translation); where the translation process plays an essential part of the biosynthetic pathway.

Post translation modifications

After a protein is built during translation, it is often like a "raw" product fresh off an assembly line. **Post-translational modification (PTM)** is the process of refining, folding, and chemically tweaking that protein so it can actually do its job.

Without these modifications, many proteins would remain inactive or end up in the wrong part of the cell

Common Types of Modifications

Cells use a variety of chemical "tags" to change how a protein behaves. Here are the most frequent types:

- **Phosphorylation:** The addition of a phosphate group. This acts like an **on/off switch**, frequently used to activate enzymes or send signals within a cell.
- **Glycosylation:** The attachment of carbohydrate (sugar) chains. These help with **cell-to-cell recognition** and are a hallmark of proteins found on the cell membrane.
- **Lipidation:** Attaching a lipid (fat) molecule. This usually acts as an **anchor**, tethering the protein to the fatty cell membrane.
- **Ubiquitination:** Adding a small protein called ubiquitin. This is essentially a **"death tag"** that marks a damaged or unneeded protein for destruction.
- **Proteolysis:** The "clipping" of the protein. Some proteins are created in an inactive, long form and must be cut by enzymes to become active (e.g., insulin).

Where does this happen?

Most PTMs occur in two specific organelles:

1. **Endoplasmic Reticulum (ER):** Mainly handles folding and initial sugar attachments.
2. **Golgi Apparatus:** Acts as the "post office," adding final chemical tags and sorting proteins for their final destination.

Why PTMs Matter: A Comparison

Feature	Before Modification	After Modification
Structure	Linear or loosely folded chain.	Complex, 3D functional shape.
Activity	Usually inactive (pro-protein).	Fully functional and active.
Location	Floating in the cytoplasm/ER.	Targeted to a specific organelle or secreted.
Lifespan	Unregulated.	Regulated (can be marked for recycling).

Significance of studying PTMs:

Addiction

A major feature of addiction is its persistence. The addictive phenotype can be lifelong, with drug craving and relapse occurring even after decades of abstinence.

Yet, post-translational modifications in addiction involve epigenetic alterations of histone protein tails in specific regions of the brain of the addictive person. Once particular post-translational epigenetic modifications occur, they appear to be long lasting "molecular scars" that may account for the persistence of addictions.

Inhibitors of translation

A translation or a protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins.

However, there are numbers of antibiotics act by inhibiting translation. These include clindamycin, anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, and puromycin.

Prokaryotic ribosomes have a different structure from that of eukaryotic ribosomes, and thus some of these antibiotics can specifically target bacterial infections without any harm to a eukaryotic host's cells.

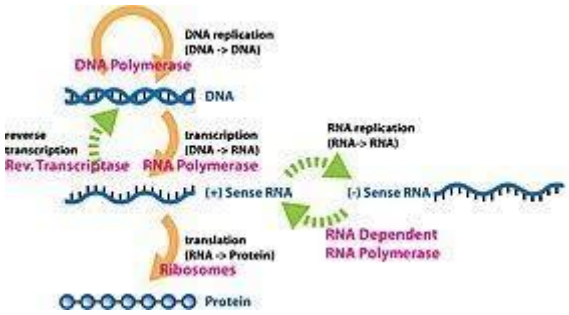
On the other hand, a natural product, namely (nagilactone C) produced from Podocarpus trees, were identified and characterized as novel protein synthesis inhibitors in humans and animals. This compound is specific for the eukaryotic translation apparatus inhibition and interfere with translation elongation.

Lecture No.8:

Title of the lecture:

Gene Expression

Expression Gene expression term refers to the process of converting the genetic information stored in the gene into a functional protein. This process is accomplished throughout two basic stages in both eukaryotes and prokaryotes; named (transcription and translation) where the genetic information present in the DNA are transcribed into mRNA molecule and then, translated into proteins by the ribosomes.



At the simplest level, a gene could be defined as a unit of information that encodes a genetic characteristic. It is about a specific nucleotide sequences present in the DNA molecule representing the genetic unit in living organisms.

However, there are differences in gene expression between prokaryotes and eukaryotes where prokaryotic gene expression (both transcription and translation) occurs within the cytoplasm of a cell due to the lack of a defined nucleus; thus, the DNA is freely located within the cytoplasm. While, Eukaryotic gene expression occurs in two sites; the nucleus (transcription) and cytoplasm (translation).

Moreover, since the genetic information is encoded in the molecular structure of the DNA (genes). The difference in gene arrangement between prokaryotes and eukaryotes was also situated considering gene expression:

In prokaryotes, the genome is composed of a single, double-stranded DNA molecule in the form of a loop or circle. The region in the cell containing this genetic material is called a nucleoid. Some prokaryotes also have smaller loops of DNA called plasmids that are not essential for normal growth. In addition, the prokaryotic gene structure consists of operons and clusters of several functionally-related genes, whereas the eukaryotic gene structure does not contain operons. Figure (7.1)

Figure (7.1): Gene structure in prokaryotes

While, in eukaryotes, the store house of genetic information within the cell are chromosomes, which consist of DNA and associated proteins, figure (7.2). The cells of each species have a characteristic number of chromosomes each carries a large number of genes. Many genes encode traits by specifying the structure of proteins. Genetic information is first transcribed from DNA into RNA, and then RNA is translated into **the amino acid sequence of a protein. In a process called "the Central Dogma of Life".**

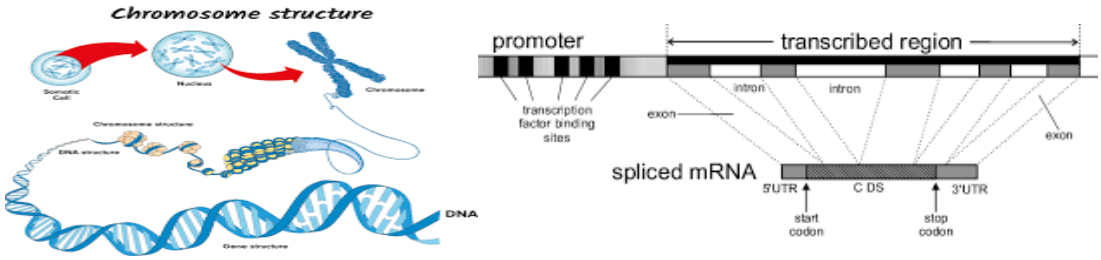


Figure (7.2): Gene assembly in eukaryotes

Characteristics of Eukaryotic gene structure:

All genes contain a coding region, which is split on the upstream of what is conventionally called promoter which is (by definition, the site for the assembly of transcriptional apparatus and its accessory factors, where they bind to it and open the DNA to initiate transcription) see figure (7.3).

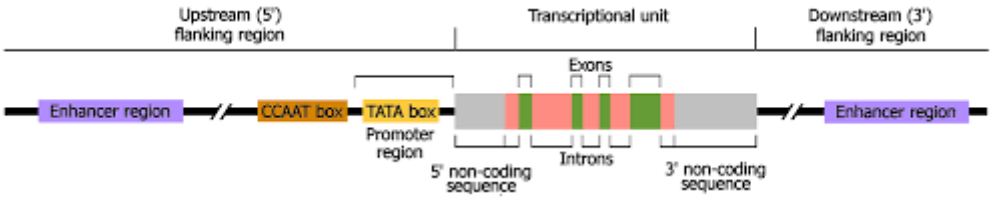


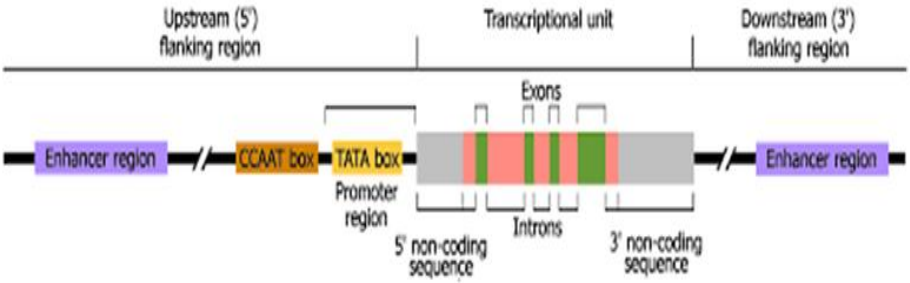
Figure (7.3): Organization of eukaryotic gene region

The genomes of most eukaryotes are larger and more complex than those of prokaryotes (Figure 4.1). This larger size of eukaryotic genomes is not inherently surprising, since one would expect to find more genes in organisms that are more complex. However, the genome size of many eukaryotes does not appear to be related to genetic complexity. For example, the genomes of salamanders and lilies contain more than ten times the amount of DNA that is in the human genome. There are however, certain regions in the DNA which contribute to the large size of DNA:

1- large amounts of noncoding DNA are also found within most eukaryotic genes. Such genes have a split structure in which segments of coding sequence (called exons) are separated by noncoding sequences (intervening sequences, or introns).
2- Another factor contributing to the large size of eukaryotic genomes is that some genes are repeated many times. Whereas most prokaryotic genes are represented only once in the genome, many eukaryotic genes are present in multiple copies, called **gene families**.
3- A substantial portion of eukaryotic genomes consists of highly repeated noncoding DNA sequences. These sequences, sometimes present in hundreds of thousands of copies per genome
4- Other repetitive DNA sequences are scattered throughout the genome rather than being clustered as tandem repeats. These sequences are classified as **SINEs** (short interspersed elements) or **LINEs** (long interspersed elements).

In the same context, in eukaryotes, structural genes are not sequentially placed. Each gene is instead composed of coding exons and interspersed non-coding introns. Regulatory sequences are typically found in non-coding regions upstream and downstream from the gene.

As figure (7.3) showed,



Outside the transcriptional area, the **5' flanking region** ("***upstream***") gene region includes the **'CAT box'** and **'TATA box' promoters** required for **RNA polymerase recognition** prior to transcription. **Enhancers** that regulate occurrence,

timing, and amount of transcription occur in both the upstream region and the **3' flanking region** ("***downstream***") region; multiple enhancers may occur many hundreds of nucleotides upstream.

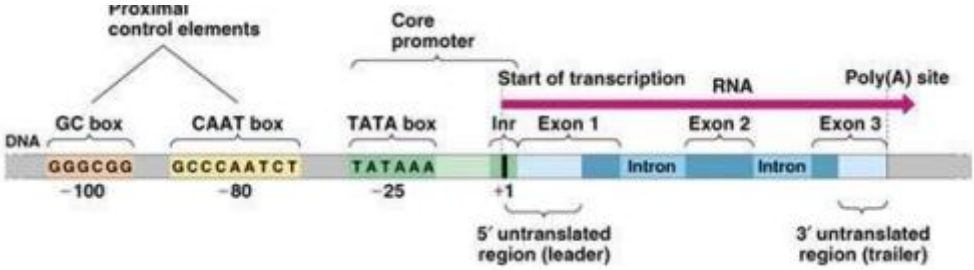


Figure (7.4): Schematic representation of eukaryotic gene

The components of the promoter, depending upon, which acts as first recognition sequence for the assembly of RNA-polymerase complex, without which enzymes won't assemble. The start point itself is bracketed by a set of sequences called "the TATA box" which is a sequence within the promoter core. Besides, there are other factors binding to specific sequence boxes increase the efficiency of transcription depending on the type of RNA polymerase enzyme as Eukaryotes have three different classes of RNA polymerases, such as RNA polymerase I, RNA polymerase II and RNA polymerase III and each of them transcribe specific groups of RNAs, like rRNA genes and tRNA gene.

The beginning and the end of each structural gene is linked to a specific sequence of nucleotides called "the control elements" that participate in the regulation of the transcription process through their reaction with the RNA polymerase and the other regulatory proteins. The other most important control element is called "the terminator" that is located downstream of the structural gene which signals the RNA polymerase to detach from the DNA template and terminate the transcription process. Though, structural genes in eukaryotes comprise the majority of the genes present in the chromosomes. However, eukaryotic cells also include other types of genes that their product is RNA molecules other than proteins; such as structural genes responsible for the assembly of" rRNA", others are responsible for the creation of" tRNA" which both play an important role in the translation of the transcribed mRNA.
Therefore, the components of the promoter in both prokaryotes and eukaryotes vary as figure (7.5) shows where many factors and components acts as first recognition sequence for the assembly of RNA-polymerase complex, without which this crucial enzyme won't assemble and no gene expression products.

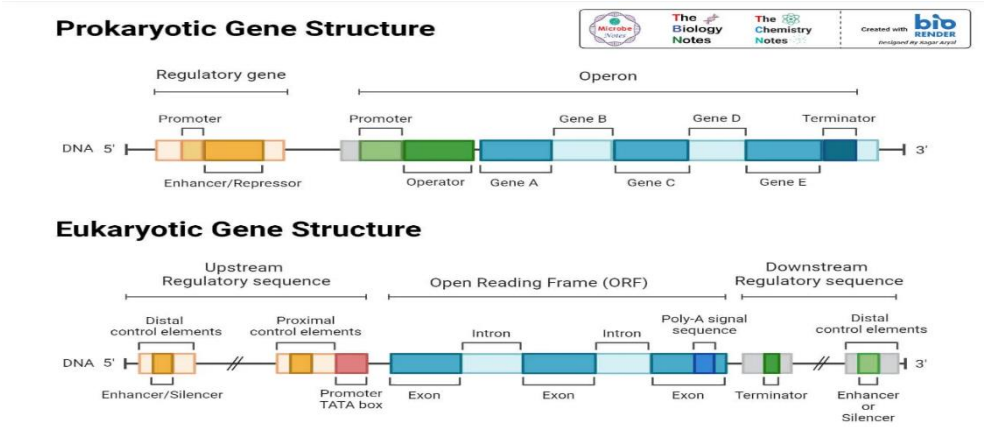


Figure (7.4): Schematic representation of eukaryotic gene

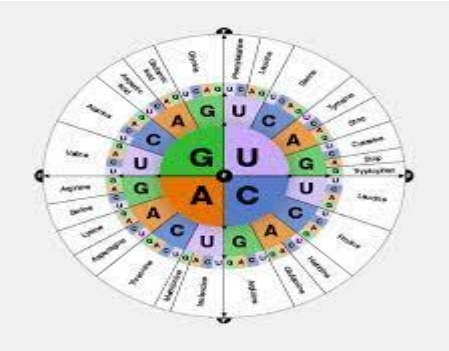
The components of the promoter, depending upon, which acts as first recognition sequence for the assembly of RNA-polymerase complex, without which enzymes won't assemble. The start point itself is bracketed by a set of sequences called "the TATA box" Besides the binding of RNA polymerase complex to TATA box, other factors binding to specific sequence boxes increase the efficiency of transcription. The promoter components vary depending upon the type of the RNA polymerase that is involved. Eukaryotes have three different classes of RNA polymerases, such as RNA polymerase I, RNA polymerase II and RNA polymerase III and each of them transcribe specific groups of RNAs, like rRNA genes and tRNA gene.

Table 1: Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms

Prokaryotic organisms	**Eukaryotic organisms**
Lack nucleus	Contain nucleus
RNA transcription and protein translation occur almost simultaneously	RNA transcription occurs prior to protein translation, and it takes place in the nucleus. RNA translation to protein occurs in the cytoplasm. RNA post-processing includes addition of a 5′ cap, poly-A tail, and excision of introns and splicing of exons.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, post-transcriptional, translational, and posttranslational)

Lecture No.9:

Title of the Lecture:
The Genetic code and It's applications



The Genetic code

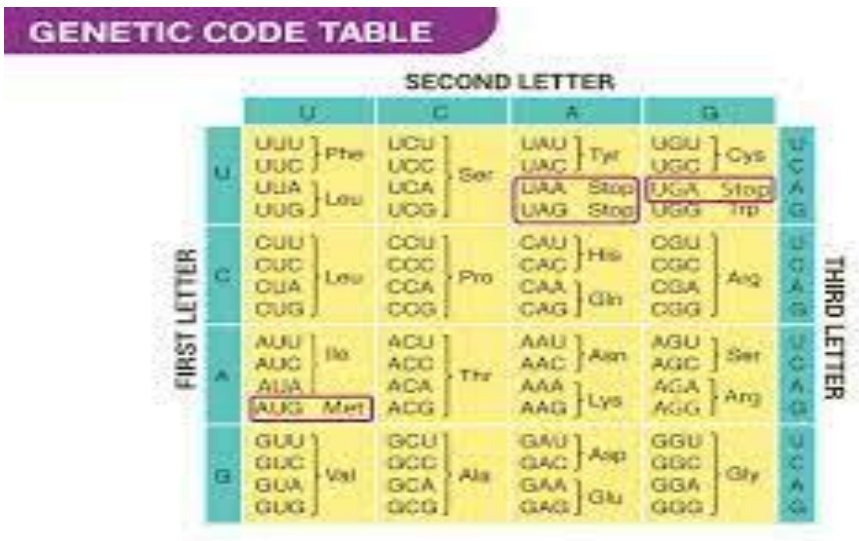
The genetic code is the code our body uses to convert the instructions contained in our DNA the essential materials of life. It is typically discussed using the "codons" found in mRNA, as mRNA is the messenger that carries information from the DNA to the site of protein synthesis.

What is the definition of the genetic code?

Genetic code refers to the instructions contained in a gene that tell a cell how to make a specific protein.

What is genetic code and its types?

The genetic code is of two types. The genetic code can be expressed as either RNA codons or DNA codons. RNA codons occur in messenger RNA (mRNA) and are the codons that are actually "read" during the synthesis of polypeptides (the process called translation).



How is genetic code created?

The genetic code is made up of codons, which are three-letter chains of nucleotides. Each codon codes for one specific amino acid. The code determines the order in which amino acids are added to a polypeptide chain during protein synthesis. Therefore, the genetic code dictates the sequence of amino acids in a protein.

What are the four bases of genetic code?

ACGT is an acronym for the four types of bases found in a DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T)

Is the genetic code universal?

It is considered universal because humans, animals, plants and bacteria all have the exact same genetic code. All known organisms have the same four nucleotide bases (adenine, cytosine, guanine and thymine) but are different due to different arrangements of these nucleotide bases.

What is the application of the genetic code?

The genetic code is the set of rules used by living cells to translate information encoded within genetic material (DNA or RNA sequences of nucleotide triplets, or codons) into proteins.

Why is genetic code important?

Without the genetic code, cells would not be able to make the proteins that are necessary for life. The genetic code is also important in the study of genetics. By understanding how the genetic code works, scientists are able to manipulate it to produce desired traits in organisms.

The genetic code uses what language?

The genetic code uses a four-letter language known as nucleotides, specifically adenine (A), thymine (T), cytosine (C), and guanine (G). These nucleotides form the building blocks of DNA and RNA, and their sequence determines the genetic information that is passed on from one generation to the next.

Why is it called genetic?

The word genetic comes from the Greek word genetikos, which comes from the word “genesis” meaning - origin. It is used to refer to “origin code” on in another word “ origin of life code”.

Lecture no.10:

Title of the lecture:

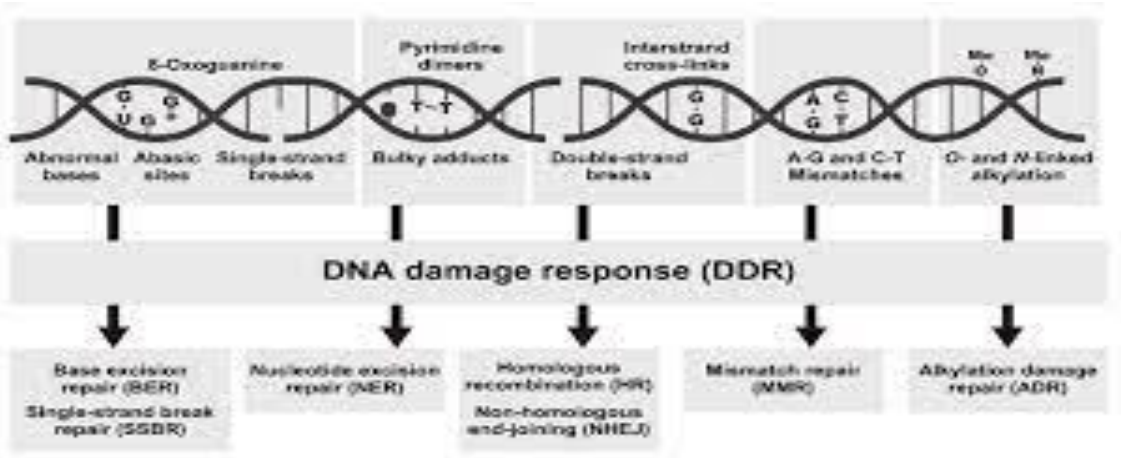
DNA Damage and repair mechanism

Since human DNA contains basically three components (deoxy- pentose sugar, nitrogen base and phosphate group) where these components govern the growth and development processes in his body. Therefore, any change or damage in these components might lead to the impairment of the DNA and affect the life of the individual. Still, changes do occur in the DNA due to many factors which could be environmental or sometimes due to normal metabolic processes inside the cell. These changes could occur at a rate of 10,000 to 1000,000 molecular lesions per cell per day.

DNA damage may be however, modifying the nucleotide sequence in a variety of ways, causing changes in its coding properties or normal function in transcription or replication which can ultimately lead to mutations and genomic instability. This could result in the development of a variety of cancers including colon, breast, and prostate.

What are types of DNA damage?

DNA bases can be damaged by:
(1) oxidative processes,
(2) alkylation of bases,
(3) base loss caused by the hydrolysis of bases,
(4) DNA crosslinking,
(5) DNA strand breaks, including single and double stranded breaks.



What is the best example of DNA damage?

Perhaps the best-known example of the link between environmental-induced DNA damage and disease is that of skin cancer, which can be caused by excessive exposure to UV radiation in the form of sunlight (and, to a lesser degree, the tanning beds).

What is the most common damage on DNA?

One of the most common causes of damage to DNA is oxidative damage. Oxidative damage takes place when hydroxyl radicals are introduced into the cell. They're typically introduced via UV radiation from excess sunshine or other sources.

However, the two major sources of DNA damage include:

1. DNA damage due to Endogenous sources such as:
   attack by reactive oxygen species (free radicals) produced from normal metabolic byproducts especially the process of oxidative deamination beside damage due to the replication errors.
2. DNA damage due to Exogenous sources by external agents such as:
1. ultraviolet [UV-A 200–400 nm] radiation from the sun
2. visible light energy (up to 670–700 nm)
3. other radiation frequencies, including x-rays and gamma rays
4. hydrolysis or thermal disruption
5. certain drugs (anticancer agents in clinical use); these include:
   a. alkylating agents (e.g., cyclophosphamide, cisplatin),
   b. antimetabolites (e.g., 5-fluorouracil),
   c. topoisomerase inhibitors (e.g., etoposide), and
   d. cytotoxic antibiotics (e.g., bleomycin)

Moreover, Quinolones are a key group of antibiotics that interfere with DNA synthesis by inhibiting topoisomerase, most frequently topoisomerase II (DNA gyrase), an enzyme involved in DNA replication.

Nowadays, quinolones are widely used for treating a variety of infections. Quinolones are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria, including mycobacteria, and anaerobes. It is particularly active

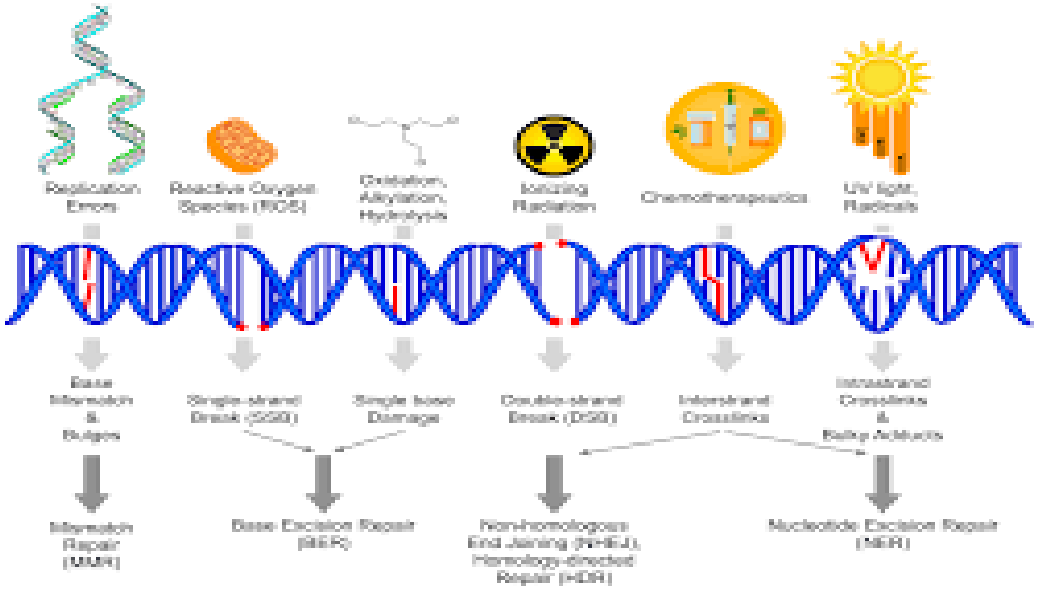
against Gram-negative bacteria, including Salmonella, Shigella, Campylobacter, Neisseria, and Pseudomonas.

Common fluoroquinolones include:

- Ciprofloxacin.
- Delafloxacin.
- Gemifloxacin (recently removed from markets)
- Levofloxacin.
- Moxifloxacin.
- Norfloxacin (discontinued in 2023)
- Ofloxacin.

On the other hand, Ciprofloxacin and other quinolones at >20 µg/ml inhibit peripheral blood lymphocyte (PBL) cell growth by 30 to 35%, causing impaired cell cycle progression through the S phase. Cell cycle analysis thus, indicates DNA synthesis to be inhibited by fluoroquinolones at these concentrations. In addition, ciprofloxacin increases DNA cleavage by topoisomerase IV leading to an increase in DNA nicks.

6. human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
7. infection with certain viruses.
8. excessive exposure to water "many researches showed that; there is a large loss of DNA in human remains that have been immersed for 72 hours. Freshwater, swamp water, and saltwater all showed a large loss of DNA over the 72-hour period.



Consequences of DNA Damage in human cells: -

• DNA damage in the reproductive cells

The damage in mammalian germ cells can be almost completely repaired in short period to provide maintenance to genomic heredity, if not it could lead to:

a. defects of spermatogenesis and male infertility.
b. Recurrent abortions in females.

• DNA damage in somatic cells lead to: -

1. Ageing:

Accumulation of DNA damage as a consequence of ageing leads to loss of homeostasis and increasing probability of illness and death followed by a progressive organic functional decline.

On the other hand, deficiency in DNA repair due to illness cause tissue degeneration and premature ageing which is indicated by number of human genetic defects such as patients having skin and eye photosensitivity exhibit premature cutaneous ageing, increased incidence of basal cell carcinoma and melanoma.

2. Neurodegenerative disorders:

Accumulation of DNA lesions in neurons is associated with neurodegenerative disorders including ataxias together with Alzheimer's, Huntington's and Parkinson's disease.

3. Genome instability and heritable diseases:

DNA repeat instability causes some 40 known diseases that result from expansions or contractions of genetically unstable DNA repeat sequences usually a tri-nucleotide motif within a specific locus. For each disease this instability is thought to arise through repetitive nature of these regions allowing aberrant DNA secondary structure formation during DNA replication or its repair.

4. Cardiovascular diseases
5. Cancer

PCR is one of the most reliably used techniques for detecting DNA damage as the amplification stops at the site of the damage.

DNA repair mechanisms

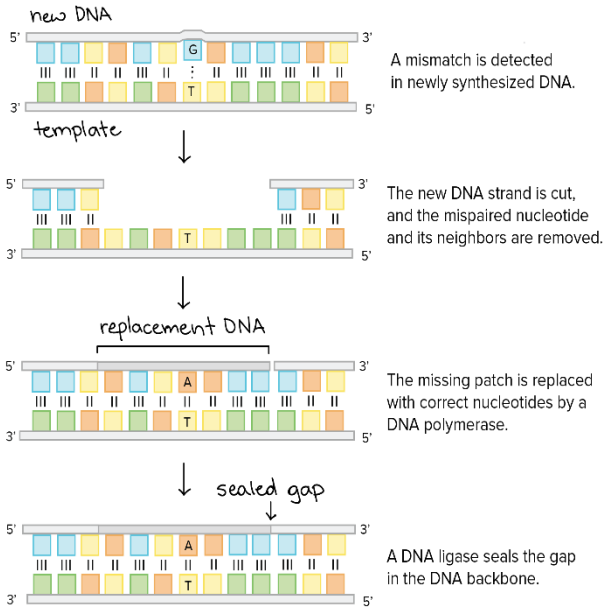
DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In human cells, both normal metabolic activities and environmental factors such as radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes.

Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA cross linkages (interstrand crosslinks). However, DNA repair systems utilize four-step mechanisms for the repair of the damaged DNA strands.

Steps of DNA repair mechanism

1. Detection, the damaged section of the DNA is recognized. (See figure below)
2. Excision, DNA repair endonucleases nick the phosphodiester bond backbone one or both sides of the DNA damage.
3. Polymerization, DNA polymerase adds nucleotides to the newly exposed 3-OH group by using the other strand as a template and replacing the damaged nucleotide.
4. Ligation, DNA ligase seals the nicks in the sugar phosphate backbone

Figure (8.1):
Steps of DNA repair mechanism



The rate of DNA repair is dependent on many factors, including:

1. The cell type,
2. The age of the cell,
3. The extracellular environment.

A cell that has accumulated a large amount of DNA damage, or one that no longer effectively repairs damage acquired to its DNA, can enter one of three possible states:

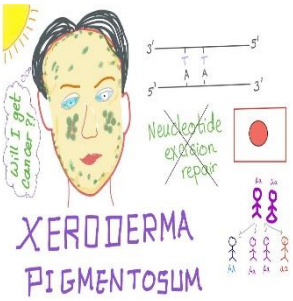
1. an irreversible state of dormancy, known as senescence
2. cell suicide, also known as apoptosis or programmed cell death
3. unregulated cell division, which can lead to the formation of a tumor that is cancerous

The DNA repair ability of a cell is vital to the integrity of its genome and thus to the normal functionality of that organism. Many genes that were initially shown to influence life span have turned out to be involved in DNA damage repair and protection. Cells cannot function if DNA damage corrupts the integrity and accessibility of essential information in the genome (but cells remain superficially functional when non-essential genes are missing or damaged).

Depending on the type of damage inflicted on the DNA's double helical structure, a variety of repair strategies have evolved to restore lost information. If possible, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to recover the original information.

When DNA damage escape repair mechanisms; the damage alters the spatial configuration of the helix, and such alterations can be reflected by the cell. Among the best studied of the human DNA repair diseases is “Xeroderma pigmentosum”, a rare

autosomal recessive condition that includes abnormal skin pigmentation and acute sensitivity to sunlight. (figure-8.2)



In the case of XP affected individuals, there is a deficit in this nucleotide (DNA)excision repair that makes them suffer from extreme sensitivity to UV rays/ sunlight. It mostly affects the skin areas that are exposed to the sun and eyes.
However, there are certain vitamins that play an important role in maintaining DNA integrity, stability and probably helps with DNA repair. Key vitamins to eat include beta-carotene, Vitamin B12, folate (B9), Vitamin D, and Vitamin E.

DNA Repair pathways

A. Single stranded damage

1. Base excision repair (BER) pathway:

It is a multi-step process that corrects non-bulky damage to bases resulting from oxidation, methylation, deamination, or spontaneous loss of the DNA base itself. These alterations, although simple in nature, are highly mutagenic and therefore represent a significant threat to genome fidelity and stability.

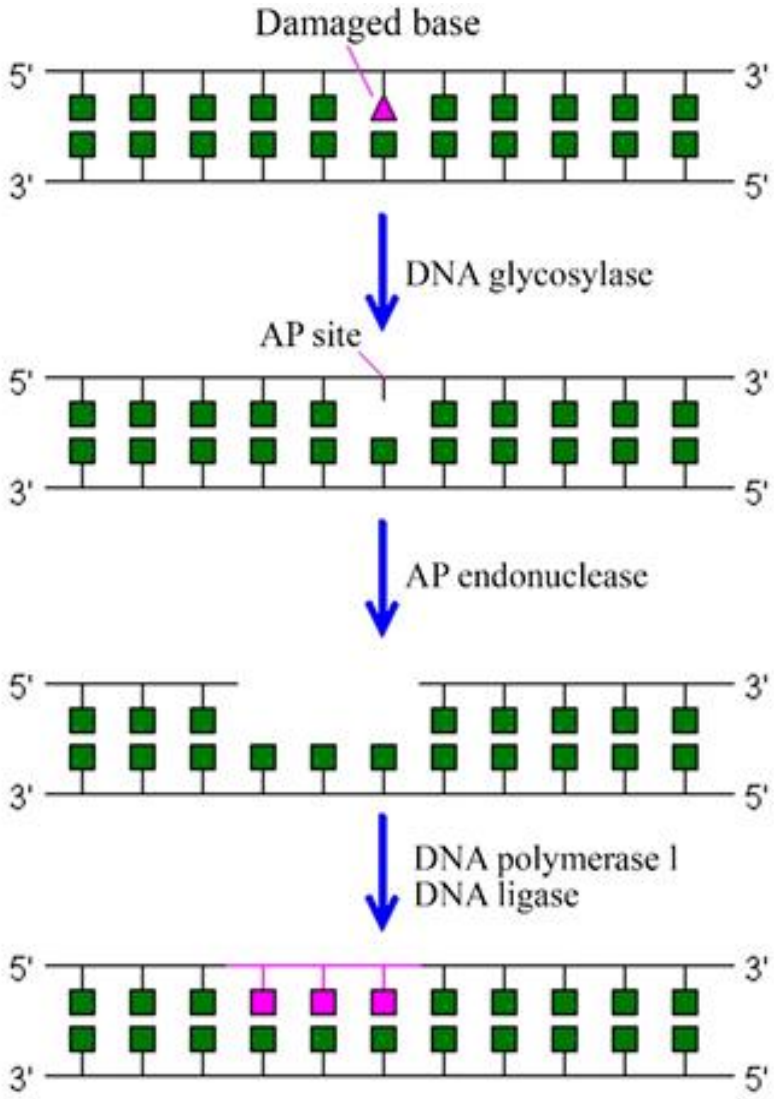


Figure (8.2): Base excision repair (BER) pathway

2.Nucleotide excision repair (NER) pathway:

It is perhaps the most flexible of the DNA repair pathways considering the diversity of DNA lesions it acts upon. The most significant of these lesions are pyrimidine dimers caused by the UV component of sunlight.

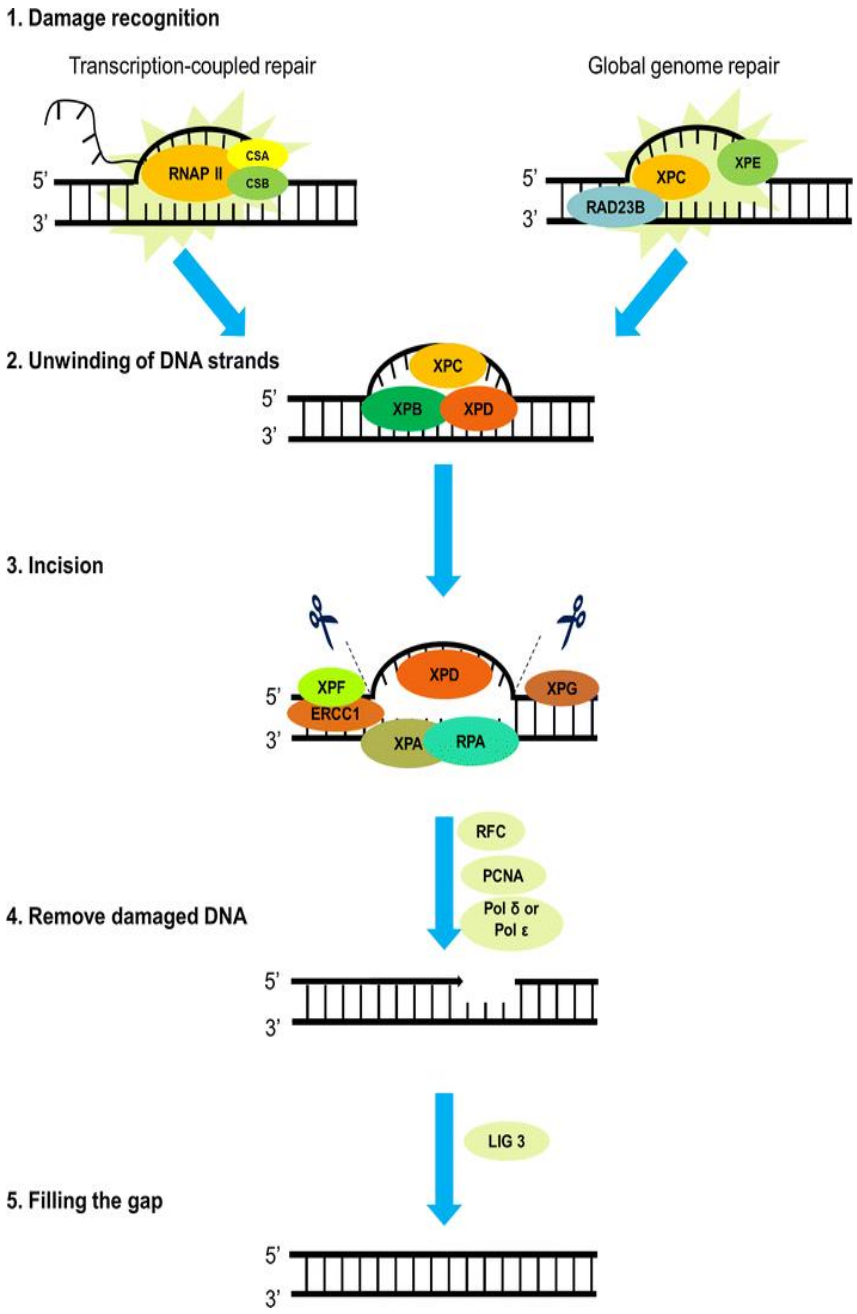


Figure (8.3): Nucleotide excision repair (NER) pathway

• The DNA mismatch repair (MMR) pathway:

This pathway plays an essential role in the correction of replication errors such as base-base mismatches and insertion/deletion loops that result from DNA polymerase misincorporation of nucleotides and template slippage.

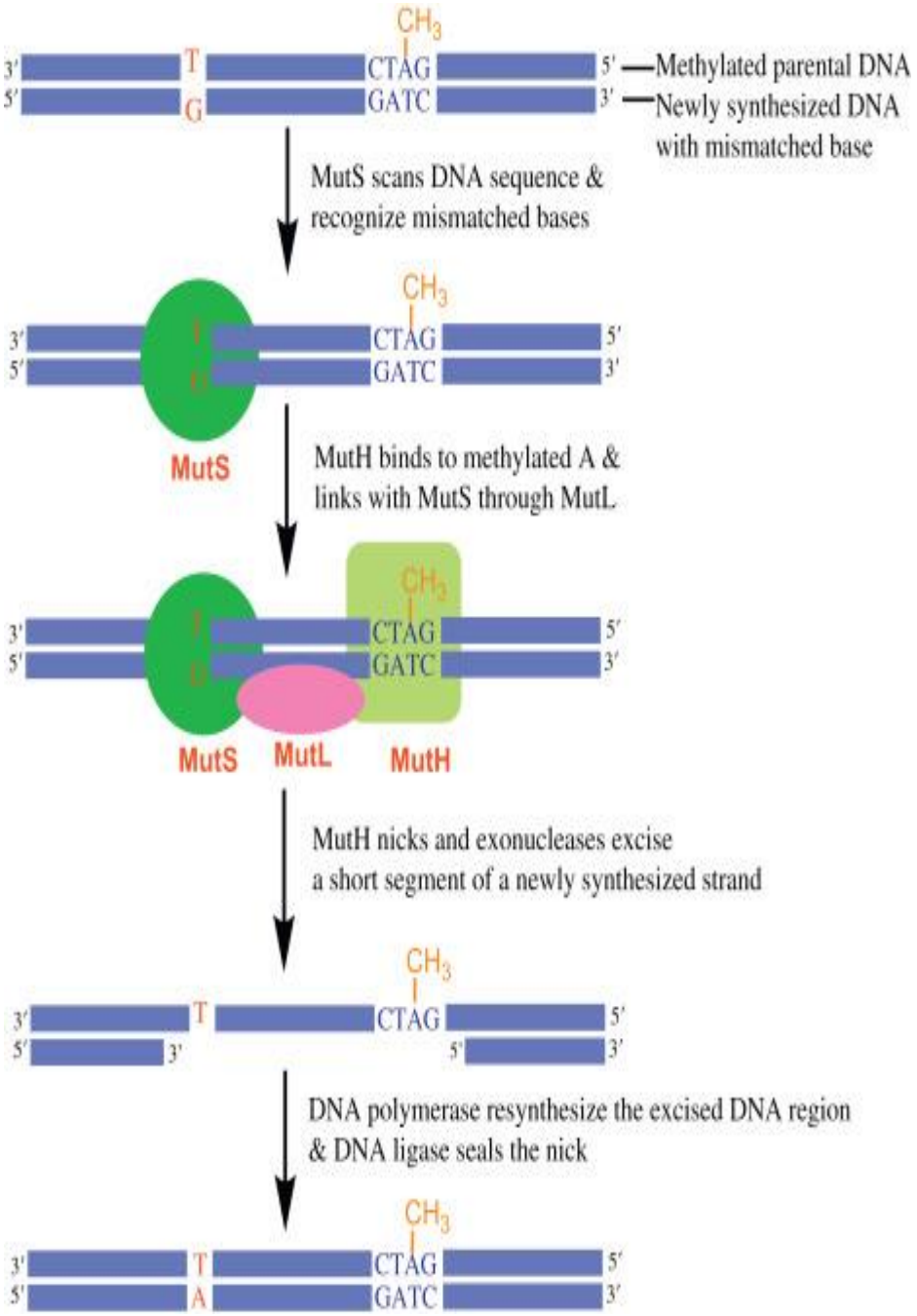


Figure (8.4): DNA mismatch repair (MMR) pathway

B. Double stranded DNA damage

Double-strand breaks (DSBs) in DNA form as a result of exposure to exogenous agents such as radiation and certain chemicals, as well as through endogenous processes, including DNA replication and repair. Radiations such as Gamma- ray or X-ray actually

could sever the DNA which causes double strand breaks (the most dangerous damaging effects on DNA); even one break in the double strand could result in cell death.
In addition, DNA Helicase is the enzyme which creates a double strand DNA break during catalysis.

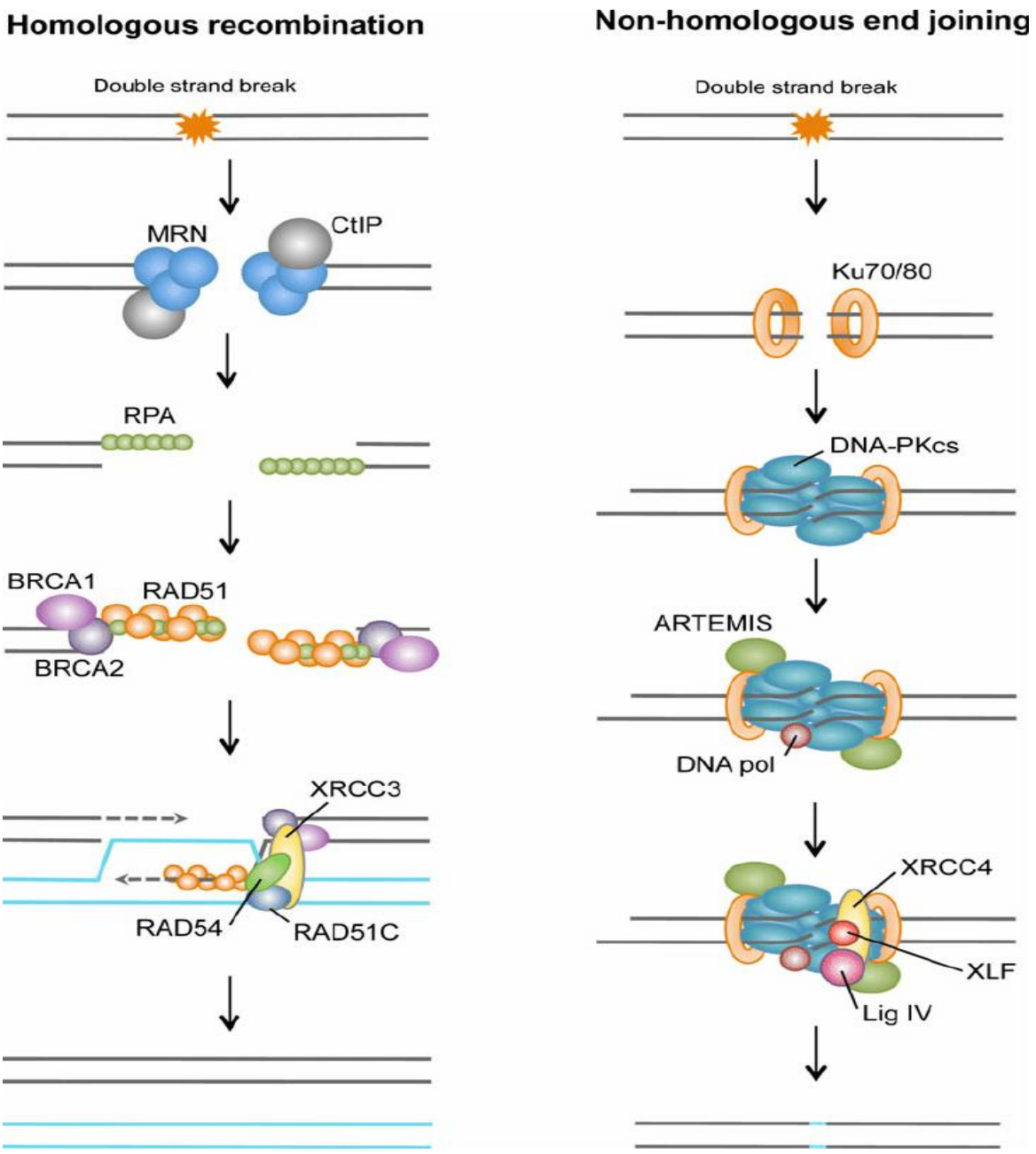


Figure (8.5): Base excision repair (BER) pathway

• The **Homologous recombination repair system:**

In this repair pathway; enzymes use an undamaged section of DNA as a template to repair the damaged one, enzymes interlace (net) the damaged nucleotides with the template to fill the gaps resulting from strand breaks are perhaps the most serious form of DNA damage because they pose problems for transcription, replication, and chromosome segregation.
Damage of this type is caused by a variety of sources including exogenous agents such as ionizing radiation and certain genotoxic chemicals.

• **The Non-Homologous recombination repair system:**

On the other hand, the non-homologous end joining repair system do not relay on template. Instead, a series of enzymes trims out a few nucleotides at the break point and then fuses the broken ends back together.
This process isn't accurate and could cause genes to mix up; but it is useful when sister DNA is damaged. Now the one could realize the damaging effect of recurrent exposure to the X-ray.
Non-homologous end joining (NHEJ) is a pathway that repairs double-strand breaks in DNA. NHEJ is referred to as "non-homologous" because the break ends are directly ligated without the need for a homologous template.

The general mechanism of NHEJ can be broken down into individual and sequential steps which are:

(I) DNA end recognition and assembly and stabilization of the NHEJ complex at the DNA double strand break;
(II) Bridging of the DNA ends and promotion of end stability;
(III) DNA end processing; and
(IV) Ligation of the broken ends and dissolution of the NHEJ complex

Lecture No.11:

Title of the lecture: DNA and gene Mutations

DNA mutation is a change in the DNA sequence of an organism. Whereas, gene mutation is a change to a gene's DNA sequence to produce something different. It creates a permanent change to that gene's DNA sequence. Genetic variations are important for humans to evolve, which is the process of change over generations. In biology, a mutation is an alteration in the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA.

Mutations may or may not produce detectable changes in the observable characteristics (phenotype) of an organism. Mutations play a part in both normal and abnormal biological processes including: evolution, cancer, and the development of the immune system. Mutation is the ultimate source of all genetic variation, providing the raw material on which evolutionary forces such as natural selection can act. Mutation can result in many different types of change in sequences; alter the product of a gene, or prevent the gene from functioning properly or completely. Mutations can also occur in non-genic regions where no effect could be detected.

Classification of mutation

Since the sequence of a gene can be altered in a number of ways. Thus, there are different classification modes of mutation displayed; for example:
Four classes of mutations are:
(1) spontaneous mutations (molecular decay),
(2) mutations due to error-prone replication bypass of naturally occurring DNA damage (also called error-prone translation synthesis),
(3) errors introduced during DNA repair, and
(4) induced mutations caused by mutagens

Or could be classified according to the mutation effect on health

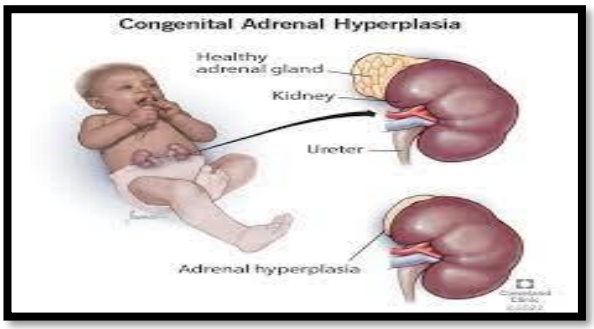
depending on where they occur and whether they alter the function of essential proteins:

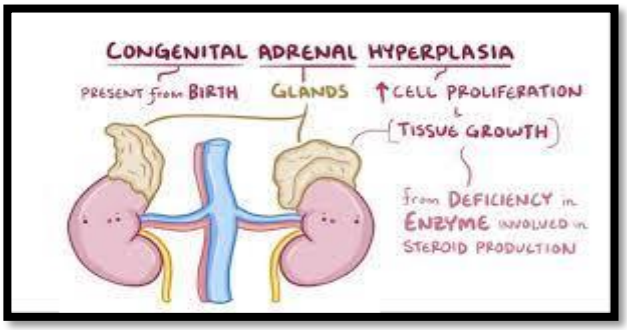
1- By effect on structure

(Large scale mutation/chromosomal abnormality) include, amplification and polyploidy (small scale mutations) include, insertion, deletion and substitution.

2- By impact on protein sequence

This kind of mutations (Whether it occurs in coding or non- coding sequence) include, frameshift mutation, missense mutation (nonfunctional protein) and A nonsense mutation is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product. This sort of mutation has been linked to different diseases, such as congenital adrenal hyperplasia.



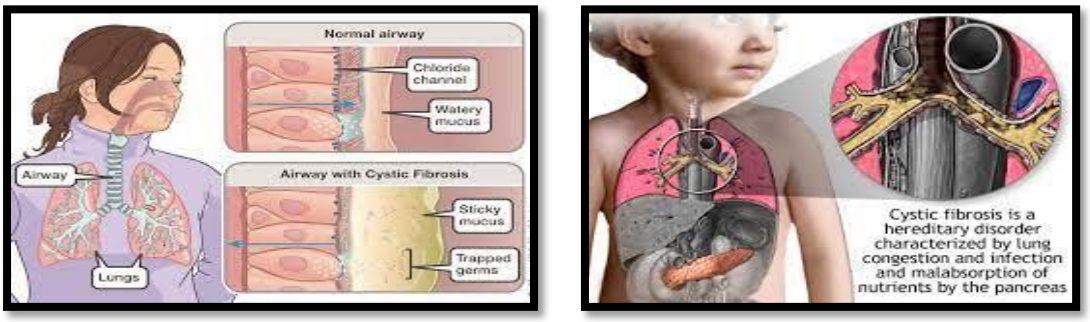


Congenital adrenal hyperplasia

3- By effect on function

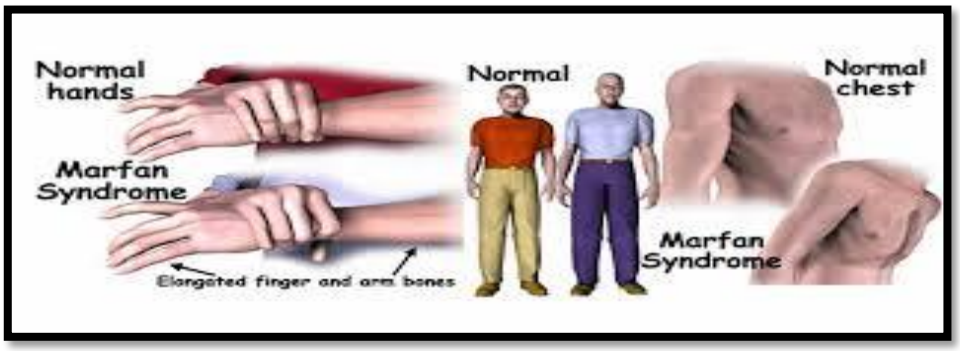
A mutation becomes an effect on function mutation when the exactitude of functions between a mutated protein and its direct interactor undergoes change such as:

- Loss-of-function mutations, also called inactivating mutations, result in the gene product having less or no function (being partially or wholly inactivated). A disease that is caused by a loss-of-function mutation is Gitelman syndrome (Muscle weakness, spasms, and affected individuals may experiences episodes of fatigue, dizziness, fainting (due to low blood pressure), muscle weakness, muscle aches, cramps and spasms). Another disease is cystic fibroses.



Cystic fibrosis

- Dominant negative mutations have an altered gene product that acts antagonistically to the wild-type allele. Marfan syndrome is caused by mutations in the FBN1 gene, located on chromosome 15.



4- By effect on fitness

In genetics, it is sometimes useful to classify mutations as either **harmful or beneficial** (or **neutral**):

- A harmful, or deleterious, mutation decreases the fitness of the organism. Many, but not all mutations in essential genes are harmful (if a mutation does not change the amino acid sequence in an essential protein, it is harmless in most cases).
- A beneficial, or advantageous mutation increases the fitness of the organism. Examples are mutations that lead to antibiotic resistance in bacteria (which are beneficial for bacteria but usually not for humans).
- A neutral mutation has no harmful or beneficial effect on the organism. In animals and plants, most mutations are neutral, given that the vast majority of their

genomes is either non-coding or consists of repetitive sequences that have no obvious function ("junk DNA")

5- By inheritance

In multicellular organisms with dedicated reproductive cells, mutations can be subdivided into germline mutations, which can be passed on to descendants through their reproductive cells, and somatic mutations (also called acquired mutations), which involve cells outside the dedicated reproductive group and which are not usually transmitted to descendants.
Diploid organisms (e.g., humans) contain two copies of each gene—a paternal and a maternal allele. Based on the occurrence of mutation on each chromosome, we may classify mutations into three types:

- A heterozygous mutation is a mutation of only one allele.
- A homozygous mutation is an identical mutation of both the paternal and maternal alleles.
- Compound heterozygous mutations or a genetic compound consists of two different mutations in the paternal and maternal alleles.

(A wild type or non-mutated organism is one in which neither allele is mutated).

Causes of Mutations

Mutation causes were generally categorized into two main groups: **Spontaneous** (internal errors) and **Induced** (external damage):

1. Spontaneous Mutations (Internal Errors)
These occur naturally within the cell without any outside influence.

- **DNA Replication Errors:** Despite being incredibly accurate, the enzyme **DNA polymerase** occasionally makes mistakes, inserting the wrong base
- **Tautomeric Shifts:** Bases can momentarily change their chemical shape, causing them to pair incorrectly during replication.
- **Deamination/Depurination:** Chemical reactions within the cell can cause parts of a DNA base to simply fall off or transform, changing its "meaning" before the cell can divide.

2. Induced Mutations (External Mutagens)
These are caused by environmental factors, known as **mutagens**, that physically or chemically break the DNA.

- **Radiation:**
  - **UV Light:** Causes "Thymine dimers," where two adjacent bases bond to each other instead of the opposite strand, creating a kink in the DNA.
  - **Ionizing Radiation (X-rays/Gamma rays):** These have enough energy to physically snap the DNA backbone, causing double-strand breaks.
- **Chemical Mutagens:**
  - **Base Analogs:** Chemicals that look so much like DNA bases that the cell accidentally uses them during replication.
  - **Intercalating Agents:** Flat molecules (like ethidium bromide) that slide between DNA rungs, stretching the helix and causing "insertion" or "deletion" errors.
- **Biological Agents:**
  - **Viruses:** Some viruses (like HPV) can insert their own genetic material into the host's DNA, disrupting healthy genes.

Summary of Causes

Type	Cause	Example
Spontaneous	Biological "Typos"	A mistake during DNA replication before a cell divides.
Physical	High-energy waves	Skin cell damage from excessive Sun (UV) exposure.
Chemical	Toxins/Pollutants	Carcinogens found in tobacco smoke or charred meats.
Biological	Pathogens	Viral integration into the human genome.

Why aren't we constanty mutating?

The cell has a sophisticated "Repair Crew" of enzymes. They constantly scan the DNA for bumps or incorrect pairings. Most mutations are caught and fixed immediately. A permanent mutation only occurs if the repair crew **misses the error** or if the damage is so extensive the cell can't keep up

Lecture No.12:

Title of the lecture:

Chromosomal Aberrations and Carcinogenesis

Chromosomal Aberrations

A chromosomal aberration is a morphological or numerical alteration in one or more chromosomes, affecting autosomes, sex chromosomes, or both. These are large-scale mutations that typically involve thousands of base pairs and are visible under a light microscope via **karyotyping**.

Classification of Aberrations

1. Numerical Aberrations (Ploidy Changes)

These involve a change in the total number of chromosomes in a cell.

- **Aneuploidy:** A condition where the chromosome number is not an exact multiple of the haploid set (e.g., 2n±12 n plus or minus 12n±1).
  - **Trisomy:** Gain of one chromosome (e.g., **Down Syndrome**, Trisomy 21).
  - **Monosomy:** Loss of one chromosome (e.g., **Turner Syndrome**, 45,X).
- **Euploidy/Polyploidy:** Changes involving entire sets of chromosomes (e.g., n3 n 3n Triploidy, 4n4 n4nTetraploidy). In humans, polyploidy is typically lethal.

2. Structural Aberrations

Changes in the physical architecture of the chromosome itself.

- **Deletions:** Loss of a segment (e.g., **Cri-du-chat syndrome** on chromosome 5).
- **Duplications:** Repetition of a segment (e.g., **Charcot-Marie-Tooth disease** on chromosome 17).
- **Inversions:** A segment breaks, flips 180°, and reattaches.
  - *Paracentric:* Does not include the centromere.
  - *Pericentric:* Includes the centromere.
- **Translocations:** Exchange of segments between non-homologous chromosomes.
  - **Reciprocal:** Balanced exchange with no loss of material.
  - **Robertsonian:** Fusion of two acrocentric chromosomes, often resulting in the loss of short arms.
- **Ring Chromosomes:** Ends of a chromosome break off and the "sticky" ends fuse into a circle.

Molecular Mechanisms of Formation

Aberrations primarily arise from errors during cell division or DNA maintenance.

1. **Nondisjunction:** Failure of homologous chromosomes or sister chromatids to separate properly during meiosis or mitosis, leading to **aneuploidy**.
2. **Unequal Crossing Over:** Misalignment of homologous chromosomes during meiosis leads to simultaneous **deletion** in one strand and **duplication** in the other.
3. **DNA Repair Errors:** Incorrect rejoining of double-strand breaks by pathways like Non-Homologous End Joining (NHEJ).

Clinical and Biological Significance

- **Developmental Impacts:** Aberrations are a leading cause of spontaneous miscarriages, congenital malformations, and intellectual disabilities.
- **Oncogenesis:** Specific aberrations drive cancer. The **Philadelphia Chromosome** (translocation between 9 and 22) is a diagnostic marker for **Chronic Myeloid Leukemia**.
- **Mosaicism:** Some individuals have two or more cell lines with different karyotypes, often resulting in milder symptoms of a syndrome.
- **Environmental Markers:** Frequency of aberrations in lymphocytes can act as a **biomarker** for exposure to radiation or genotoxic chemicals.

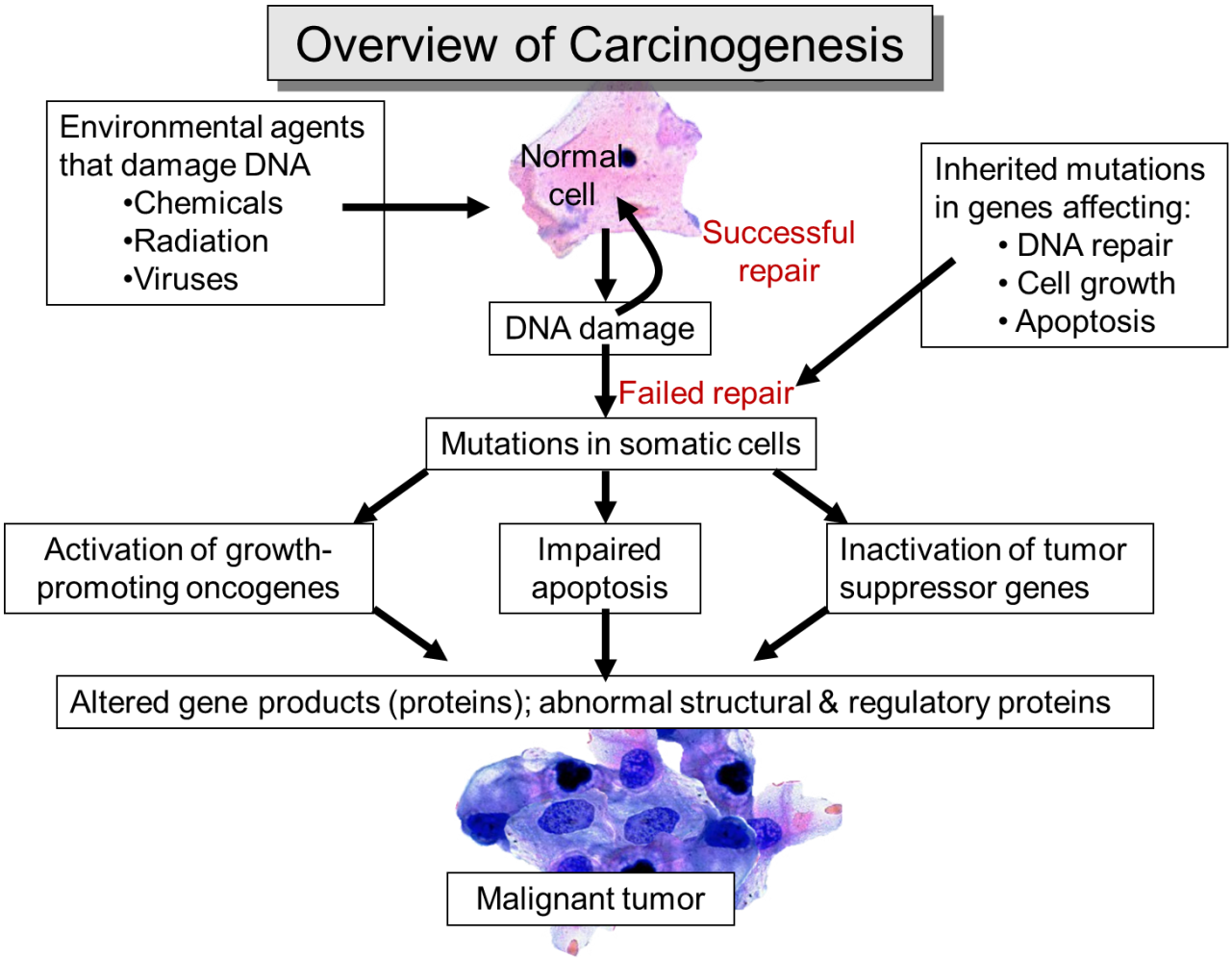
Common Human Syndromes due to chromosomal abberations

Syndrome	**Type of Aberration**	**Karyotype**	**Key Clinical Feature**
Down Syndrome	Trisomy 21	47, XX/XY, +21	Distinct facial features, cognitive delays
Patau Syndrome	Trisomy 13	47, XX/XY, +13	Severe organ defects, cleft palate
Turner Syndrome	Monosomy X	45, X	Short stature, infertility in females
Klinefelter Syndrome	Trisomy (Sex Chr)	47, XXY	Reduced testosterone, infertility in males
Cri-du-chat	Deletion	46, XX/XY, 5p-	High-pitched "cat-like" cry in infants

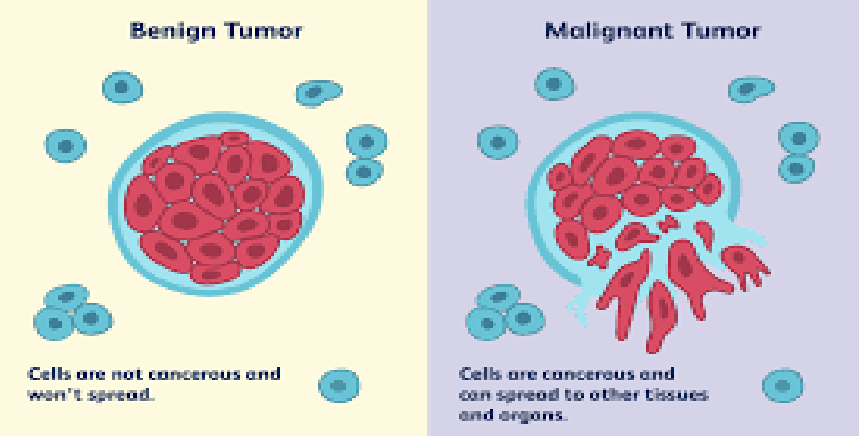
Carcinogenesis

The process by which normal, healthy cells transform into cancer cells is termed carcinogenesis. Cancer has existed for all of human history. The earliest written record regarding cancer is as old as Hippocrates descriptions made in (460 BC) as he described several kinds of cancer, referring to them with the Greek word karkinos (crab or crayfish). Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely.

On the other hand, the development of a malignant tumor in otherwise healthy tissue is the result of a complex series of events beginning with a single cell that has acquired malignant properties through cellular DNA damage.



However, many people mistakenly believe that a tumor and cancer are the same thing, but they are two very different conditions/diseases. A tumor is an abnormal growth or mass of tissue. It is also known as lump, lesion, or neoplasm. Cancer is a group of diseases caused by the uncontrolled growth and spread of abnormal cells.
But, when a tumor cells show the six hallmarks of cancer, then, those cells will be characterized as malignant tumors.



The six Marks of cancer/malignant tumor are:-

1• Absence of the proper signals of Cell growth and division
2• Continuous growth and division even given contrary signals
3• Avoidance of programmed cell death
4• Limitless number of cell divisions
5• Promoting blood vessel construction
6• Invasion of tissue and formation of metastases

The progression from normal cell to cancer cell is of three stages called carcinogenesis stages, including (initiation, promotion and progression). See figure (12.1).

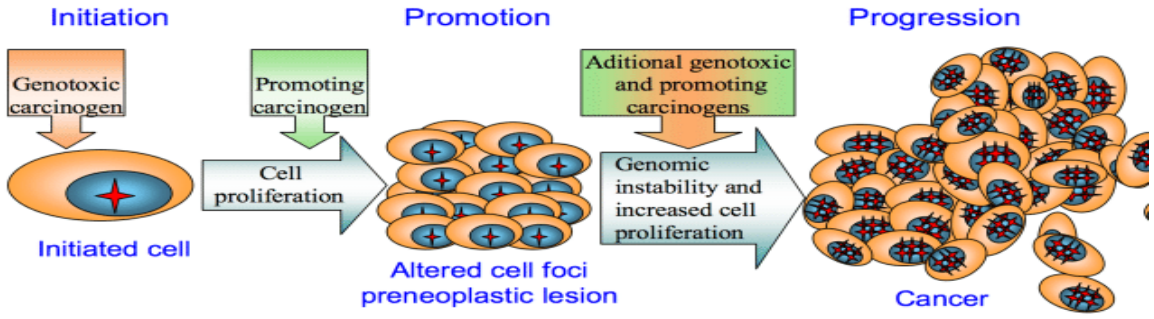


Figure (12.1): Carcinogenesis stages in a human cell.

Cancer signs and symptoms

Symptoms of cancer metastasis depend on the location of the tumor. When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows or ulcerates. The findings that result depend on the cancer's type and location. Few symptoms are specific. Many frequently occur in individuals who have other conditions. Cancer is a "great imitator". Thus, it is common for people diagnosed with cancer to have been treated for other diseases.

Though, **The main types of cancer**

- carcinoma – this cancer begins in the skin or in tissues that line or cover internal organs.
- sarcoma – this cancer begins in the connective or supportive tissues such as bone, cartilage, fat, muscle or blood vessels.
- leukemia – this is cancer of the white blood cells.

However, signs of cancer could be classified into:

(A)- Local symptoms

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can block the bronchus resulting in cough or pneumonia; esophageal cancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, affecting bowel habits. Masses in breasts or testicles may produce observable lumps. Ulceration can cause bleeding that, if it occurs in the lung, will lead to coughing up blood, in the bowels to anemia or rectal bleeding, in the bladder to blood in the urine and in the uterus to vaginal bleeding. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause a buildup of fluid within the chest or abdomen.

(B)- Systemic symptoms

General symptoms occur due to effects that are not related to direct or metastatic spread. These may include: unintentional weight loss, fever, excessive fatigue and changes to the skin. leukemias and cancers of the liver or kidney can cause a persistent fever. Some cancers may cause specific groups of systemic symptoms, termed paraneoplastic syndrome. Examples include the appearance of myasthenia gravis in thymoma and clubbing in lung cancer.

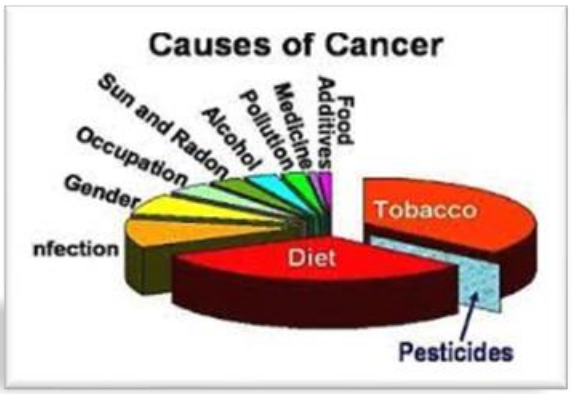
(C)- Metastasis

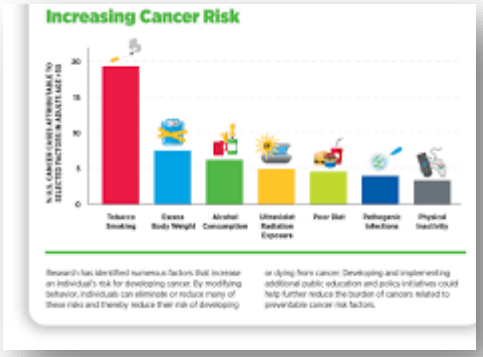
Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by hematogenous spread via the blood to distant sites, known as metastasis. When cancer spreads by a hematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as hypothesized in the soil and seed hypothesis of cancer metastasis.

The symptoms of metastatic cancers depend on the tumor location and can include enlarged lymph nodes (which can be felt or sometimes seen under the skin and are typically hard), enlarged liver or enlarged spleen, which can be felt in the abdomen, pain or fracture of affected bones and neurological symptoms.

Causes of cancer

The majority of cancers, some 90–95% of cases, are due to genetic mutations from environmental and lifestyle factors. The remaining 5–10% are due to inherited genetic diseases. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15– 20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and pollution.





However, it is not generally possible to prove what caused a particular cancer because the various causes do not have specific fingerprints. For example, if a person who uses tobacco heavily develops lung cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung cancer as a result of air pollution or radiation, the cancer may have developed for one of those reasons. Excepting the rare transmissions that occur with pregnancies and occasional organ donors, cancer is generally not a transmissible disease.

However, several causes might lead to the development of cancer

1-Chemicals (alcohol &smoking) 

The incidence of lung cancer is highly correlated with smoking. Exposure to particular substances have been linked to specific types of cancer. These substances are called carcinogens. Tobacco smoke, for example, causes 90% of lung cancer. It also causes cancer in the larynx, head, neck, stomach, bladder, kidney, esophagus and pancreas. Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Tobacco is responsible for about one in five cancer deaths worldwide and about one in three in the developed world.

2-Diet and exercise 

Diet, physical inactivity and obesity are related to up to 30–35% of cancer deaths. In the United States, excess body weight is associated with the development of many types of cancer and is a factor in 14–20% of cancer deaths. A UK study including data on over 5 million people showed higher body mass index to be related to at least 10 types of cancer and responsible for around 12,000 cases each year in that country.

Physical inactivity is believed to contribute to cancer risk, not only through its effect on body weight but also through negative effects on the immune system and endocrine system. More than half of the effect from diet is due to overnutrition (eating too much), rather than from eating too few vegetables or other healthful foods.

3-Infections

Worldwide approximately 18% of cancer deaths are related to infectious diseases. This proportion ranges from a high of 25% in Africa to less than 10% in the developed world. (1) Viruses are the usual infectious agents that cause cancer but cancer bacteria and parasites may also play a role.

a. Oncoviruses include human papillomavirus (cervical cancer),

b. Epstein–Barr virus (B-cell lymphoproliferative disease and nasopharyngeal carcinoma),

c. Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas),

d. Hepatitis B and hepatitis C viruses (hepatocellular carcinoma) and human Tcell leukemia virus-1 (T-cell leukemias).

On the other hand,
(2) Bacterial infection may also increase the risk of cancer, as seen in Helicobacter pylori-induced gastric carcinoma.
(3) Parasitic infections associated with cancer include Schistosoma haematobium (squamous cell carcinoma of the bladder).

4- Radiation 

Up to 10% of invasive cancers are related to radiation exposure, including both ionizing radiation and non-ionizing ultraviolet radiation. Additionally, the majority of non-invasive cancers are non-melanoma skin cancers caused by nonionizing ultraviolet radiation, mostly from sunlight. Sources of ionizing radiation include medical imaging and radon gas.
Ionizing radiation is not a particularly strong mutagen. Residential exposure to radon gas, for example, has similar cancer risks as passive smoking. Radiation is a more potent source of cancer when combined with other cancer-causing agents, such as radon plus tobacco smoke. Radiation can cause cancer in most parts of the body, in all animals and at any age. Children and adolescents are twice as likely to develop radiation-induced leukemia as adults; radiation exposure before birth has ten times the effect.
Medical use of ionizing radiation is a small but growing source of radiationinduced cancers. Ionizing radiation may be used to treat other cancers, but this may, in some cases, induce a second form of cancer.
Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies. Clear evidence establishes ultraviolet radiation, especially the non-ionizing medium wave UVB, as the cause of most non-melanoma skin cancers, which are the most common forms of cancer in the world.
Non-ionizing radio frequency radiation from mobile phones, electric power transmission and other similar sources have been described as a possible carcinogen by the World Health Organization's International Agency for Research on Cancer. However, studies have not found a consistent link between mobile phone radiation and cancer risk.

5-Heredity 

The vast majority of cancers are non-hereditary (sporadic). Hereditary cancers are primarily caused by an inherited genetic defect. Less than 0.3% of the population are carriers of a genetic mutation that has a large effect on cancer risk and these causes less than 3–10% of cancer. Some of these syndromes include: certain inherited mutations in

the genes BRCA1 and BRCA2 with a more than 75% risk of breast cancer and ovarian cancer, and hereditary nonpolyposis colorectal cancer(HNPCC or Lynch syndrome), which is present in about 3% of people with colorectal cancer, among others.

6-Hormones

Some hormones play a role in the development of cancer by promoting cell proliferation suggesting possible involvement in carcinogenesis. However, many examples are present to recognize the involvement of hormones with cancer:

1- Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary and testis and also of thyroid cancer and bone cancer.

2- The daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain their higher risk of breast cancer, even in the absence of a breast-cancer gene.

3- Similarly, men of African ancestry have significantly higher levels of testosterone than men of European ancestry and have a correspondingly higher level of prostate cancer. Men of Asian ancestry, with the lowest levels of testosterone-activating, have the lowest levels of prostate cancer.

4- Other factors are relevant: obese people have higher levels of some hormones associated with cancer and a higher rate of those cancers.

5- Women who take hormone replacement therapy have a higher risk of developing cancers associated with those hormones.

6- On the other hand, people who exercise far more than average have lower levels of these hormones and lower risk of cancer.

7-Autoimmune diseases

There is an association between celiac disease and an increased risk of all cancers. People with untreated celiac disease have a higher risk, but this risk decreases with time after diagnosis and strict treatment, probably due to the adoption of a gluten free diet, which seems to have a protective role against development of malignancy in people with celiac disease. However, the delay in diagnosis and initiation of a gluten-free diet seems to increase the risk of malignancies. Rates of gastrointestinal cancers are increased in people with Crohn's disease and ulcerative colitis, due to chronic inflammation. Also, immunomodulators and biologic agents used to treat these diseases may promote developing extra-intestinal malignancies.

Lecture No.13:

Title of the lecture:

The Molecular Clock: Telomeres and Telomerase

Introduction: The Problem of Linear DNA

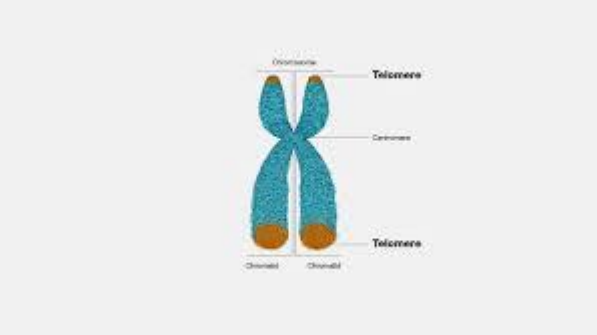
In eukaryotic cells, DNA is organized into linear chromosomes. This structure presents a physical challenge during replication. Unlike circular bacterial DNA, linear chromosomes have "ends."

Every time a cell divides, it must copy its DNA. However, because of how **DNA Polymerase** works (requiring an RNA primer and only building in a 5′→3′5 prime right arrow 3 prime 5′→3′ direction), the very tip of the "lagging strand" cannot be copied.

The Result: Chromosomes shorten slightly with every single cell division. This is known as the **End Replication Problem**.

What are Telomeres?

To prevent the loss of vital genetic information during this shortening, chromosomes are capped with **Telomeres**.



- **Structure:** Telomeres are repetitive, non-coding DNA sequences at the terminal ends of chromosomes. In humans, the sequence is typically **TTAGGG** repeated thousands of times.
- **The "Aglet" Analogy:** Think of telomeres like the plastic tips on shoelaces. They don't contain "instructions" for the shoe, but they prevent the lace (the chromosome) from fraying or sticking to other laces.
- **The Shelterin Complex:** A group of specialized proteins binds to these repeats to "hide" the ends of the DNA from the cell's repair machinery, which might

otherwise mistake a chromosome end for a broken piece of DNA and try to "fix" it by fusing it to another chromosome.

Telomerase: The Cellular Fountain of Youth

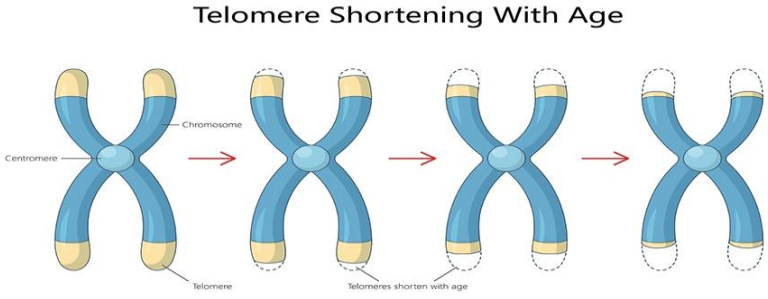
If cells simply kept shortening, they would eventually hit a limit and die. To counter this, some cells use an enzyme called **Telomerase**.

1. **Composition:** Telomerase is a **ribonucleoprotein**. It carries its own internal RNA template that matches the TTAGGG repeat.
2. **Mechanism:**
    - It binds to the overhanging end of the telomere.
    - It uses its RNA as a template to synthesize new DNA (acting as a **reverse transcriptase**).
    - This extends the parent strand, providing enough "room" for DNA polymerase to complete the lagging strand.
3. **Expression:** Telomerase is **not** active in most adult somatic (body) cells. It is primarily active in:
    - Germ cells (sperm/eggs).
    - Stem cells.
    - Cancer cells (which use it to become "immortal").

The Hayflick Limit and Aging

Most of our cells have a "fuse" that is slowly burning down.

- **The Hayflick Limit:** This is the number of times a normal human cell population will divide before cell division stops.
- **Senescence:** When telomeres become critically short, the cell enters a state of "retirement" called senescence. It is still alive but can no longer divide. This contributes significantly to the biological process of aging.



Clinical Significance: Cancer and Therapeutics

The study of telomeres sits at the intersection of aging and oncology.

- **Cancer's Secret Weapon:** Approximately **85–90% of all cancers** abnormally reactivate telomerase. This allows the tumor to divide indefinitely without ever running out of telomere "buffer."
- **Therapeutic Potential:**
  - **Telomerase Inhibitors:** Being studied as a way to "turn off" the immortality of cancer cells.
  - **Telomere Extension:** Being researched (cautiously) as a way to treat age-related degenerative diseases.

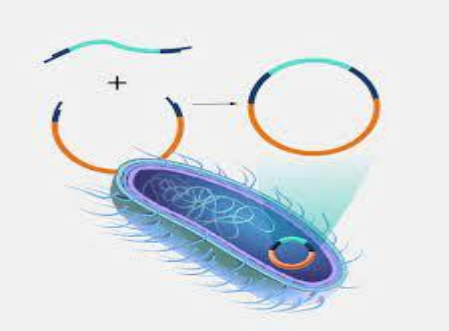
In summary,

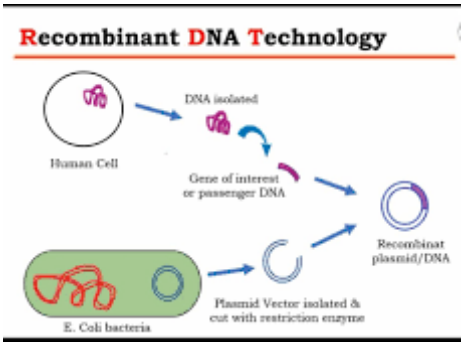
- **Telomeres are protective caps of repetitive DNA.**
- **The End Replication Problem causes these caps to shrink over time.**
- **Telomerase is the enzyme that can rebuild these caps, but it is usually turned off in adult cells.**
- **The balance between telomere shortening (aging) and lengthening (cancer/immortality) is a central focus of modern molecular research.**

Lecture No.14:

Title of the lecture:

Introduction to Recombinant DNA technology





Recombinant DNA technology involves using enzymes and various laboratory techniques to manipulate and isolate DNA segments of interest. This method can be used to combine (or splice) DNA from different species or to create genes with new functions.

Why is recombinant DNA used?

Recombinant DNA technology enables the manufacture of proteins and antibodies with a defined specificity and uniformity, which is a vast improvement over previous methods of production by extraction and purification from human or animal blood and tissues.

What is recombinant DNA used for?

Recombinant DNA technology is an extremely important research tool in biology. It allows scientists to manipulate DNA fragments in order to study them in the lab. It involves using a variety of laboratory methods to put a piece of DNA into a bacterial or yeast cell.

How is recombinant DNA formed?

Recombinant DNA is the method of joining two or more DNA molecules to create a hybrid. The technology is made possible by two types of enzymes, restriction endonucleases and ligase. A restriction endonuclease recognizes a specific sequence of DNA and cuts within, or close to, that sequence.

The process of recombination DNA technology consists of the following steps:

- Isolation of genetic material (DNA) DNA is enclosed within the membrane. ...
- Cutting of DNA at specific locations. ...
- Joining of DNA fragment. ...
- Insertion of DNA into the host cell. ...
- Selection and screening of transformed cells.

What is recombinant DNA also called?

Recombinant DNA molecules are sometimes called chimeric DNA because they can be made of material from two different species like the mythical chimera. rDNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

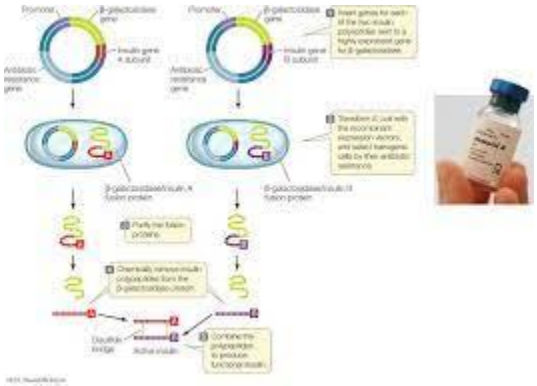
What are the advantages and disadvantages of recombinant DNA technology?

Recombinant DNA technology, also called "genetic engineering," has many benefits, such as the ability to improve health and improve the quality of food. But there are downsides as well, such as the potential for using personal genetic information without consent.

What are some examples of recombinant DNA technology?

5 recombinant DNA examples

- 1 Insulin production with recombinant DNA technology.
- 2 Recombinant DNA for human growth hormone production.
- 3 Recombinant Vaccines.
- 4 Gene therapies and recombinant DNA.
- 5 Enzyme production with recombinant DNA technology.
- Recombinant DNA technology for protein production.



Tools involved in Recombinant DNA technology

What are the five main tools of recombinant DNA technology?

The five key tools involved in recombinant DNA technology are as follows:

- Enzymes involved in DNA manipulation.
- Cloning vectors.
- Gel electrophoresis.
- PCR.
- Host organism.

Recombinant DNA technology alters the phenotype of an organism (host) through a genetically altered vector. This cloning vector is introduced and integrated into the genome of the organism. So, basically, the process involves the introduction of a foreign piece of DNA into the genome which contains our gene of interest. The gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. Here we will learn about key tools of recombinant DNA technology.

Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. This recombinant DNA then has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring. Recombinant DNA technology can be complete and achieved with the help of some elemental tools. The different tools used for the purpose are discussed below:

Restriction Enzymes

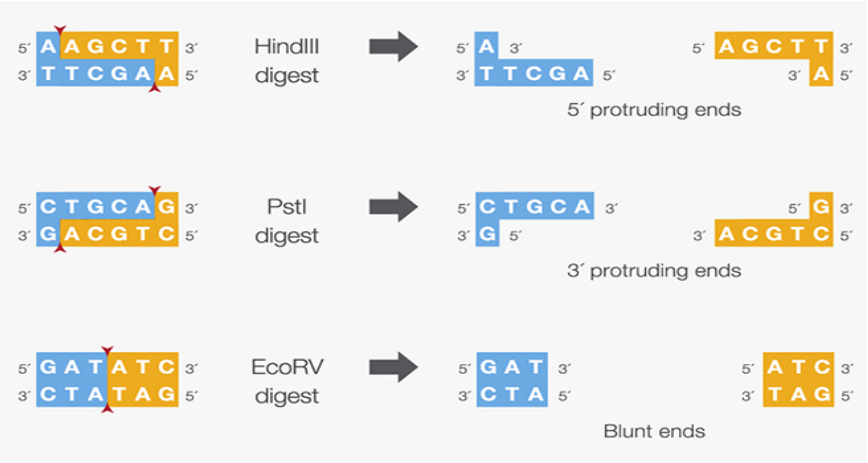
The restriction enzymes – help to cut, the polymerases- help to synthesize and the ligases- help to bind.

The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely endonucleases and exonucleases. The endonucleases cut within the DNA strand whereas the exonucleases cut the nucleotides from the ends of the DNA strands. The restriction endonucleases are sequence-specific which is usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same

restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

How many types of restriction enzymes are there?

There are three types of restriction enzymes- Type I, II, III.



Vectors

The vectors help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology.

Host Organism

Host organism is the organism into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes. There are a number of ways in which this recombinant DNA's are inserted into the host, namely – microinjection, biolistic or gene gun, alternate cooling and heating, use of calcium ions, etc.

Lecture No.15:

Title of the lecture:

Introduction to genetic engineering

Genetic engineering (also called genetic modification) is a process that uses laboratory-based technologies to alter the DNA makeup of an organism. This may involve changing a single base pair (A-T or C-G), deleting a region of DNA or adding a new segment of DNA.

What is the purpose of genetic engineering?

Genetic engineering aims to modify the genes to enhance the capabilities of the organism beyond what is normal. Ethical controversy surrounds possible use of the both of these technologies in plants, nonhuman animals, and humans.

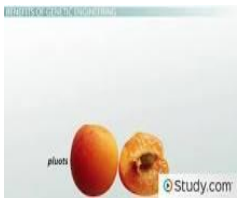
What are the 7 steps of genetic engineering?

Stages of genetic engineering to include:

- identify section of DNA that contains required gene from source chromosome,
- extract required gene,
- insert required gene into vector/bacterial plasmid,
- insert plasmid into host cell,
- grow transformed cells to produce a GM organism.

Where has genetic engineering been used?

Used in research and industry, genetic engineering has been applied to the production of cancer therapies, brewing yeasts, genetically modified plants and livestock, and more.
Which is the best example of genetic engineering?



What is an example of a genetically engineered organism?

Genetic engineering is commonly used in agriculture to modify crops such as corn, soybeans, and cotton. By inserting the genetic material from bacteria into the plants, the crops resist insects when eaten

What are the advantages of genetic engineering?

The possible benefits of genetic engineering include:

- More nutritious food.
- Tastier food.
- Disease- and drought-resistant plants that require fewer environmental resources (such as water and fertilizer)
- Less use of pesticides.
- Increased supply of food with reduced cost and longer shelf life.
- Faster growing plants and animals.

What is the principle of genetic engineering?

The principle of genetic engineering is to manipulate and modify the genetic material of an organism to incorporate desirable traits. Recombinant DNA technology is the main pillar of genetic engineering. Recombinant DNA Technology is a technique to alter the genes of an organism.

What are the tools of genetic engineering?

The basic tools are enzymes, vectors and host organisms. Now we know from the foregoing discussion that in order to generate recombinant DNA molecule, certain basic tools are necessary for the process. The basic tools are enzymes, vectors and host organisms

Restriction enzyme	Microbial source	Recognition sequence	Fragments
Alu I	Arthrobacter luteus	5'AG↓CT3' 3'TC↑GA5'	A-G C-T T-C G-A Blunt ends
BamHI	Bacillus amyloliquefaciens	5'G↓GATCC3' 3'CCTAG↑G5'	G G-A-T-C-C C-C-T-A-G G Sticky ends
EcoRI	Escherichia coli	5'G↓AATTC3' 3'CCTAG↑G5'	G A-A-T-T-C C-T-T-A-A G Sticky ends
HaeIII	Haemophilus aegyptus	5'GG↓CC3' 3'CC↑GG5'	G-G C-C C-C G-G Blunt ends
HindIII	Haemophilus influenza	5'A↓AGCTT3' 3'TTCGA↑A5'	A A-G-C-T-T T-T-C-G-A A Sticky ends

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