B- Differential stain:

Using two or more types of dye successfully to differentiate between the bacteria according to their response to these dyes.
Example for these stains:

1- Differential staining of bacteria (Gram staining)

Is one of the most important methods widely used in bacteriology differentiating bacterial species into two large groups (gram-positive and gram-negative). Discovered in 1884 by Christian gram (a Danish physician), using two dyes in sequence, each of different color; he found bacteria fall into two different categories.
a- Those that retained the first dye (crystal violet) throughout the staining procedure, are known as "gram – positive".
b- Those that lost the first dye (crystal violet) after washing with a decolorizing solution and stained with the second dye (diluted carbol fuchsine) are known as "gram – negative".
In conclusion, the gram – positive bacteria appear violet, while gram – negative bacteria are red in colour.

- Gram +ve had thick layer of Peptidoglycan which give rigidity and strong integrity to the cell wall and not affected by alcohol. Also it contains tiechoic acid which are the source of mg+2.
- Gram-ve head thin layer of Peptidoglycan with thick layer of lipid containing layer (lipoprotein, Lipopolysaccharid and phospholipids) and those it affect more by alcohol.
- It is almost always the first step in the identification of a bacterial organism.
- Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria.

Requirements:

1. Slides.

2. Burner.

3. Loop.

4. Distilled water.

5. Light microscope.

6. Sample.

7. Cedar wood oil.

8. Reagents.

A- **Crystal violate** (primary stain): that stains all bacteria purple.

B- **Gram s iodine (Mordant):** combines with crystal violet to form a CVI complex

C- **Decolorizing agent (95% alcohol or acetone**): washes out the CVI complex from some bacteria (dissolves the lipid outer membrane of Gram negative bacteria).

D- **Safranin** (secondary stain): to stain the decolorized bacteria.

Procedure:

I. Prepare and heat fix smears on clean glass slides.

2. Place the slide on the staining rack and cover the smear with **crystal violet** and leave it for **one min**. Wash the slide with tap water gently.

3. Cover the smear with **Gram's iodine** for **one min**.

4. Wash off the iodine by tilting the slide and pour water over the smear.

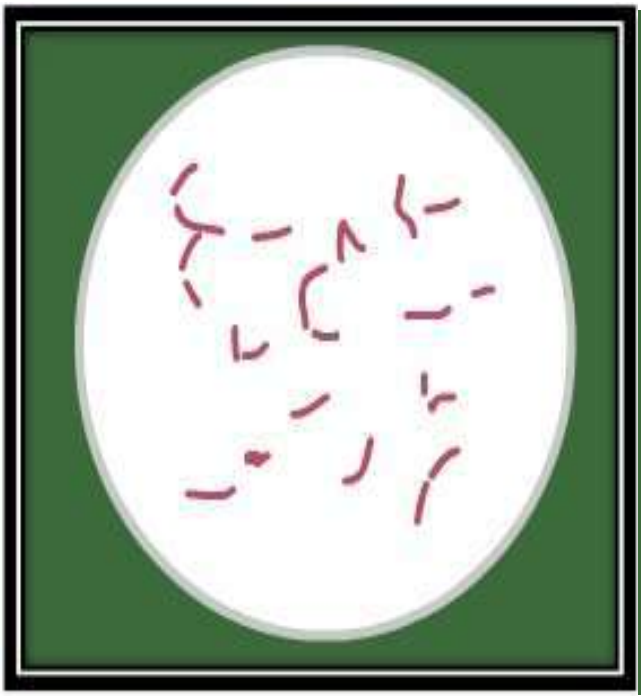
5. Decolorize with **ethyl alcohol or acetone** for about **10-15 sec**. or until no large amount of purple color wash out. However, do not over or under decolorize. Immediately wash with water.

6. Cover the smear with **safranine** for **30 seconds**. Wash with water.

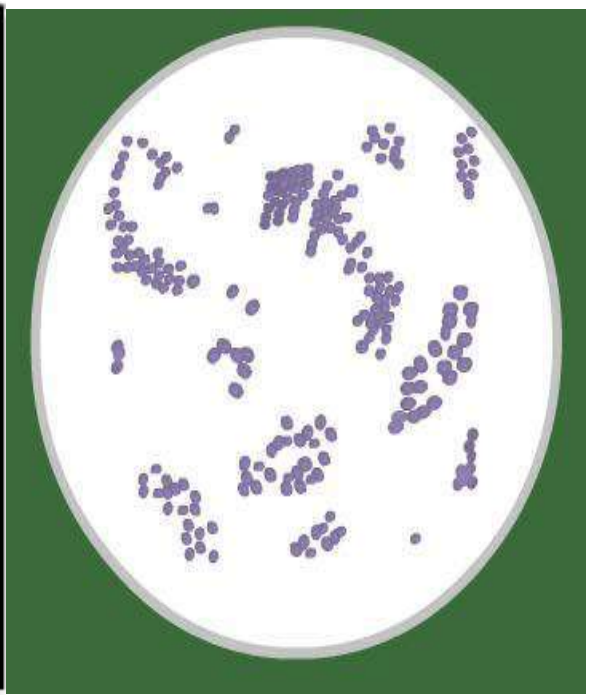
7. dry and examine under oil immersion objective, and record the observations indicating the Gram character and morphological arrangement of the microorganisms in each culture.

Results

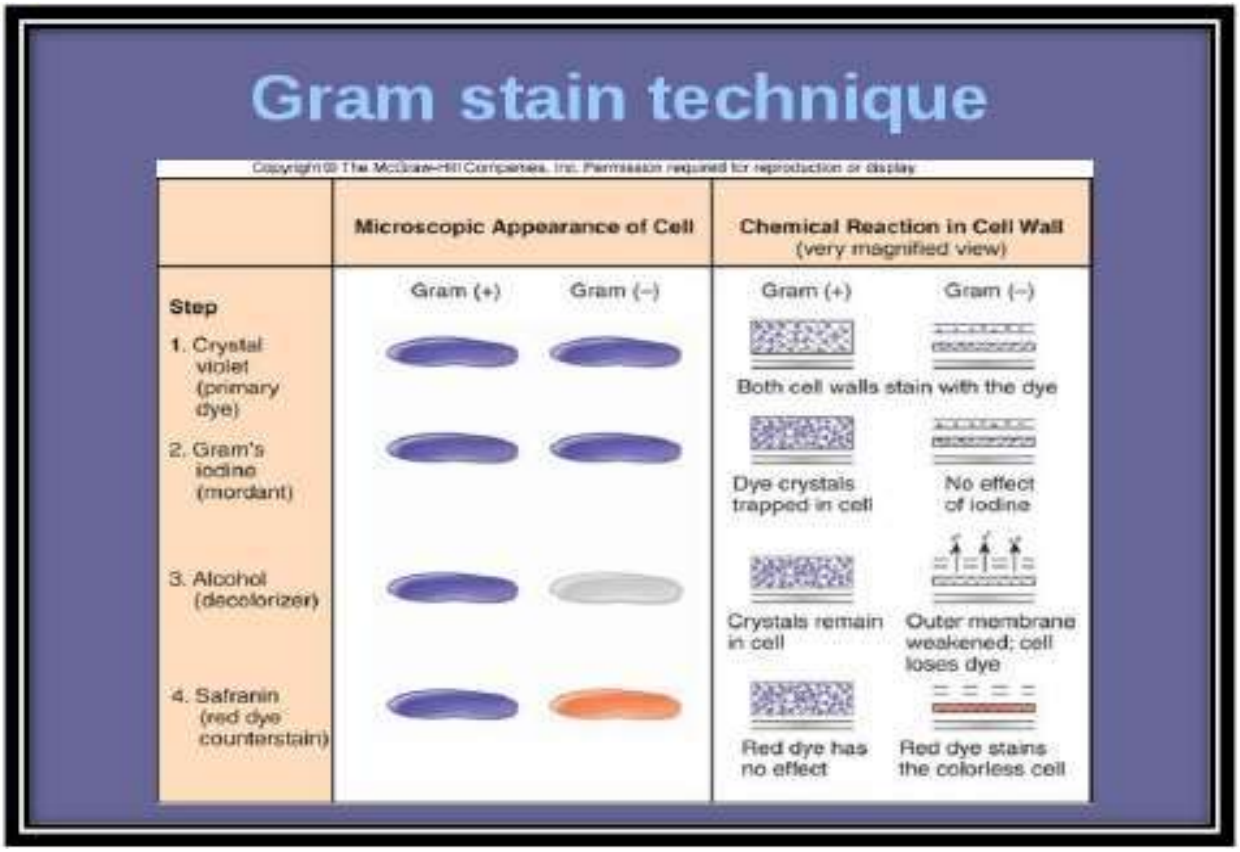
Gram positive ----------dark purple
Gram negative ----------light pink or red
Yeast cells ----------dark purple
Red blood cells ----------pink
White blood cells ----------pink
Epithelial cells ----------pale red



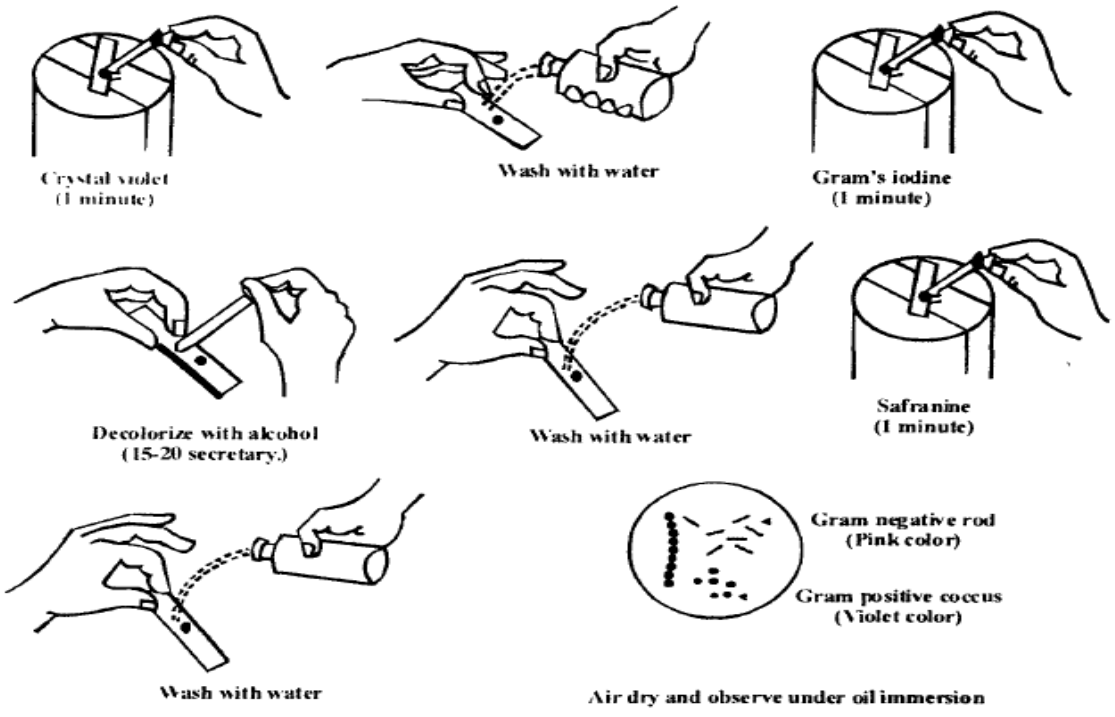
Gram negative bacteria



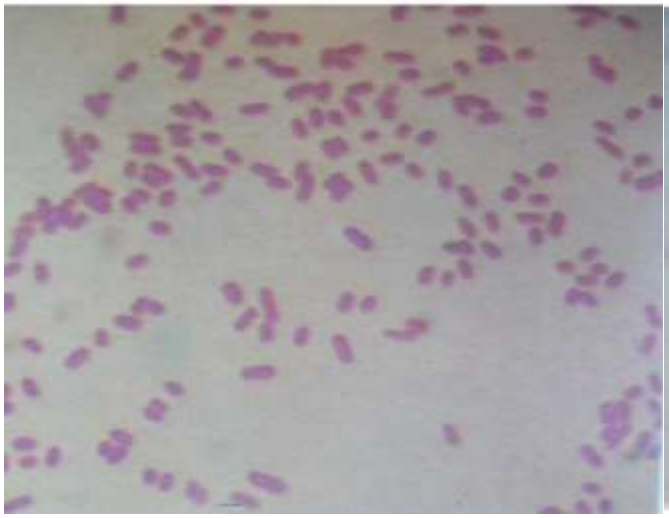
Gram positive bacteria



Differential staining of bacteria (Gram staining)

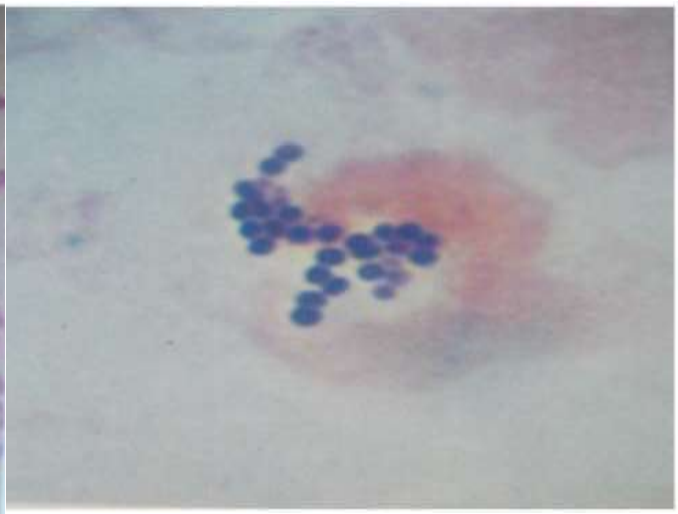


Gram staining



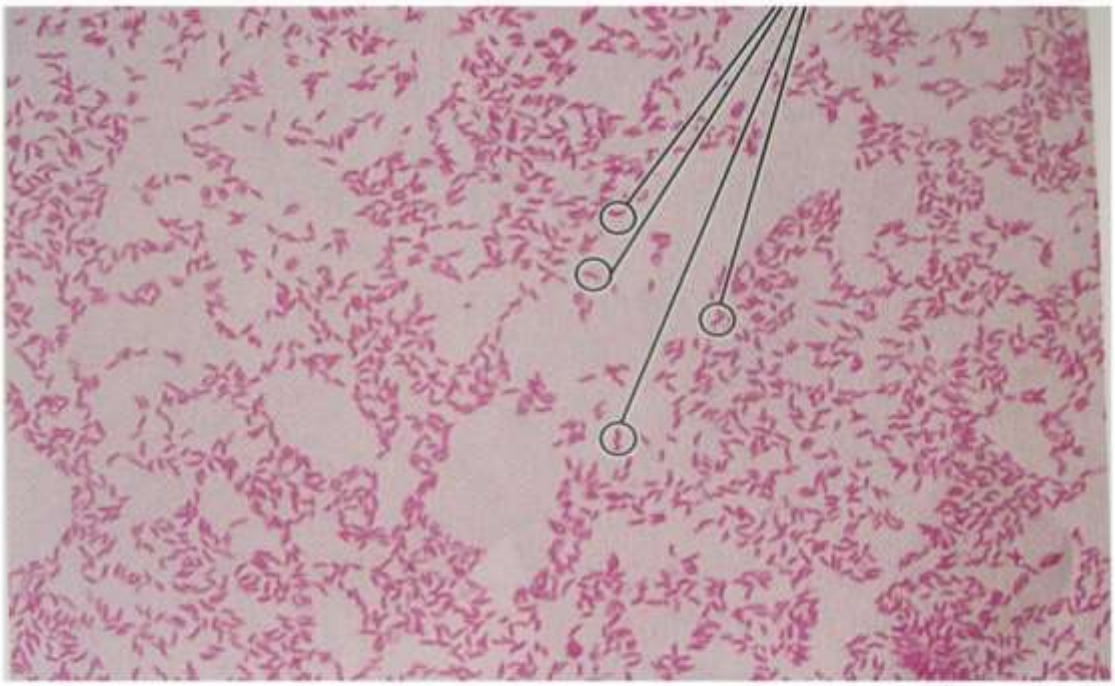
Gram stain of *E.coli*

The *E.coli* Appear as gram negative bacilli.



Gram stain of Staphylococcus.

the gram positive coccus in grape like clusters



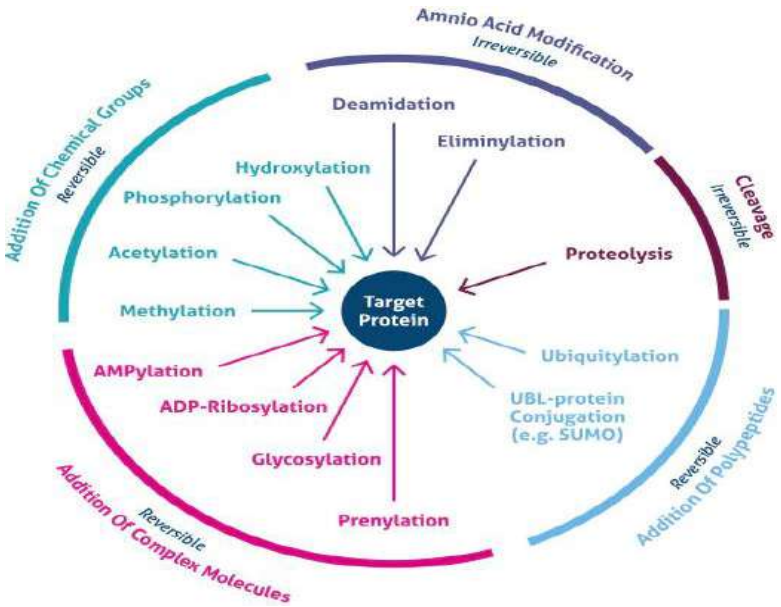
Simple stain of the Gram-negative rod *Vibrio cholerae*, the causative agent of cholera.

post Translation modification:

(**PTMs**) are covalent processing events that change the properties of a protein by proteolytic cleavage and adding a modifying group, such as acetyl, phosphoryl, glycosyl and methyl, to one or more amino acids . PTMs play a key role innumerous biological processes by significantly affecting the structure and dynamics of proteins .

Generally, a PTM can be reversible or irreversible . The reversible reactions contain covalent modifications, and the irreversible ones, which proceed in one direction, include proteolytic modifications . PTMs occur in a single type of amino acid or multiple amino acids and lead to changes in the chemical properties of modified sites .

PTMs usually are seen in the proteins with important structures/functions such as secretory proteins, membrane proteins and histones. These modifications affect a wide range of protein behaviors and characteristics, including enzyme function and assembly , protein lifespan, protein–protein interactions , cell–cell and cell–matrix interactions, molecular trafficking, receptor activation, protein solubility , protein folding and protein localization. Therefore, these modifications are involved in various biological processes such as signal transduction, gene expression regulation, gene activation, DNA repair and cell cycle control PTMs occur in various cellular organelles including the nucleus, cytoplasm, endoplasmic reticulum and Golgi apparatus .



Types of post-translational modification:

1- Phosphorylation

This process is an important reversible regulatory mechanism that plays a key role in the activities of many enzymes, membrane channels and many other proteins in prokaryotic and eukaryotic organisms . Phosphorylation target sites are Ser, Thr, Asp and Cys , but this modification mainly happens on Ser, Thr, Tyr . This PTM includes transferring a phosphate group from (ATP) to the receptor residues by kinase enzymes . Conversely, removal of a phosphate group is an enzymatic reaction catalyzed by different phosphatases.

Phosphorylation is the most studied PTM and one of the essential types of PTM, which often happens in cytosol or nucleus on the target proteins . This modification can change the function of proteins in a short time via one of the two principal ways: by allostery or by binding to interaction domains

Phosphorylation has a vital role in significant cellular processes such as replication, transcription, environmental stress response, cell movement, cell metabolism, apoptosis and immunological responsiveness . It has been shown that disruption in the pathway of phosphorylation can lead to various diseases such as cancer, Alzheimer's disease, Parkinson's disease and heart disease.

2- Acetylation

Acetylation is catalyzed via lysine acetyltransferase (KAT) and histone acetyltransferase (HAT) enzymes. Acetyltransferases use acetyl CoA as a cofactor for adding an acetyl group ($COCH_3$) to the ε-amino group of lysine side chains, whereas deacetylases (HDACs) remove an acetyl group on lysine side chains . There are three forms of acetylation: Nα-acetylation, Nε-acetylation and O-acetylation. Nα-acetylation is an irreversible modification, and the other two types of acetylation are reversible . Nε-acetylation is more biologically significant compared to the other types of acetylation.

Acetylation has an essential role in biological processes such as chromatin stability, protein–protein interaction, cell cycle control, cell metabolism, nuclear transport and actin nucleation ,

acetylated lysine is vital for cell development, and its dysregulation would lead to serious diseases such as cancer, aging, immune disorders, neurological diseases (Huntington's disease and Parkinson's disease) and cardiovascular diseases.

3- Methylation:

Methylation is a reversible PTM, which often occurs in the cell nucleus and on the nuclear proteins such as histone proteins . Methylation occurs on the Lys, Arg, Met, Phe and Pro residues in target proteins . However, lysine and arginine are the two main target residues in methylation, at least in eukaryotic cells. Nε-lysine methylation is one of the most abundant histone modifications in eukaryotic chromatin, which includes transferring the methyl groups from S-adenosylmethionine to histone proteins via methyltransferase enzyme .

Recent studies have shown tDefect in this modification can lead to various diseases such as cancer, mental retardation (Angelman syndrome), diabetes mellitus, lipofuscinosis and occlusive disease .

Lab1/ partial molecular biology / 2nd stage / Lec :Evan Hussein

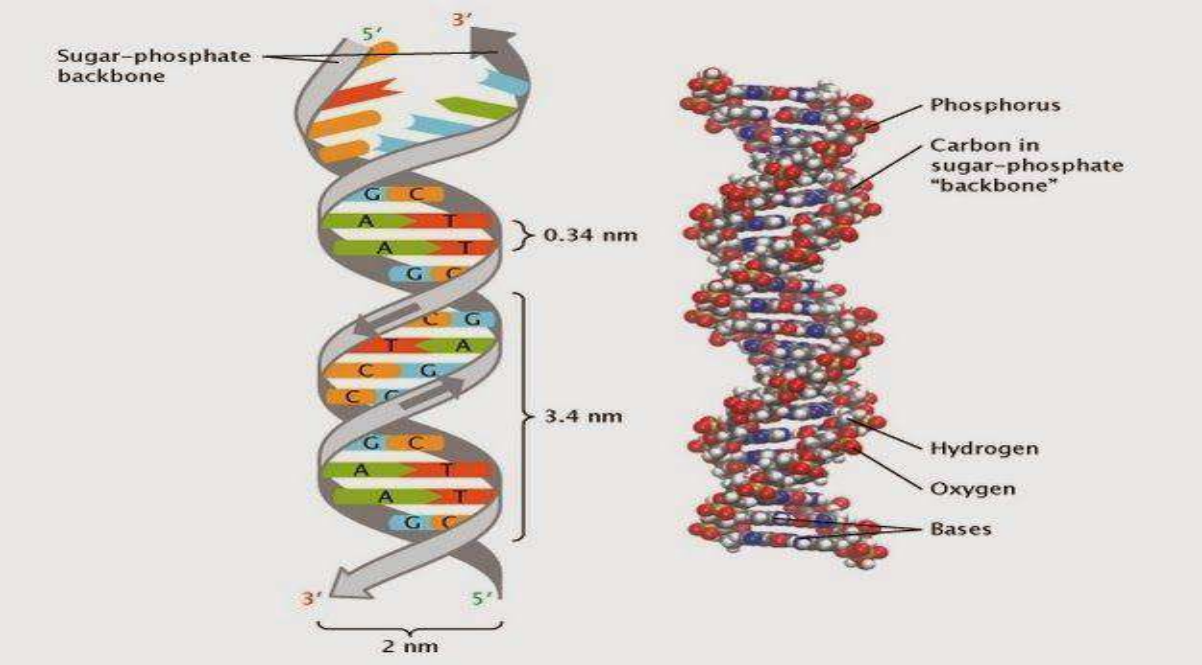
Introduction:

The molecular biology: is a branch of biology, It focuses on studying living organisms at the molecular level, It focuses on the interrelationships between the cellular molecules (Macro & Micro molecules) ,that the living cell consist of .

The cellular molecules are divided into two major groups:-

A- Macro molecules: nucleic acids (ribonucleic acid (**RNA**) and deoxyribonucleic acids (**DNA**)), Proteins, Carbohydrates and Lipids .

***B-Micro molecules*:** such as adenosine tri phosphate ATP, Nitrogenous bases (uracil, adenine, thymine, guanine and cytosine), Ions and others.



cellular molecules (Macro & Micro molecules) consist

History :

In 1945 the term molecular biology was used by physicist William Astbury. In 1953 Francis Crick, James Watson, and colleagues, working at Medical Research Council unit, Cavendish laboratory, Cambridge , made a double helix model of DNA which changed the entire research . They proposed the DNA structure based on previous research done by Rosalind Franklin and Maurice Wilkins. This research then lead to finding DNA material in other microorganisms, plants and animals.

Molecular biology is not simply the study of biological molecules and their interactions; it is also a collection of techniques developed since the field's genesis which have enabled scientists to learn about molecular processes. One notable technique which has revolutionized the field is the polymerase chain reaction (PCR), which was developed in 1983 and it is used in many applications across scientific disciplines (It will be studied later).

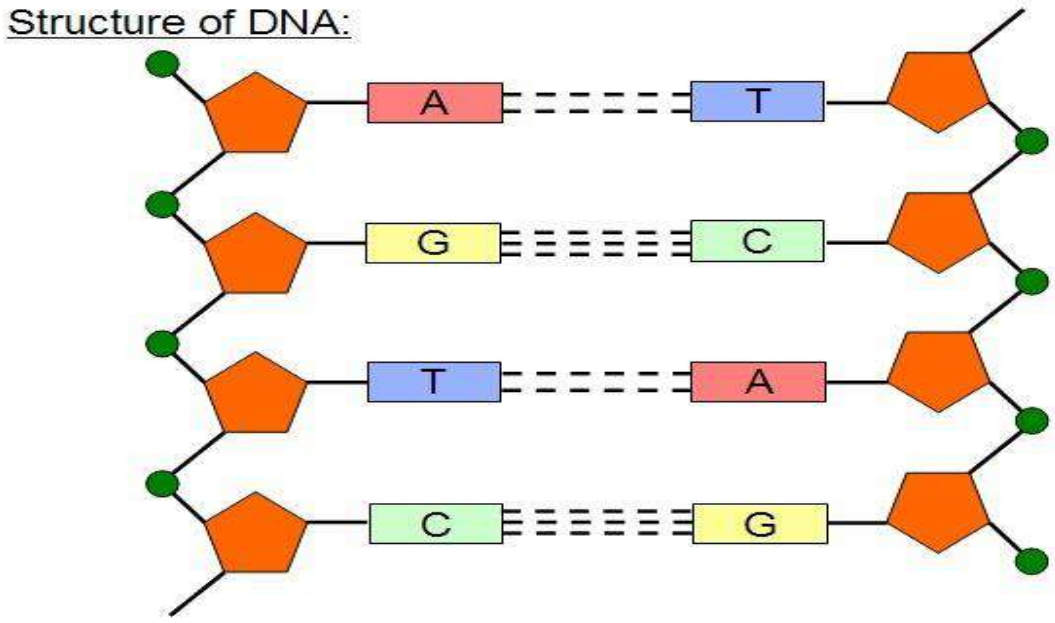
Molecular biology also plays a critical role in the understanding of structures, functions, and internal controls within cells, all of which can be used to efficiently target new drugs, diagnose disease, and better understand cell physiology. Some clinical research and medical therapies arising from molecular biology are covered under gene therapy and also the use in diagnosis.

William Astbury, Francis Crick, James Watson and Maurice Wilkins

What is DNA?

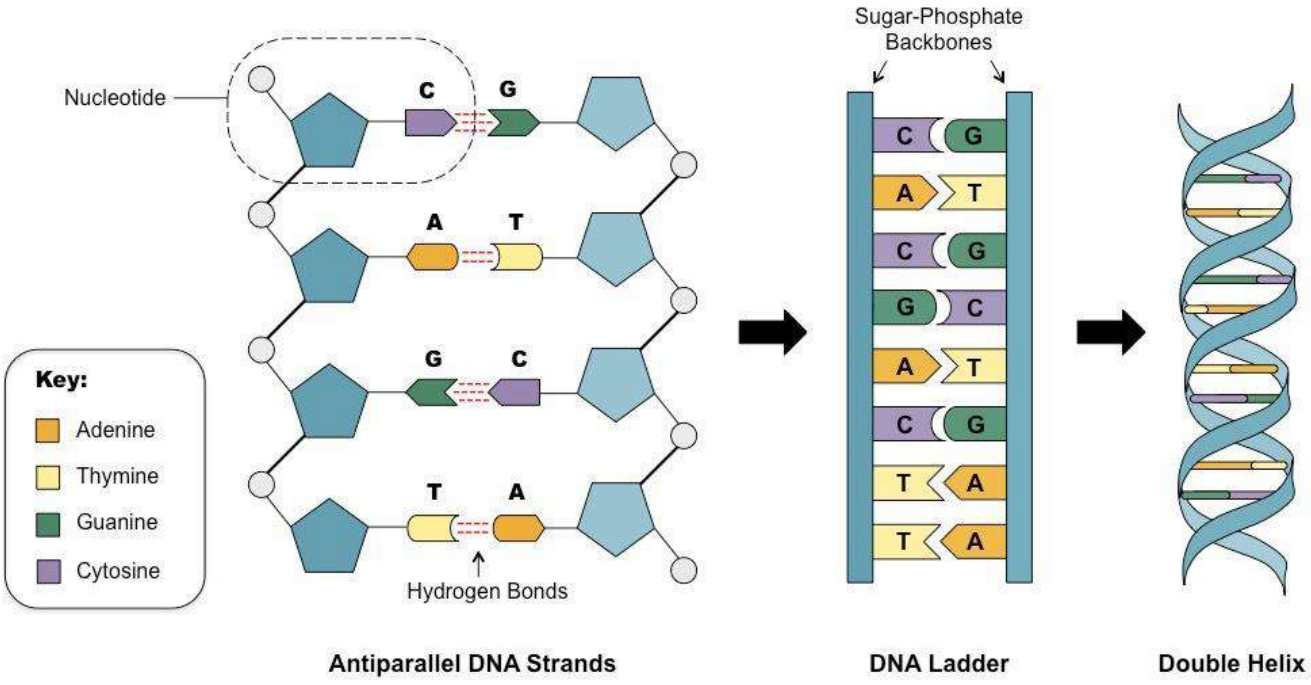
DNA, or deoxyribonucleic acid, is the hereditary material found in almost all other organisms (prokaryotic (bacteria) and eukaryotic cells (human, fungi) and in many viruses). Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

The information in DNA is stored as a code made up of four chemical bases: **adenine (A), guanine (G), cytosine (C), and thymine (T).** Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism.



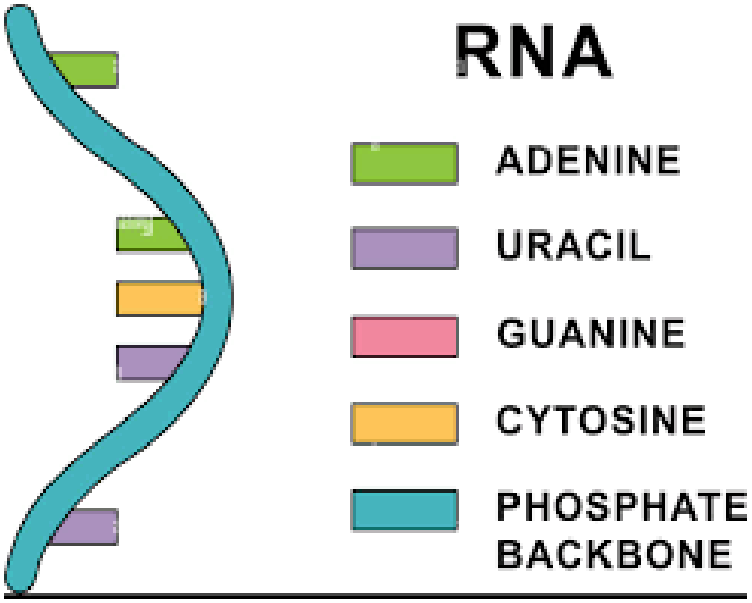
DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.



What is RNA?

RNA(ribonucleic acid), complex compound of 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 phosphodiesterase bonds, forming strands of varying lengths. The nitrogenous bases in RNA are **adenine, guanine, cytosine,** and **uracil,** which replaces **thymine** in DNA. The structure of the RNA molecule was described by R.W. Holley in 1965.



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. These and other types of RNAs 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.

1- mRNA(are messenger RNA) : carries genetic codes from the DNA in the nucleus to ribosomes(the sites of protein translation)in the cytoplasm.

2- tRNA (transfer RNA): 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.

3- rRNA (ribosomal RNA): In protein synthesis, 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.

4- In addition, RNAcan be broadly divided into coding (cRNA) and noncoding RNA (ncRNAs). There are two types of ncRNAs, housekeeping ncRNAs (tRNA and rRNA) and regulatory ncRNAs, which are further classified according to their size.

A-Long ncRNAs: ncRNAs, have at least 200 nucleotides.

B- Small ncRNAs : miRNA , snoRNA , snRNA , siRNA and piRNA, have fewer than 200 nucleotides.

DNA &RNA structure difference:

criteria	DNA	RNA
structure	a **double-stranded** molecule ,its contain deoxyribonucleic acid and its contain (U,A,G,C)	a **Single-strand molecule** ,its contain ribonucleic acid and its contain (T,A,G,C)
Function	The genetic and hereditary material of the cell	Synthesis of protein
Site	Present in nucleus of cell	Present in both nucleus and cytoplasm
Types	non	Many type.

Lab 3& 4 / DNA replication and transcription / 2 nd stage / par. Biology molecular / Asst. Lec :Evan Hussein

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DNA replication : is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. Replication is an essential process because, whenever a cell divides, the two new daughter cells must contain the same genetic information, or DNA, as the parent cell.

Replication Process :

Step 1: Replication Fork Formation: Before DNA can be replicated, the double stranded molecule must be "unzipped" into two single strands. In order to unwind DNA, these interactions between base pairs must be broken. **DNA helicase** disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin, DNA is directional in both strands, signified by **a 5' and 3' end**.

Step 2: Primer Binding: The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called **a primer** binds to the strand. The primer always binds as the starting point for replication. Primers are generated by the enzyme **DNA primase**.

Step 3: Elongation: **DNA polymerases** are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases in bacteria and human cells. **DNA polymerase III** binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication.

The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called **Okazaki fragments**, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

Step 4: Termination: Once both the continuous and discontinuous strands are formed, an enzyme called **exonuclease** removes all RNA primers from the original strands. These primers are then replaced with appropriate bases. **Another exonuclease** "proofreads" the newly formed DNA to check, remove and replace any errors. Another enzyme called **DNA ligase** joins Okazaki fragments together forming a single unified strand. The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction.

The ends of the parent strands consist of repeated DNA sequences called **telomeres**. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing.

Transcription : is the process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA).

DNA safely and stably stores genetic material in the nuclei of cells as a reference, or template. Meanwhile, mRNA is comparable to a copy from a reference book because it carries the same information as DNA but is not used for long-term storage and can freely exit the nucleus.

Steps of Transcription:

Step 1: Initiation: It occurs when the enzyme RNA polymerase binds to a region of a gene called the promoter. This signals the DNA to unwind so the enzyme can ‘‘read’’ the bases in one of the DNA strands. The enzyme is now ready to make a strand of mRNA with a complementary sequence of bases.

Step 2: Elongation: is the addition of nucleotides to the mRNA strand. RNA polymerase reads the unwound DNA strand and builds the mRNA molecule, using complementary base pairs. There is a brief time during this process when the newly formed RNA is bound to the unwound DNA. During this process, an adenine (A) in the DNA binds to an uracil (U) in the RNA.

Step 3: Termination: is the ending of transcription, and occurs when RNA polymerase crosses a stop (termination) sequence in the gene. The mRNA strand is complete, and it detaches from DNA.

Lab 5 / Translation / 2 nd stage / par. Biology molecular / Asst. Lec :Evan Hussein

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Translation: is the process by which the genetic code contained within a messenger RNA (mRNA) molecule is decoded to produce a specific sequence of amino acids in a polypeptide chain. It occurs in the cytoplasm following DNA transcription and, like transcription.

translation process:

Step 1: Initiation: For translation to begin, the start codon (5'AUG) must be recognised. This codon is specific to the amino acid methionine, which is nearly always the first amino acid in a polypeptide chain. At the 5' cap of mRNA, the small 40s subunit of the ribosome binds. Subsequently, the larger 60s subunit binds to complete the initiation complex.

Step 2: Elongation: The ribosome then translocates along the mRNA molecule to the next codon, again using energy yielded from the hydrolysis of GTP. The polypeptide chain is built up in the direction from the N terminal (methionine) to the C terminal (the final amino acid).

Step 3: Termination: When the ribosome reaches a stop codon, such as UAA, UAG, or UGA, translation is finished since these codons lack tRNAs. When this happens, the translation stops, and the newly produced polypeptide chain is released.

There are a group of devices in medical laboratories in general and in the molecular biology laboratory in particular, and these devices include the following:

1- **microscope:** use to magnify or enlarge micro spacimen to study structure ,size &shape of cellular particles.

2-centrifuge: Use to sepration of plasma /serum From blood &blood compenantsPurification /sepration of biological Mixture sample i.e.DNA ,RNA ,proteins,RBC,WBC,pus cell.

3- Hot air oven : Hot air oven are electrical devices which is use dry heat to sterilize of glassware ,cotton ,swab ,culturemedia ,they can be operated from

(50-300 C).

4- Autoclave : Used to sterilize (removing or killing of bacteria) i.e.culture media ,glassware ,chemical ,at 121C,15Ibs pressure for 20 minutes.

5- incubator: Incubator is device used to grow & maintain microbiological culture or cell culture ,the optimal temp.humidity.

6- Laminar air flow (hood): Use to sterilize specific working environment while pour culture plate .

7- water bath : Its used to incubate samples in water at aconstant temperture(20-60 C), Over along of time .

8- Vortex: it's used to assist in the process of shaking tubes.

9- PCR machine : PCR machine or DNA amplifier is alaboratory apparatus most commonly used to amplify segment of DNA via the polymerase chain reaction (PCR).

10- Gel electrophoresis: Used to separation or identication of DNA ,RNA ,protein .

11- Uv transiluminator: Used to molecular biology labs to veiow DNA or RNA that has esparated by electrophoresis through an agarose gel .

Lab 6 / DNA extraction / par. Biology molecular / Asst. Lec :Evan Hussein

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DNA extraction : is frequently a preliminary step in many diagnostic procedures used to identify environmental (prokaryotic &eukaryotic) which is used for scientific , medical or forensic purpose . example, diagnose illnesses and hereditary diseases, as The first isolation of DNA was done in 1869 by Friedrich Miescher.

DNA can be extracted from most any intact cellular tissue include :

Skin , blood ,saliva , semen ,mucus , bone marrow , muscle tissue, plant &microorganism (bacteria, viruses ,fungi) ,etc…

There are three basic steps of DNA extraction are 1) lysis, 2) precipitation, and 3) purification:

Step 1: Lysis

In this step, the cell and the nucleus are broken open to release the DNA inside and there are two ways to do this. **First**, mechanical disruption breaks open the cells. This can be done with a tissue homogenizer (like a small blender), with a mortar and pestle, or by cutting the tissue into small pieces. Mechanical disruption is particularly important when using plant cells because they have a tough cell wall. **Second,** lysis uses detergents and enzymes such as Proteinase K to free the DNA and dissolve cellular proteins.

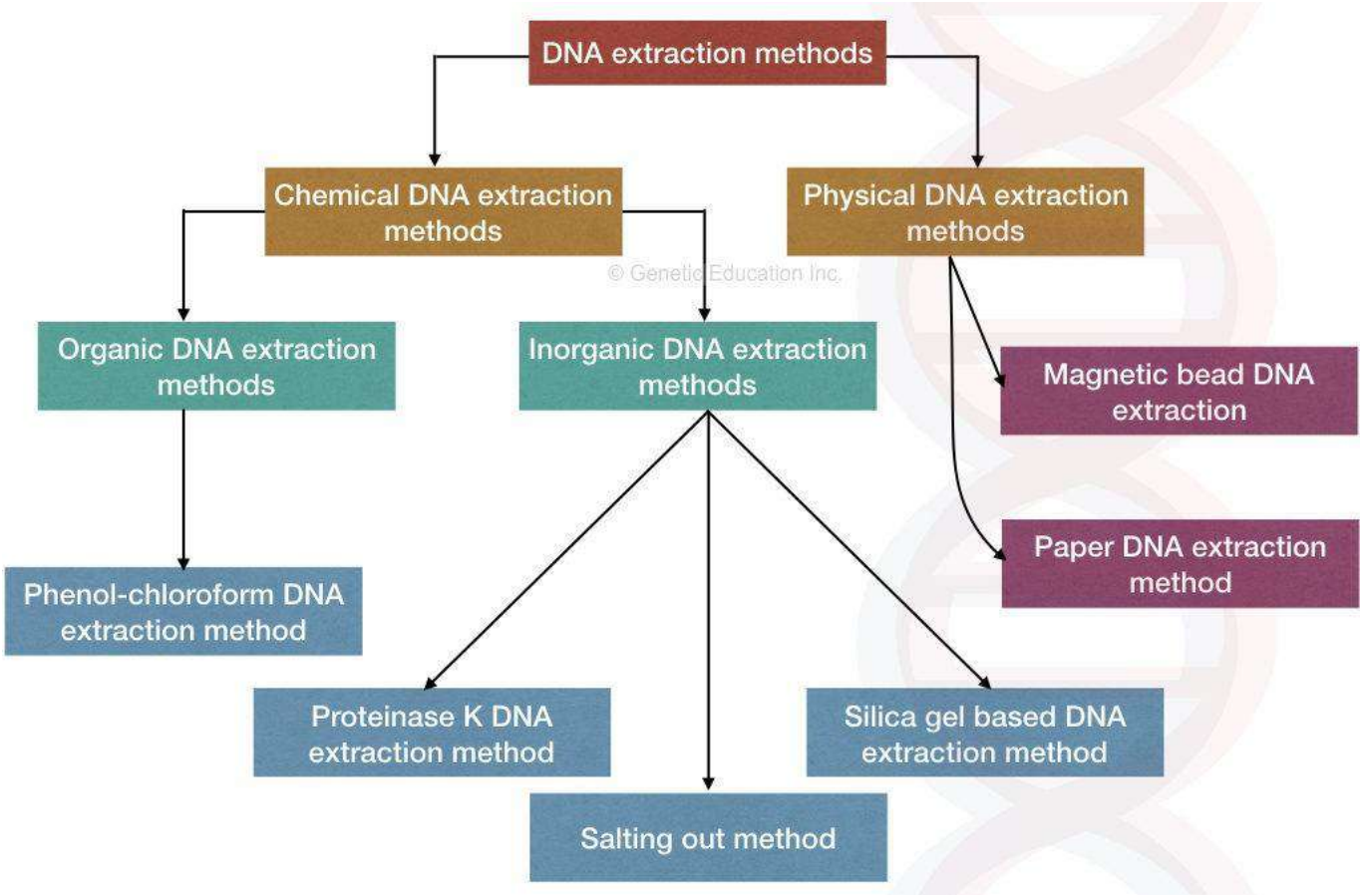
Step 2: Precipitation

When you complete the lysis step, the DNA has been freed from the nucleus, but it is now mixed with mashed up cell parts. Precipitation separates DNA from this cellular debris. **First,** Na+ ions (sodium) neutralize the negative charges on the DNA molecules, which makes them more stable and less water soluble. **Second,** alcohol (such as ethanol or isopropanol) is added and causes the DNA to precipitate out of the aqueous solution because it is not soluble in alcohol.

Step 3: Purification

Now that DNA has been separated from the aqueous phase, it can be rinsed with alcohol to remove any remaining unwanted material and cellular debris. At this point the purified DNA is usually re-dissolved in water for easy handling and storage.

There are many methods of DNA extraction of the following :



Introduction :

Regulation of gene expression(gene regulation) , includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA). For a cell to function properly, necessary proteins must be synthesized at the proper time. All cells control or regulate the synthesis of proteins from information encoded in their DNA. The process of "turning on" a gene to produce mRNA and protein is called gene expression. Whether in a simple unicellular organism (prokaryotic) or a complex multi-cellular organism(Eukaryotic), each cell controls when its genes are expressed, how much of the protein is made, and when it is time to stop making that protein because it is no longer needed.

The regulation of gene expression conserves energy and space. It is more energy efficient to turn on the genes only when they are required. The control of gene expression is extremely complex. Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases, including cancer.

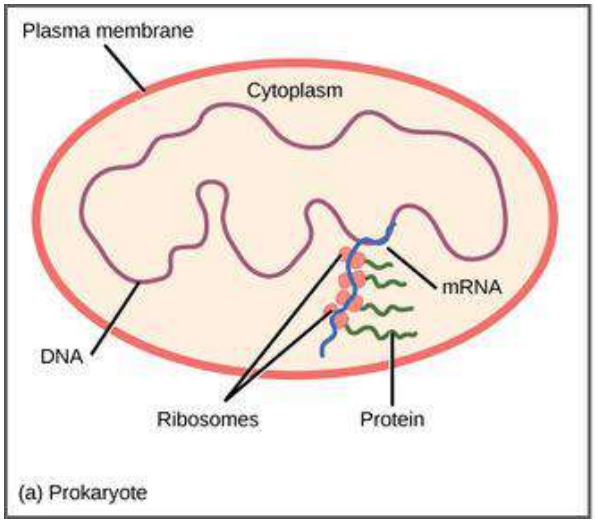
the first discovery of a gene regulation system is widely considered to be the identification in 1961 of the lac operon, discovered by Francois Jacob and Jacques Monod, in which some enzymes involved in lactose metabolism are expressed by E. coli only in the presence of lactose and absence of glucose.

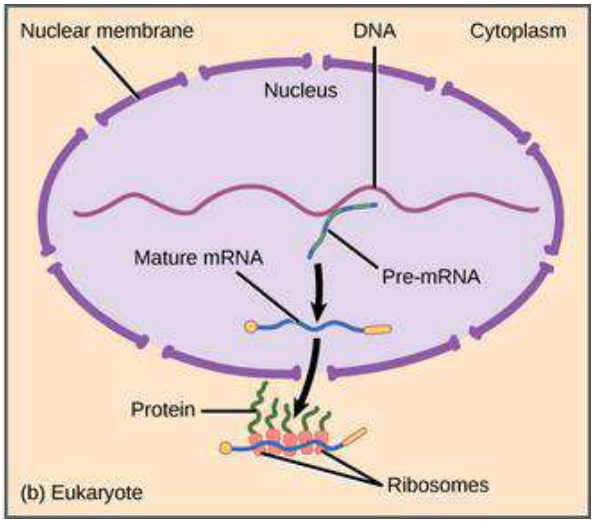
In multicellular organisms, gene regulation drives cellular differentiation and morphogenesis in the embryo, leading to the creation of different cell types that possess different gene expression profiles from the same genome sequence. Although this does not explain how gene regulation originated.

Prokaryotic & Eukaryotic Gene Expression:

Since **prokaryotic organisms** are single-celled organisms that lack a cell nucleus, their DNA floats freely in the cell's cytoplasm. When a particular protein is needed, the gene that codes for it is transcribed in mRNA, which is simultaneously translated into protein. When the protein is no longer needed, transcription stops. As a result, the primary method to control how much of each protein is expressed in a prokaryotic cell is the regulation of transcription.

Eukaryotic cells, in contrast, have intracellular organelles that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus, where it is transcribed into mRNA. The newly synthesized mRNA is then modified and transported out of the nucleus into the cytoplasm, where ribosomes translate the mRNA into protein. The processes of transcription and translation are physically separated by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only in the cytoplasm. The regulation of gene expression in eukaryotes can occur at all stages of the process.





A/Prokaryotic Gene Regulation:

The DNA of prokaryotes is organized into a circular chromosome that resides in the cell's cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are often encoded together in blocks called operons. For example, all five of the genes needed to make the amino acid tryptophan in the bacterium E. coli are located next to each other in the trp operon. The genes in an operon are transcribed into a single mRNA molecule. This allows the genes to be controlled as a unit: either all are expressed, or none is expressed. Each operon needs only one regulatory region, including a promoter, where RNA polymerase binds, and an operator, where other regulatory proteins bind.

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons.

1-Activators are proteins that increase the transcription of a gene.

2-Repressors are proteins that suppress transcription of a gene.

3- inducers are molecules that bind to repressors and inactivate them.

Below the example of how these molecules regulate different operons:

the trp Operon: A Repressor Operon:

Like all cells, bacteria need amino acids to survive. Tryptophan is one amino acid that the bacterium E. coli can either ingest from the environment or synthesize. When E. coli needs to synthesize tryptophan, it must express a set of five proteins that are encoded by five genes. These five genes are located next to each other in the tryptophan (trp) operon

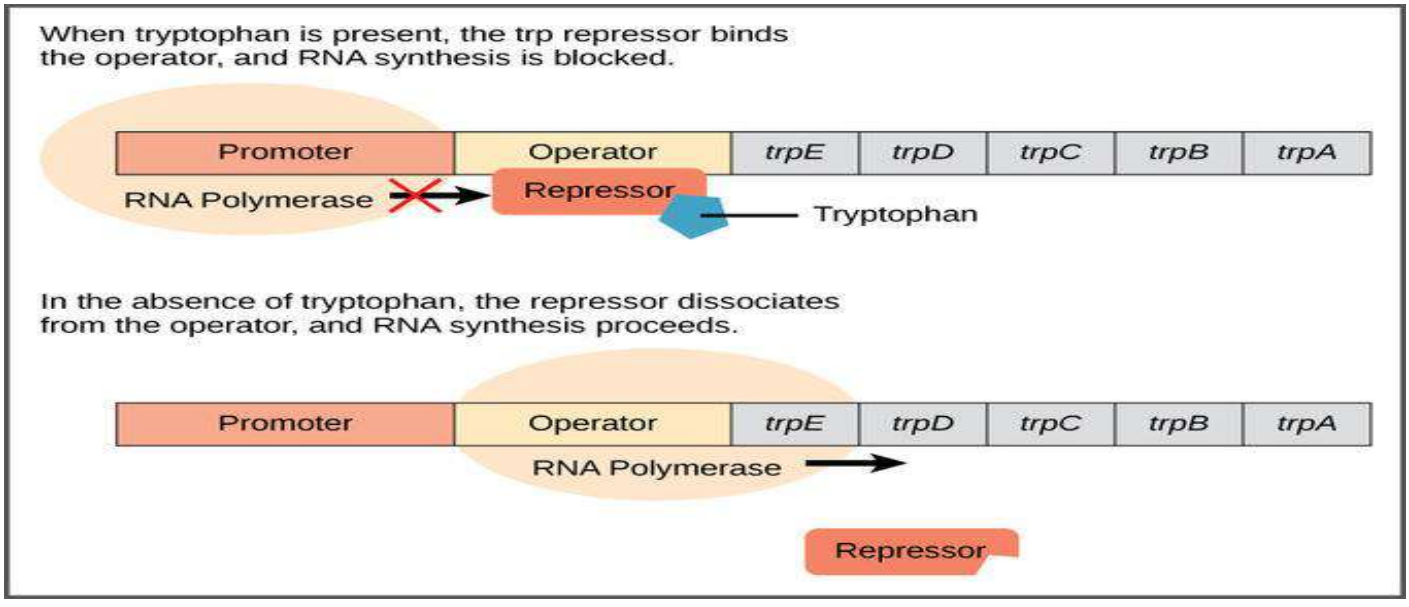
.

When tryptophan is present in the environment, E. coli does not need to synthesize it, and the trp operon is switched off. However, when tryptophan availability is low, the trp operon is turned on so that the genes are transcribed, the proteins are made, and tryptophan can be synthesized.

A DNA sequence called the operator is located between the promoter and the first trp gene. The operator contains the DNA code to which the repressor protein can bind. The repressor protein is regulated by levels of tryptophan in the cell.

When tryptophan is present in the cell, two tryptophan molecules bind to the trp repressor. This causes the repressor to change shape and bind to the trp operator. Binding of the tryptophan–repressor complex at the operator physically blocks the RNA polymerase from binding, and transcribing the downstream genes. Thus, when the cell has enough tryptophan, it is preventing from making more.

When tryptophan is not present in the cell, the repressor has no tryptophan to bind to it. The repressor is not activated and it does not bind to the operator. Therefore, RNA polymerase can transcribe the operon and make the enzymes to synthesize tryptophan. Thus, when the cell does not have enough tryptophan, it synthesizes it.



Post-transcriptional modifications:

Transcriptional modification or co-transcriptional modification is a set of biological processes common to most eukaryotic cells by which an RNA primary transcript is chemically altered following transcription from a gene to produce a mature, functional RNA molecule that can then leave the nucleus and perform any of a variety of different functions in the cell. There are many types of post-transcriptional modifications achieved through a diverse class of molecular mechanisms.

in Prokaryotic mRNA does not require any significant post-transcriptional modification as translation can occur immediately which prevents degradation of the mRNA .

In eukaryotes, transcription and translation occur in separate parts of the cell, allowing for significant post-transcriptional modification to occur, and the immediate product of an mRNA transcript is called pre-mRNA which needs to be modified to form mature mRNA.

Three post-transcriptional events must occur:

1- CAPPING (5’ END) : A methylated cap is added to the 5' end to protect against degradation by Exonuclease.{ addition of 7-methylguanosine to the 5’end. This is achieved by the removal of terminal 5’ phosphate that is done with the help of phosphate enzymes. This reaction is accelerated by the enzyme adenosyltransferase, which at last produces diphosphate 5‘end}.

2- A poly-A tail (long chain of adenine nucleotides (**AAUAAA**) is added to the 3' end for further protection and to help the transcript exit the nucleus, It is known as polyadenylation.

3- SPLICING Removal of introns (Splicing) or intervening sequences are the RNA sequences which do not code for the proteins. These introns are removed from the primary transcript in the nucleus, exons (coding sequences) are ligated to form the mRNA molecule, and the mRNA molecule is transported to the cytoplasm. The

molecular machine that accomplishes the task of splicing is known as the spliceosomes. Small nuclear RNA molecules that recognize splice sites in the pre-mRNA sequence. The excised intron is released as a "lariat" structure, which is degraded.

What are Exons?

Exons are nucleotide sequences in DNA and RNA that are conserved in the creation of mature RNA. The process by which DNA is used as a template to create mRNA is called transcription. mRNA then works in conjunction with ribosomes and transfer RNA (tRNA), both present in the cytoplasm, to create proteins in a process known as translation. Exons usually include both the 5'- and 3'- untranslated regions of mRNA, which contain start and stop codons, in addition to any protein coding sequences.

What are Introns?

Introns are nucleotide sequences in DNA and RNA that do not directly code for proteins, and are removed during the precursor messenger RNA (pre-mRNA) stage of maturation of mRNA by RNA splicing. Introns can range in size from 10's of base pairs to 1000's of base pairs, and can be found in a wide variety of genes that generate RNA in most living organisms, including viruses.

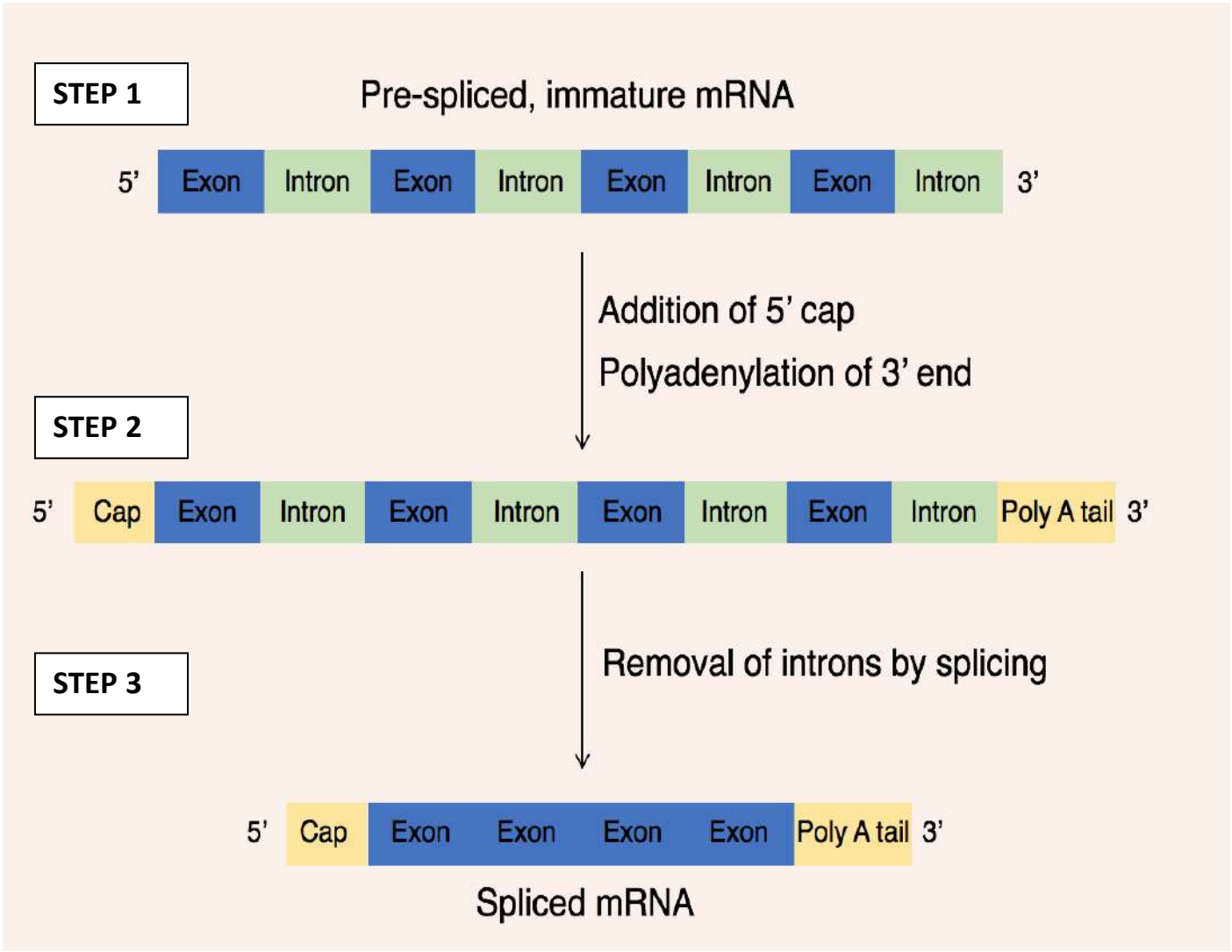
three distinct types of introns have been identified:

1-Introns in protein coding genes, removed by spliceosomes

2- Self-splicing introns, which catalyze their own removal from mRNA, tRNA, and rRNA precursors using guanosine-5'-triphosphate (GTP), or another nucleotide cofactor.

3- Self-splicing introns, which do not require GTP in order to remove themselves.

It is vital for the introns to be removed precisely, as any left-over intron nucleotides, or deletion of exon nucleotides, may result in a faulty protein being produced. This is because the amino acids that make up proteins are joined together based on codons, which consist of three nucleotides. An imprecise intron removal thus may result in a frame shift, which means that the genetic code would be read incorrectly.

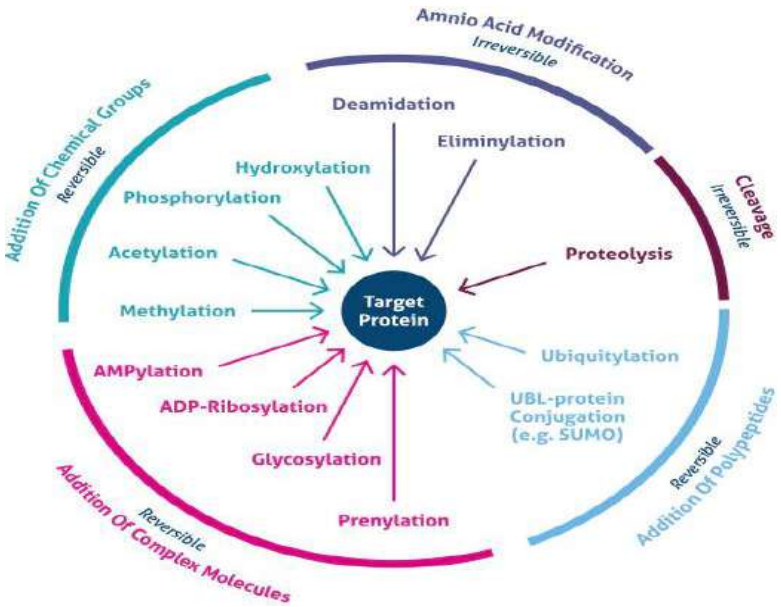

STEP 1
Pre-spliced, immature mRNA
5'
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
3'
Addition of 5' cap
Polyadenylation of 3' end
STEP 2
5'
Cap
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
Poly A tail
3'
STEP 3
Removal of introns by splicing
5'
Cap
Exon
Exon
Exon
Exon
Poly A tail
3'
Spliced mRNA

post Translation modification:

(**PTMs**) are covalent processing events that change the properties of a protein by proteolytic cleavage and adding a modifying group, such as acetyl, phosphoryl, glycosyl and methyl, to one or more amino acids . PTMs play a key role innumerous biological processes by significantly affecting the structure and dynamics of proteins .

Generally, a PTM can be reversible or irreversible . The reversible reactions contain covalent modifications, and the irreversible ones, which proceed in one direction, include proteolytic modifications . PTMs occur in a single type of amino acid or multiple amino acids and lead to changes in the chemical properties of modified sites .

PTMs usually are seen in the proteins with important structures/functions such as secretory proteins, membrane proteins and histones. These modifications affect a wide range of protein behaviors and characteristics, including enzyme function and assembly , protein lifespan, protein–protein interactions , cell–cell and cell–matrix interactions, molecular trafficking, receptor activation, protein solubility , protein folding and protein localization. Therefore, these modifications are involved in various biological processes such as signal transduction, gene expression regulation, gene activation, DNA repair and cell cycle control PTMs occur in various cellular organelles including the nucleus, cytoplasm, endoplasmic reticulum and Golgi apparatus .



Types of post-translational modification:

1- Phosphorylation

This process is an important reversible regulatory mechanism that plays a key role in the activities of many enzymes, membrane channels and many other proteins in prokaryotic and eukaryotic organisms . Phosphorylation target sites are Ser, Thr, Asp and Cys , but this modification mainly happens on Ser, Thr, Tyr . This PTM includes transferring a phosphate group from (ATP) to the receptor residues by kinase enzymes . Conversely, removal of a phosphate group is an enzymatic reaction catalyzed by different phosphatases.

Phosphorylation is the most studied PTM and one of the essential types of PTM, which often happens in cytosol or nucleus on the target proteins . This modification can change the function of proteins in a short time via one of the two principal ways: by allostery or by binding to interaction domains

Phosphorylation has a vital role in significant cellular processes such as replication, transcription, environmental stress response, cell movement, cell metabolism, apoptosis and immunological responsiveness . It has been shown that disruption in the pathway of phosphorylation can lead to various diseases such as cancer, Alzheimer's disease, Parkinson's disease and heart disease.

2- Acetylation

Acetylation is catalyzed via lysine acetyltransferase (KAT) and histone acetyltransferase (HAT) enzymes. Acetyltransferases use acetyl CoA as a cofactor for adding an acetyl group ($COCH_3$) to the ε-amino group of lysine side chains, whereas deacetylases (HDACs) remove an acetyl group on lysine side chains . There are three forms of acetylation: Nα-acetylation, Nε-acetylation and O-acetylation. Nα-acetylation is an irreversible modification, and the other two types of acetylation are reversible . Nε-acetylation is more biologically significant compared to the other types of acetylation.

Acetylation has an essential role in biological processes such as chromatin stability, protein–protein interaction, cell cycle control, cell metabolism, nuclear transport and actin nucleation ,

acetylated lysine is vital for cell development, and its dysregulation would lead to serious diseases such as cancer, aging, immune disorders, neurological diseases (Huntington's disease and Parkinson's disease) and cardiovascular diseases.

3- Methylation:

Methylation is a reversible PTM, which often occurs in the cell nucleus and on the nuclear proteins such as histone proteins . Methylation occurs on the Lys, Arg, Met, Phe and Pro residues in target proteins . However, lysine and arginine are the two main target residues in methylation, at least in eukaryotic cells. Nε-lysine methylation is one of the most abundant histone modifications in eukaryotic chromatin, which includes transferring the methyl groups from S-adenosylmethionine to histone proteins via methyltransferase enzyme .

Recent studies have shown tDefect in this modification can lead to various diseases such as cancer, mental retardation (Angelman syndrome), diabetes mellitus, lipofuscinosis and occlusive disease .