

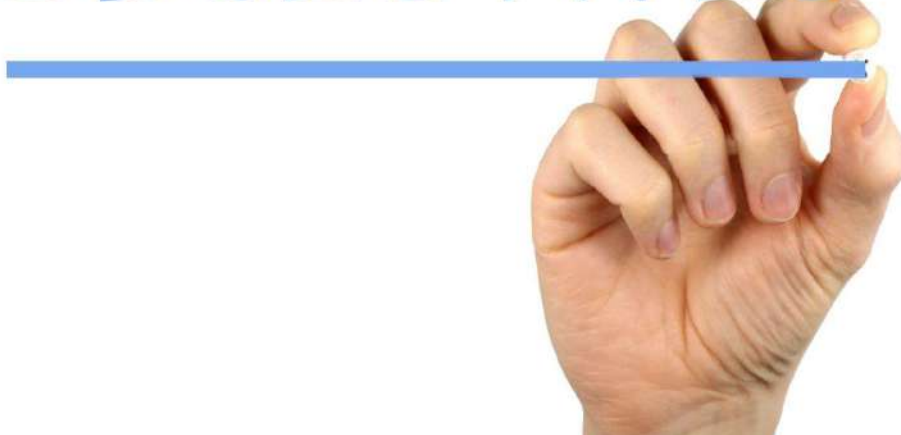


# Molecular Biology

## 1<sup>st</sup> lecture

### Introduction in Molecular Biology

# OBJECTIVES



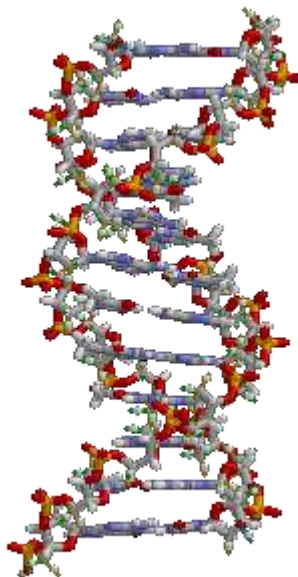
- Introduction of Molecular Biology.
- Benefits of Molecular Biology.
- Common Molecular Biology Techniques.
- Structure of DNA.
- Chemical Structure of Nucleotides.

# Introduction

Molecular biology was established as an official branch of science in the 1930s, the term wasn't invented until 1938 by **Warren Weaver**.

In 1953, **James Watson** and **Francis Crick** described the structure of DNA and the interactions within the molecule. This triggered research into molecular biology and increased interest in it.

Molecular biology is studying the molecular structures of the processes of replication, transcription, translation and cell function. It is closely related and working with Biochemistry and Genetics.





# Introduction

**Definition:** Molecular biology is the study of living organisms at the level of the molecules that control them and make them up. In addition, to understand living organisms by examining the components that make them up.

- Molecular biology is a specific branch of **biochemistry**.
- Molecular biology is the studying of the **chemistry of molecules** which are specifically connected to **living processes**.
- **DNA** and **RNA** have a particular importance to molecular biology and the **proteins** which are constructed based on the **genetic instructions** encoded in the DNA.
- Lipids and carbohydrates may also be studied for their interaction with nucleic acids and proteins.



# Benefits of Molecular Biology

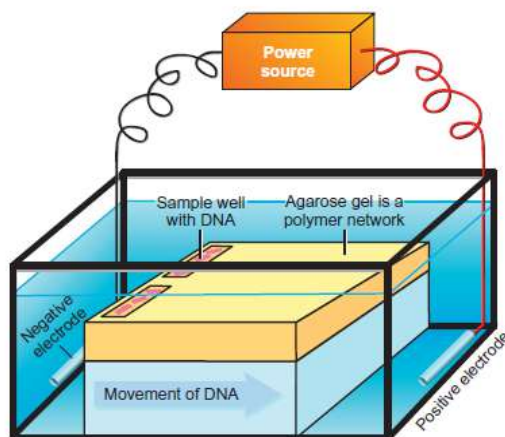
Studying molecular biology permitting us to **understand the bases of life** and provides the scientist with mechanistic tools for scientific research.

1. **Determining the function of single gene or proteins, and find out what will happen if that gene or protein was absent or defected.**
2. **Examine when and why certain genes are switched ON or OFF.**
3. **Provided scientists with a deeper understanding of how living things work.**
4. **Using molecular biology techniques in diagnosis and confirmation of certain diseases.**
5. **Using molecular biology knowledge to develop treatments.**

# Common Molecular Biology Techniques

**I. Electrophoresis:** is a process that separates and purifies molecules such as DNA or proteins out depending on their size (Agarose Gel Electrophoresis) and electrical charge.

- Electrophoresis is a **backbone** of molecular biology techniques.
- Electrophoresis is the **most used physical method** in all molecular biology.
- Knowing the **size of a molecule is very important** and it can be used to identify molecules or fragments of molecules.
- Ensuring that we have the **correct molecule** present.





# Common Molecular Biology Techniques

**II. Polymerase Chain Reaction (PCR):** is a process used to amplify minute amounts of distinct DNA sequence to large amounts of identical sufficient copies which can be used in further experiments or analysis. The PCR technique is very versatile technique for copying DNA.

- PCR is **adequately sensitive** to **amplify** the DNA from a single cell to yield amounts sufficient for analysis.
- PCR can **add, delete, and modify** the DNA.
- PCR can be used in **forensics** (identification using DNA profiling).
- PCR used in **disease diagnosis** such as Covid-19.
- PCR can be used to introduce small **point mutations** into a **gene** in a process called **site-directed mutagenesis**.

# Common Molecular Biology Techniques

## **Types of polymerase chain reaction- PCR**

Many modifications of PCR methods have been established to improve the effectiveness of PCR in diagnostic settings based on their application.

**Some of most common PCR types are:**

- 1. Real-Time PCR (Quantitative PCR, qPCR).**
- 2. Reverse-Transcriptase PCR (RT-PCR).**
- 3. Multiplex PCR**
- 4. Nested PCR.**

Other types of PCR are:

5. High Fidelity PCR.
6. Fast PCR.
7. Hot Start PCR.
8. GC-Rich PCR.
9. Long-Range PCR.
10. Arbitrary Primed PCR.



# Common Molecular Biology Techniques

**III. Restriction Digestion:** is the process of cutting DNA up into smaller pieces using restriction enzymes which only act at a particular genetic sequence.

**IV. Ligation:** is the process of connecting two pieces of DNA together. Ligation is useful when introducing a new piece of DNA into another genome.

**V. Blotting:** is a technique used to specifically identify biomolecules following electrophoresis. The molecule of interest is indicated using either a labeled probe (a complementary strand of nucleic acid) or a labeled antibody produced against a specific protein.

## Common Molecular Biology Techniques

**VI. Cloning:** is the technique of introducing a new gene or into a cell or organism. This can be used to see what effect the expression of that gene has on the organism.

For example to turn an organism into a factory which will produce large quantities of the gene or the protein it codes for, or (within the inclusion of a label) to indicate where the products of that gene are expressed in the organism.

Insertion of genetic material into a bacterium is called **transformation**, while insertion into a eukaryotic cell is called **transfection**. If a virus is used to introduce this material, the process is called **transduction**.

Transformation → تحويل- تغيير هيئة

Transfection → تعداد- تعمد التغيير

Transduction → توضيح



## Structure of DNA

DNA was first discovered in 1869 by **Frederich Miescher** who extracted it from the **pus from infected wounds**.

However, it was nearly a century before its true significance was revealed by **Oswald Avery**. Avery concluded that the genes were made of DNA and that somehow genetic information was encoded in this molecule.

the **fundamentals** of **Modern Genetics** were laid when **Mendel** found that hereditary information consists of discrete basic units now called **genes**. Each gene is responsible for a **single inherited property** or **characteristic** of the organism.

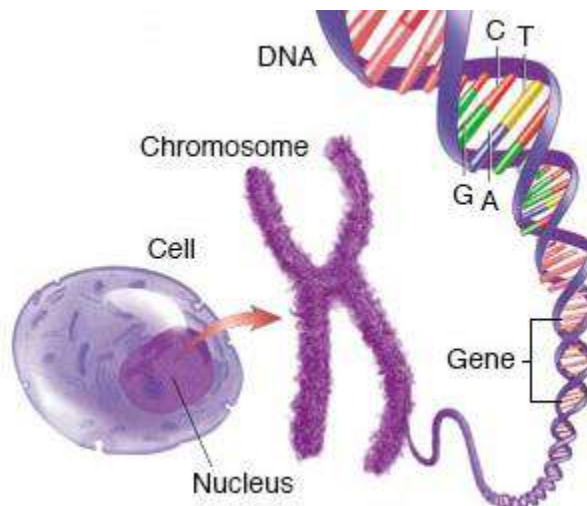
The realization that genes are made up of DNA molecules opened the way both to a **deeper understanding of life** and to its **artificial alteration** by **genetic engineering**.

# Structure of DNA

Genetic information is encoded by molecules named **nucleic acids** because they were originally isolated from the **nucleus of eukaryotic cells**.

There are **two related types** of nucleic acid, **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**.

The **master copy** of each cell genome is stored on **long molecules of DNA**, which may each contain **many thousands of genes**. Each gene is therefore, a **linear segment of long DNA molecule**.

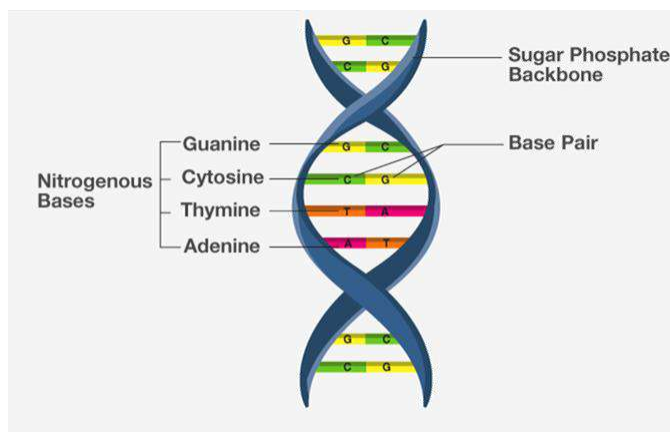


# Structure of DNA

**Definition:** Deoxyribonucleic acid (DNA) is a large molecule which stores the genetic information in an organisms. It is composed of two strands, arranged in a double helix form. Each strand is composed of a chain of molecules called **nucleotides**.

Nucleotides are composed of a **phosphate group**, a **pentose** (five carbon sugar) called deoxyribose and one of four different nitrogen containing bases (Adenine, Thymine, Cytosine, Guanine).

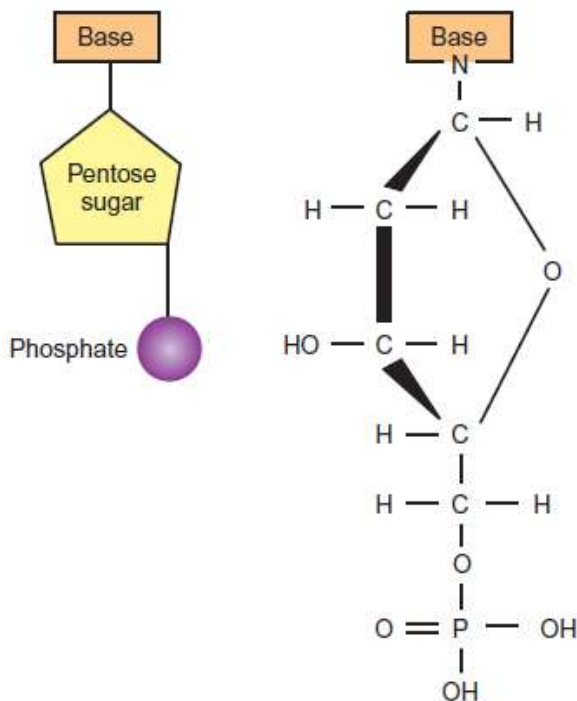
Each nucleotide is connected to the next nucleotide utilizing a **covalent bonding** between the phosphate group of one nucleotide and the third carbon in the deoxyribose ring. This gives the DNA strand a **direction from 5' (five prime end) to the 3' (three prime end)**. Conventionally, a DNA sequence is always **read from 5' → 3' ends**.



## Chemical Structure of Nucleotides

The DNA is a linear polymers made of subunits known as **nucleotides**. The **information** in each gene is determined by the **order** of the **different nucleotides**.

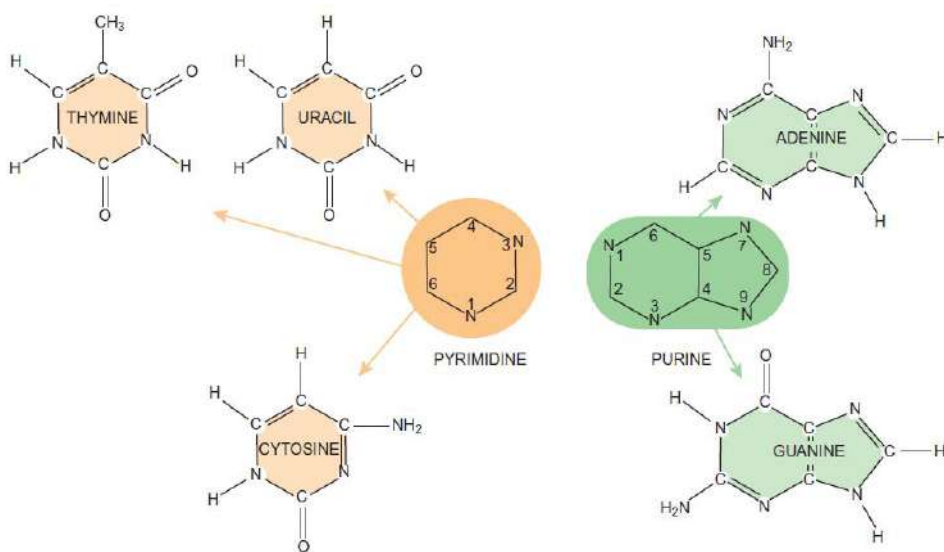
There are **four different nucleotides** in each type of nucleic acid and their order determines the genetic information. Each nucleotide has three components: **phosphate group**, **five-carbon sugar** and **nitrogen containing base**.



# Chemical Structure of Nucleotides

There are two distinct types of nitrogen containing bases:

1. **Purines** which consist of two aromatic rings such as **Adenine** and **Guanine**.
2. **Pyrimidines** which consist of single aromatic ring such as **Thymine**, **cytosine** (and **Uracil**, just found in RNA).



**BASE:** Alkaline chemical substance, in molecular biology especially refers to the cyclic nitrogen compounds found in DNA and RNA.

**DEOXYRIBOSE:** The sugar with five carbon atoms that is found in DNA.

**PENTOSE:** A five-carbon sugar, such as ribose or deoxyribose.

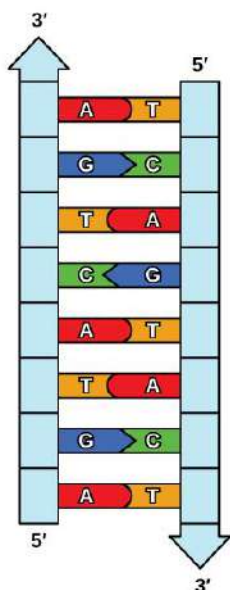
**PHOSPHATE GROUP:** Group of four oxygen atoms surrounding a central phosphorus atom found in the backbone of DNA and RNA.

**RIBOSE:** The five-carbon sugar found in RNA.

## Chemical Structure of Nucleotides

Each of the four bases (**A, T, G, C**) protrude of the sugar-phosphate backbone. If the double helix of the DNA molecule can be thought of as a twisted ladder, the sugar-phosphate backbones form the rails, while the four nitrogenous bases (**A, T, G, C**) form the steps.

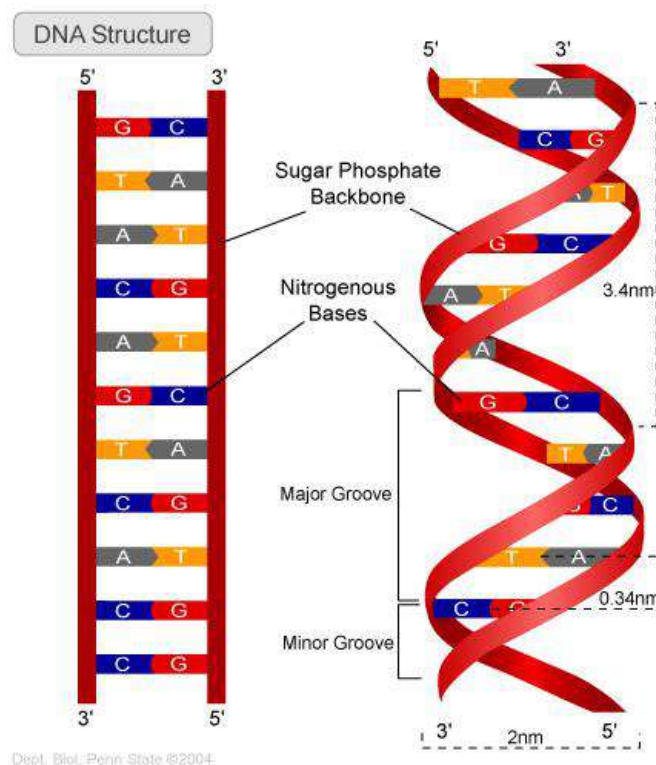
The two strands of DNA are bound together by **hydrogen bonding** between the nucleotides. Adenine always binds to thymine (**A-T**) and guanine always binds to cytosine (**G-C**). This means that the two strands of DNA are **complementary**. This complementary nature of DNA allows it to be copied and for genetic information to be passed on to the next generation. Each strand can perform as a template for the construction of its complementary strand.



# Chemical Structure of Nucleotides

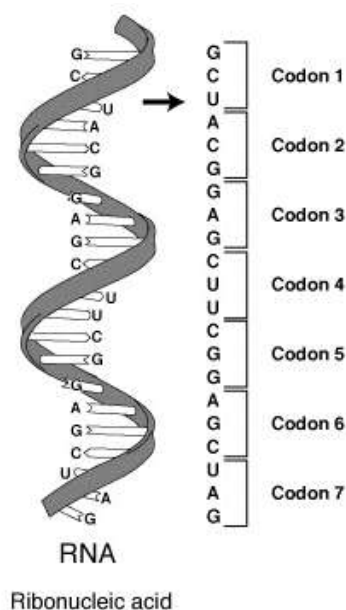
Order of the nitrogen bases along a DNA strand is called **DNA sequence** (also called **gene**). The DNA sequence contains the information needed to create **proteins** via the processes of **transcription** and **translation**.

Each strand of DNA is **anti-parallel**, this means that each strand runs in a different direction to the other. As one travels **down the DNA** duplex, the other one travels **up the DNA** duplex. One strand runs from 5' → 3', while the other runs from 3' → 5'.

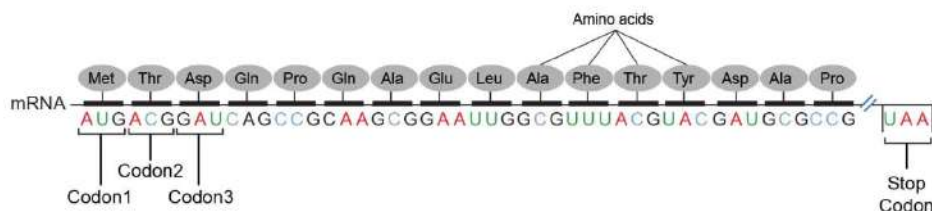


# Chemical Structure of Nucleotides

**Codon:** is a trinucleotide sequence of DNA or RNA that corresponds to a specific amino acid.



It is a genetic code describes the relationship between the sequence of DNA bases (**A, T, C, G**) in a gene and the corresponding protein sequence that encodes.





# KEY POINTS

**Definition:** Molecular biology is the study of living organisms at the level of the molecules that control them and make them up. In addition, to understand living organisms by examining the components that make them up.

## Benefits of Molecular Biology

1. Determining the function of single gene or proteins, and find out what will happen if that gene or protein was absent or defected.
2. Examine when and why certain genes are switched ON or OFF.
3. Provided scientists with a deeper understanding of how living things work.
4. Using molecular biology techniques in diagnosis and confirmation of certain diseases.
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## Common Molecular Biology Techniques

- |                             |               |
|-----------------------------|---------------|
| I. Electrophoresis.         | II. PCR.      |
| III. Restriction digestion. | IV. Ligation. |
| V. blotting.                | VI. Cloning.  |

## Structure of DNA

DNA was first discovered in 1869 by **Frederich Miescher** who extracted it from the **pus from infected wounds**.



# KEY POINTS

The **fundamentals** of **Modern Genetics** were laid when **Mendel** found that hereditary information consists of discrete basic units now called **genes**. Each gene is responsible for a **single inherited property** or **characteristic** of the organism.

There are **two related types** of nucleic acid, **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**.

**Definition:** Deoxyribonucleic acid (DNA) is a large molecule which stores the genetic information in an organisms. It is composed of two strands, arranged in a double helix form. Each strand is composed of a chain of molecules called **nucleotides**.

Nucleotides are composed of a **phosphate group**, a **pentose** (five carbon sugar) called deoxyribose and one of four different nitrogen containing bases (Adenine, Thymine, Cytosine, Guanine).

## **Chemical Structure of Nucleotides.**

There are two distinct types of nitrogen containing bases:

1. **Purines** which consist of two aromatic rings such as **Adenine** and **Guanine**.
2. **Pyrimidines** which consist of single aromatic ring such as **Thymine**, **cytosine** (and **Uracil**, just found in RNA).

Adenine always binds to thymine (**A-T**) and guanine always binds to cytosine (**G-C**).



## ***Lecture 1 Questions Practice***

### **Q1:- Define the followings:**

- a. Molecular biology.
- b. Deoxyribonucleic acid (DNA).

### **Q2:- Enumerate the followings:**

- a. Enumerate the types of nucleotides.
- b. Enumerate the types of pyrimidines.

### **Q3:- MCQ**

#### **1. Type of polymerase chain reaction (PCR)?**

- a. Wet PCR.
- b. Hot PCR.
- c. Cold PCR.
- d. Warm PCR.

#### **2. Genetic information is encoded by molecules named nucleic acids because they were originally isolated from?**

- a. Nucleus of prokaryotes.
- b. Nucleus of viruses.
- c. Nucleus of eukaryotes.
- d. Nucleus of bacteria.



# Molecular Biology

## 2<sup>nd</sup> lecture

### Introduction in Molecular Biology

# OBJECTIVES



- Structure of RNA.
- Main differences between DNA & RNA.
- Types of RNA.
- Functions of RNA.
- DNA as The Vehicle of Inheritance.

## Structure of RNA

The structure of RNA molecule was first described in 1965 by **Robert William Holley**.

**Definition:** Ribonucleic acid (RNA) is large molecule composed of single strand that functions in cell protein synthesis. It replaces DNA as carrier of genetic material in some viruses. The RNA is composed of ribose nucleotides.

- The sugar in RNA molecule is **ribose** while in DNA molecule, the sugar is always **deoxyribose**.
- RNA molecule is **shorter** than DNA molecule.
- RNA molecule **carry one or few genes** only.
- RNA molecule used to **transmit** the **genetic information** to cell protein factory, a **ribosome**.

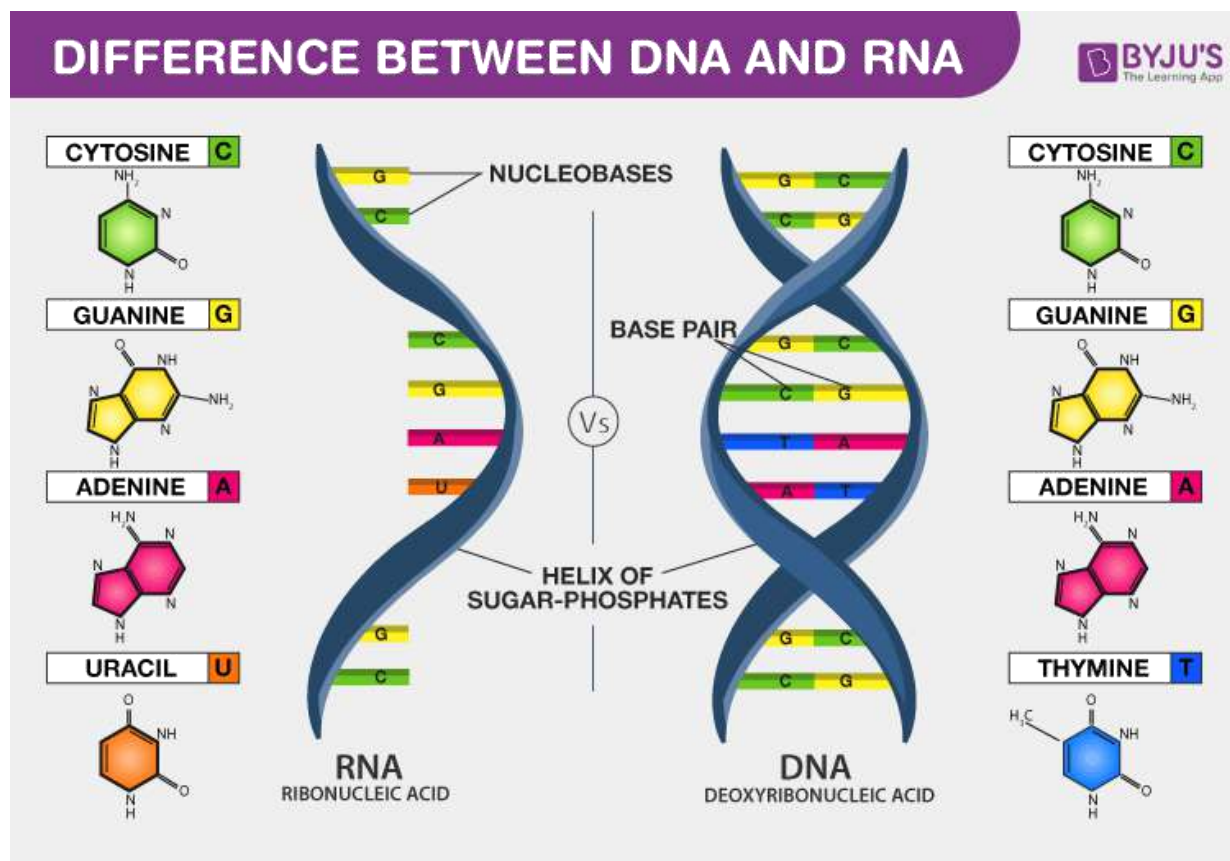


## Structure of RNA

Ribose nucleotides contains four types of nitrogen bases (**Adenine, Uracil, Guanine, Cytosine**). In RNA adenine is always bind to uracil (**A-U**), whilst guanine is always bind to cytosine (**G-C**).

The information for protein making is encoded in the base sequence of DNA (gene). However, DNA doesn't serve as the direct template for making the protein. Instead, the information in the gene is copied into the closely related molecule, named **RNA**, which leads the synthesis of proteins.

# Main Differences Between DNA and RNA Molecules



- Although RNA is single strand molecule, many RNA molecules fold up giving double stranded regions\*.
- In all above cases, the uracil in RNA will base pair with adenine.
- The genome of certain viruses consists of double-stranded RNA.

\*





## Types of RNA

The RNA molecule have many types. However, there are three main types as follow:

1. **Messenger RNA (mRNA):** is a single strand of RNA that is complementary to one of the DNA strands of gene. It carries the genetic code from the DNA in the nucleus to the ribosomes in the cytoplasm.
2. **Transfer RNA (tRNA):** is a single strand of RNA that serves as the physical link between the mRNA and the amino acids sequence of protein. It carries the amino acids from the cytoplasm into the ribosome.
3. **Ribosomal RNA (rRNA):** is a single strand of RNA that serves as ribozyme (acts as scaffold and catalyzing enzyme) which carry out protein synthesis in ribosomes.

## Types of RNA

Additionally, there are several more types of RNA such as:

- **Coding RNA (cRNA).**
- **Noncoding RNA (ncRNA),** which further subdivided into:
  - a) **housekeeping ncRNA.**
  - b) **regulatory ncRNA.**

**Functions of RNA are:**

All types of RNA carry out **common functions** such as:-

1. **Involved in protein translation and protein synthesis.**
2. **Involved in biochemical reactions similar to enzymes.**
3. **Have regulatory functions in cells.**
4. **Play important roles in both normal cellular processes and diseases.**



# DNA as The Vehicle of Inheritance

DNA (deoxyribonucleic acid) is a molecule that is vital to the survival of almost all organisms. It contains the **instructions needed** for **normal cell growth, function** and **reproduction**. The DNA dictates how an organism develop and function through its life.

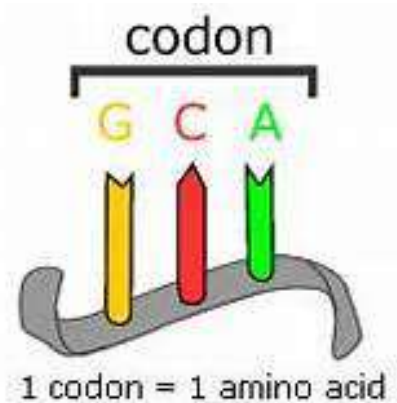
DNA, is the single molecule that carry all of the **information needed** for making all of the **proteins** in each of the body's cells.

One main function of the information stored in DNA is to provide instructions for building proteins that are built from amino acids. The information for amino acids needed is rises directly from the sequence of the bases in DNA. The sequence of bases in DNA stores instructions for building proteins.

These information that is securely stored in DNA is then copied to RNA whenever the information needs to be accessed and copied.

# DNA as The Vehicle of Inheritance

- There are only four bases (**A, T, G, C**) in DNA or RNA.
- **Proteins** are built using **20 different amino acids**.
- Using one base or two bases to built one amino acid dose not work.
- Using three bases is enough to built a protein.
- Cells use three bases which called **codon**.
- One codon = One amino acid

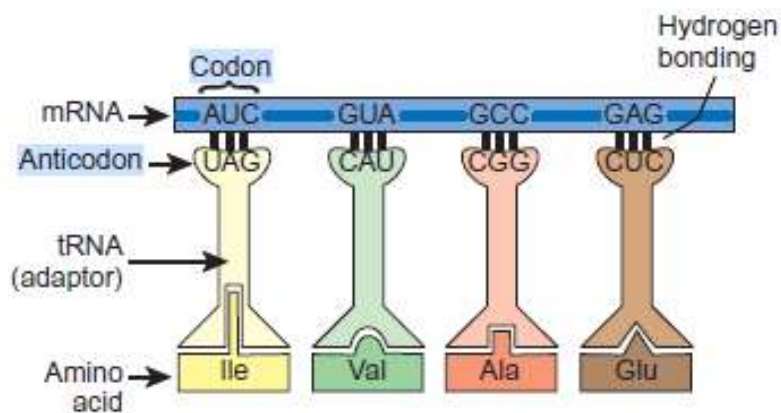


**Codon:** Group of three RNA or DNA bases that encodes a single amino acid.

**Genetic code:** The code for converting the base sequence in nucleic acids, read in groups of three, into the sequence of a polypeptide chain.

## DNA as The Vehicle of Inheritance

- In addition to coding for amino acids, a codon contains 3 punctuation marks, called **stop codon** that functions like period to signal the end of a protein coding.
- There is more than one codon for one amino acid (at least two codons).
- The more common an amino acid in a protein, the more codons it have.





# DNA as The Vehicle of Inheritance

- The codon that specifies **Methionine** is the start codon, because its almost always the first codon in protein-coding sequence.
- The rules specifying which codons represent which amino acid are referred to as the **Genetic Code**.
- The genetic code is only stored in DNA.
- Any region of DNA that contains information needed to make a protein is called a **Gene**.
- Every protein, such as hemoglobin or an enzymes of metabolic pathways is encoded in genes.
- In addition to storing information for building proteins, DNA also contains information that controls **how much and when** of each protein is made.

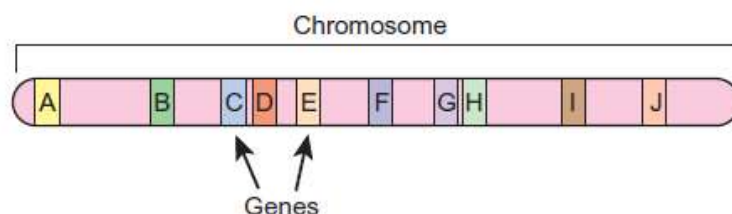
# DNA as The Vehicle of Inheritance

Chromosome is thread-like structure located inside the nucleus of a cell. It made of single molecule of DNA and protein. Chromosomes are long DNA molecules that carries genes. Near by genes on the same chromosome are tend to be inherited together.

Inheritance is due to genes, most of which encode proteins. Therefore, differences in many observable genetic traits are due to modifications in individual proteins that make up the biochemical pathways or that form cellular structures.

Other traits or characters, which are more difficult to study result from the effects of multiple genes.

Living organisms contain about several hundred to several thousand different genes carried on one or more chromosomes.





# DNA as The Vehicle of Inheritance

**Genes** are **basic units of genetic** information and each gene provides the instructions for some character of the organism.

In addition to the genes that affect the characteristics of an organism directly or indirectly, there are many **regulatory genes**, that control other genes.

Each gene exist in an alternative forms known as **alleles**. Alleles, code for different versions of a particular inherited character such as black vis red hair color. There is **dominant** and **recessive alleles**.

Different alleles of the same gene are closely related, but have minor chemical variations in their DNA that may produce significant different outcomes.



# DNA as The Vehicle of Inheritance

Any segment of DNA is called **locus** (**loci**, plural). Loci is a location on a chromosome (or molecule of DNA).

The overall nature of any living organism is a result of the sum of the effects of all its genes expressed in a particular environment. The total genetic make-up of an organism is referred to as its **genome**.

The outward (appearance) characteristic of a genome called **phenotype**, whilst the inward (genetic make-up) characteristics called **genotype**.



# DNA as The Vehicle of Inheritance

In animals, DNA between genes comprises up to 96% of the chromosome length and the functional genes comprise only 4% of the chromosome length.

In addition to DNA, chromosomes carry a variety of other proteins such as **histone proteins** (histone-like proteins in bacteria). These are important in maintaining the structure, especially of large chromosomes.

Cell usually contains two copies of each chromosome. Each pair of identical chromosomes possesses copies of the same genes arranged in the same linear order.

Identical chromosomes are truly not identical as the two members of the same pair often carry different **alleles** of the **same genes**.



# DNA as The Vehicle of Inheritance

Chromosome that carry the same set of genes in the same sequence is called **homologous chromosome**.

The cell possess two sets of its homologous copies of each of its chromosomes is called **diploid** ( $2n^*$ ). These cells are called **somatic cells** (any cell of the body except sperms and eggs).

Those cells that possess only a single copy of each of its chromosome is called **haploid** ( $n$ ). These cells are called **gametes** (reproductive cells, sperms and eggs).

*\*  $n$  refers to the number of chromosomes in one complete set.*

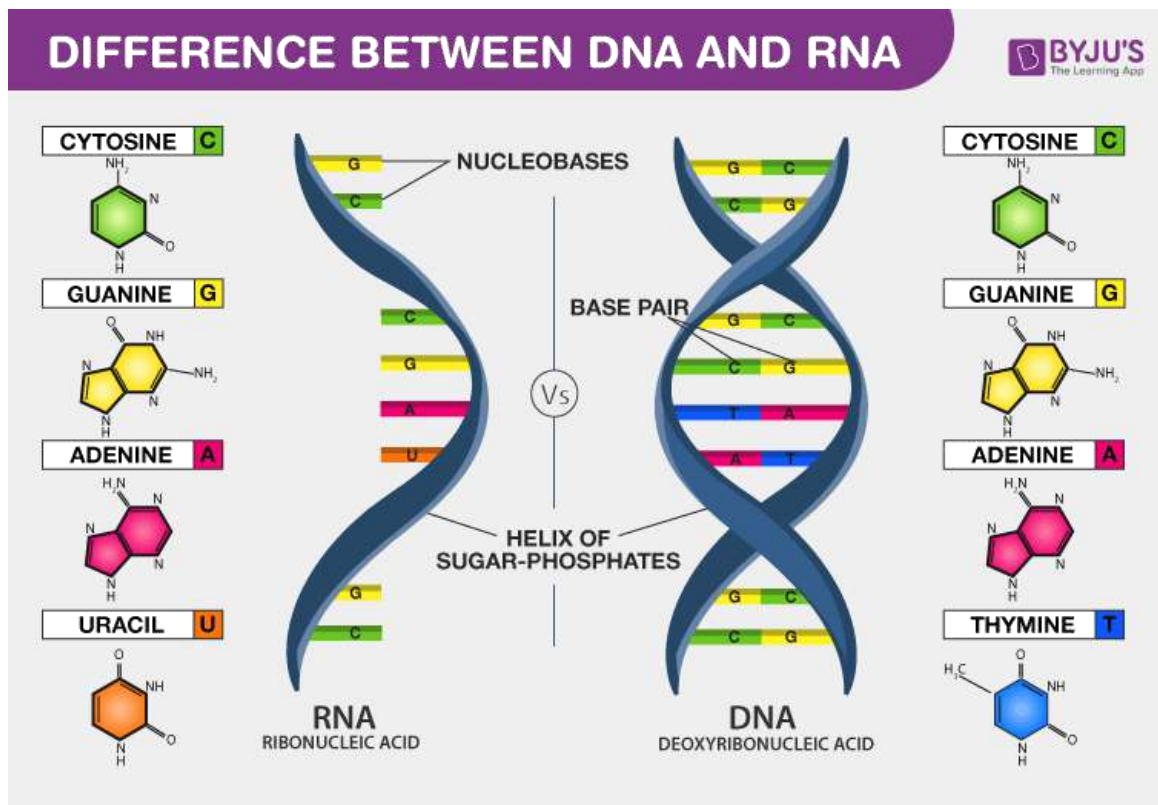


# KEY POINTS

## Structure of RNA

**Definition:** Ribonucleic acid (RNA) is large molecule composed of single strand that functions in cell protein synthesis. It replaces DNA as carrier of genetic material in some viruses. The RNA is composed of ribose nucleotides.

## Main Differences Between DNA & RNA Molecules





# KEY POINTS

## **Types of RNA**

- 1. Messenger RNA (mRNA)**
- 2. Transfer RNA (tRNA)**
- 3. Ribosomal RNA (rRNA)**

**Additionally, there are several more types of RNA such as:**

- Coding RNA (cRNA).**
- Noncoding RNA (ncRNA), which further subdivided into:**
  - a) housekeeping ncRNA.**
  - b) regulatory ncRNA.**

## **Functions of RNA are:**

- 1. Involved in protein translation and protein synthesis.**
- 2. Involved in biochemical reactions similar to enzymes.**
- 3. Have regulatory functions in cells.**
- 4. Play important roles in both normal cellular processes and diseases.**

## **DNA as Vehicle of Inheritance.**



## ***Lecture 2 Questions Practice***

### **Q1:- Define the followings:**

- a. Genotype.
- b. Ribonucleic acid (RNA).

### **Q2:- Enumerate the followings:**

- a. Enumerate the types of ribonucleic acid (RNA).
- b. Enumerate the functions of ribonucleic acid (RNA).

### **Q3:- MCQ**

- 1. The DNA doesn't serve as the direct template for making the protein. Instead, the information in the gene is copied into the closely related molecule, named?
  - a. Ribosome.
  - b. tRNA.
  - c. mRNA.
  - d. rRNA.
  
- 2. It carries the amino acids from the cytoplasm into the ribosome?
  - a. mRNA.
  - b. rRNA.
  - c. tRNA.
  - d. Ribosome.

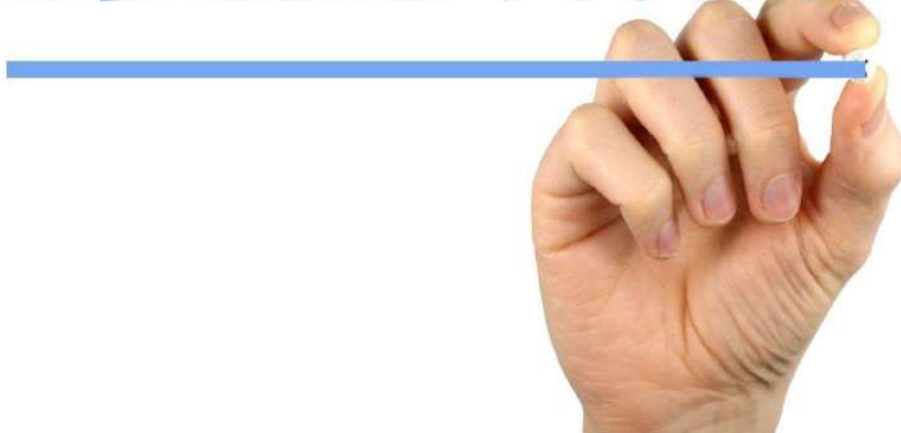


# Molecular Biology

3<sup>rd</sup> lecture

DNA Replication

# OBJECTIVES



- Replication Concept.
- DNA Replication.
- DNA Replication in Prokaryote: Bacteria.
- DNA Polymerase.
- Nucleotides, the Precursor for DNA Synthesis.



## Replication Concept

- In **unicellular organism**, **cell division** and **reproduction** occur **simultaneously**.
- In **multicellular organism**, **cell division** and **reproduction** are **two different** processes.
- **Mitosis** is type of cell division that results in two daughter cells each having the same number ( $2n$ ) and kind of chromosomes as their parent cell.
- **Meiosis** is type of cell division that results in four daughter cells each with half the number ( $n$ ) of their parent cell chromosomes as in the production of gametes.



## Replication Concept

- **Horizontal Gene Transfer (HGT).** Side way DNA transfer without cell division. It involves transferring small segment of DNA from a donor cell to a recipient cell without cell division.
- **Vertical Gene Transmission.** Is when DNA or genes transmitted to the new generation from the previous generation. Therefore, vertical transmission includes all forms of cell divisions whether **sexual** or **a sexual** to create a new copy of the genome.

# DNA Replication

**DNA Replication.** Is the process by which the DNA of the ancestral cell is duplicated prior to cell division. Upon cell division, each of descendant receives one complete copy of the DNA that is identical to its predecessor.

For DNA to be replicated properly, a variety of technical steps must take place, for example:

- DNA molecule compacted into chromosomes to fit into the **nucleus (Eukaryotes)** or the **cytoplasm of the cell (Prokaryotes)**.
- DNA must **unfold** for replication to proceed.
- Priming, **initiation** of the DNA new strands.
- Coordinate the **synthesis** of the two new strands of the DNA that run in opposite directions.
- These steps are basic principles in bacteria (Prokaryote).
- In higher organisms (Eukaryote), the presence of multiple chromosomes enveloped by the nuclear membrane complicates DNA replication and cell division.

# DNA Replication

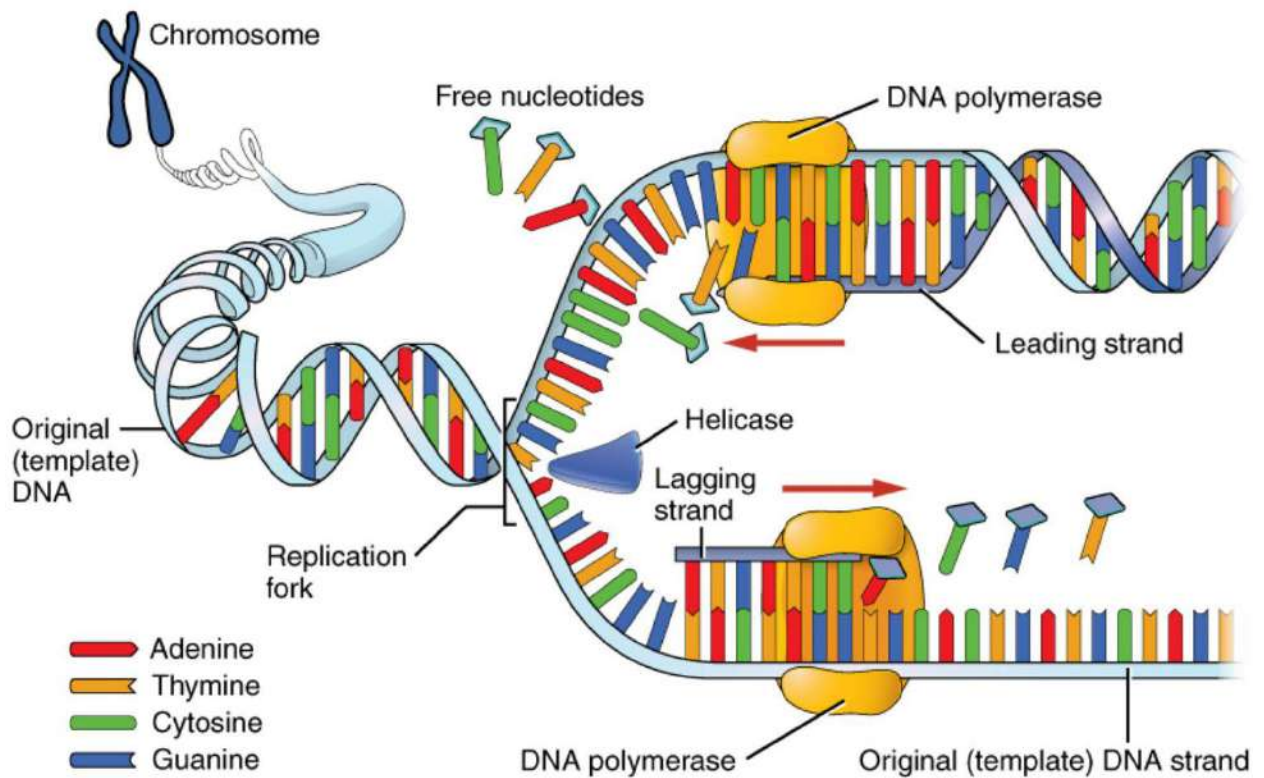
Almost all living cells have DNA that is crucial for cell division and replication. For this to take place, the DNA must be replicated either **before** or **during** cell division. Therefore, DNA replication (synthesis) and cell division must be precisely coordinated.

**Hereditary**, is the mechanism by which DNA replication is controlled.

Generally, DNA replication follows a certain order as explained below:

1. **The two strand of DNA helix undergoes separation.**
2. **Complementary strands are made using the original two strands of the DNA as a template.**
3. **This process produces two identical copies of the original DNA.**
4. **Finally, the two DNA copies distributed to the two daughter cells that produced.**

# DNA Replication

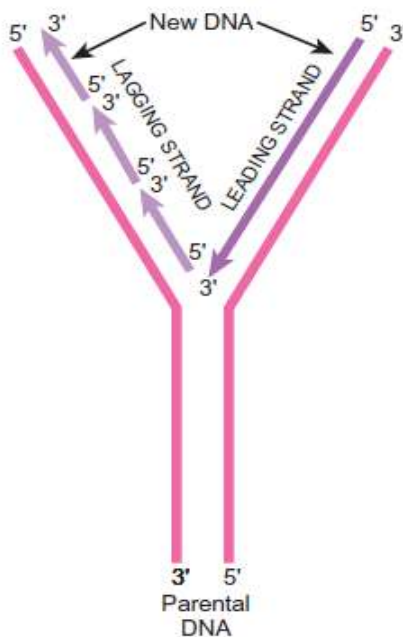
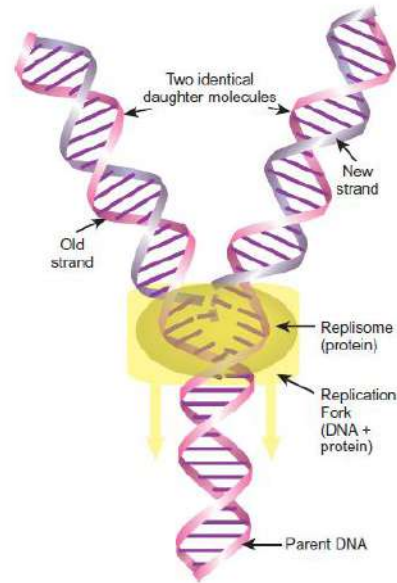




# DNA Replication

1. **Helicase** is an enzyme breaks the hydrogen bonds that hold the two DNA strands together in the DNA helix. Helicase forms the **replication fork**.
2. **Replication fork** is the structure that has two branching, each one made up of a single strand of DNA.
3. **DNA polymerase** is an enzyme that catalyze the synthesis od DNA molecule from nucleotides (**A, T, C, G**).
4. The complex consists of **helicase, replication fork and DNA polymerase** called **replisome**.
5. Synthesis of the two new strands of DNA starts at the **replication fork**.
6. Replication fork moves along the parental DNA molecule.
7. **Leading strand** is the DNA synthesized strand that runs from 5' to 3' end.
8. **Lagging strand** is the discontinuous DNA synthesized strand that runs from 3' to 5' end.

# DNA Replication



The protein at the replication fork responsible for DNA synthesis, DNA polymerase, always synthesizes DNA in the 5' to 3' direction. Therefore, one new strand (leading strand) can be made continuously, while the other (lagging strand) must be made discontinuously (i.e. in short segments).



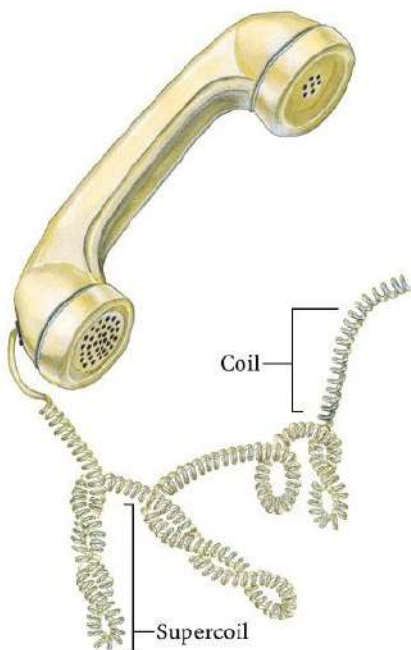
# DNA Replication

- The result of the replication is **two double stranded DNA molecules** . Both are identical to the original one (parental DNA).
- One of these daughter molecules has the **original right strand** and the other daughter has the **original left strand**.
- This form of replication is called **semi conservative**, since each of the progeny conserves half of the original DNA molecule
- Replication is similar, but not exactly the same in **Prokaryotes** and **Eukaryotes**.

# DNA Replication in Prokaryote: Bacteria

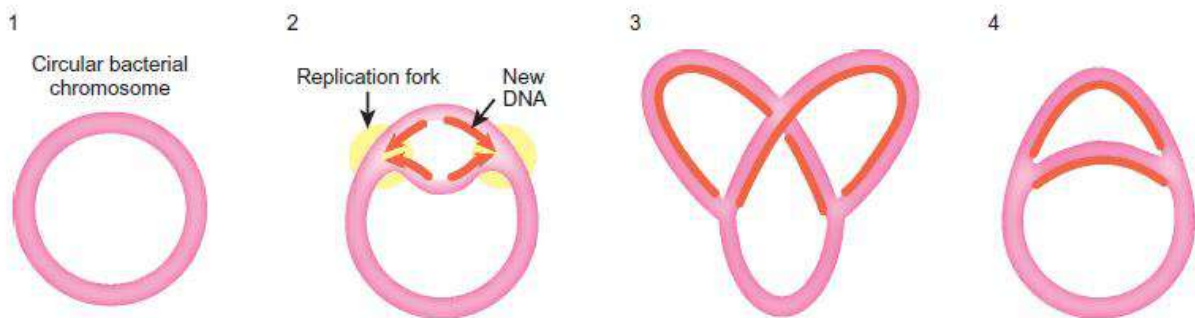
Bacterial Chromosome is double stranded and **circular** in shape. Its called **plasmid**. For bacterial DNA to be replicated, several major problems must be solved prior to the beginning of the replication process.

1. **Topological problems**, since bacterial Chromosome is not only double helix, but also super coiled, it cannot simply be pulled apart.



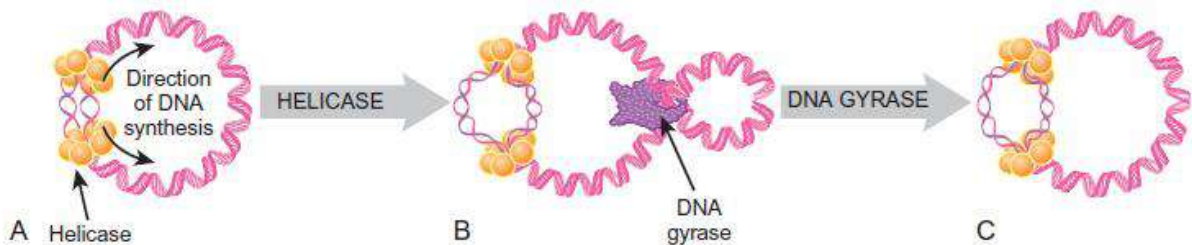
## DNA Replication in Prokaryote: Bacteria

2. Prior to new DNA can be made, the **chromosome supercoils** must be **unwound**.
3. Consequently, the **double helix** must be **untwisted**.
4. In addition, since the bacterial Chromosome is circular in shape, it is important to **untangle** the new two circles of the DNA.
5. Energy is needed for this process and it supplied from **ATP** (adenosine triphosphate).



## DNA Replication in Prokaryote: Bacteria

- Since the bacterial Chromosome is **circular in shape**, **two replication forks** proceeds in opposite directions along the circle.
- This process is called **bi-directional replication**.



- **DNA gyrase** is an enzyme that reduces or solves the topological strains (problems) in the supercoiled chromosome and unwinds it in front of the helicase (replication fork) and prior to DNA replication.
- Chromosome supercoil will be created again after the new DNA being made and reach 5% of its total size.

*The **quinolones antibiotics** (Ciprofloxacin, Nalidixic acid) inhibits DNA gyrase and prevent the movement of replication fork. The end result is cell death (bacteria) which is an advantage for treating bacterial diseases in patients.*

# DNA Polymerase

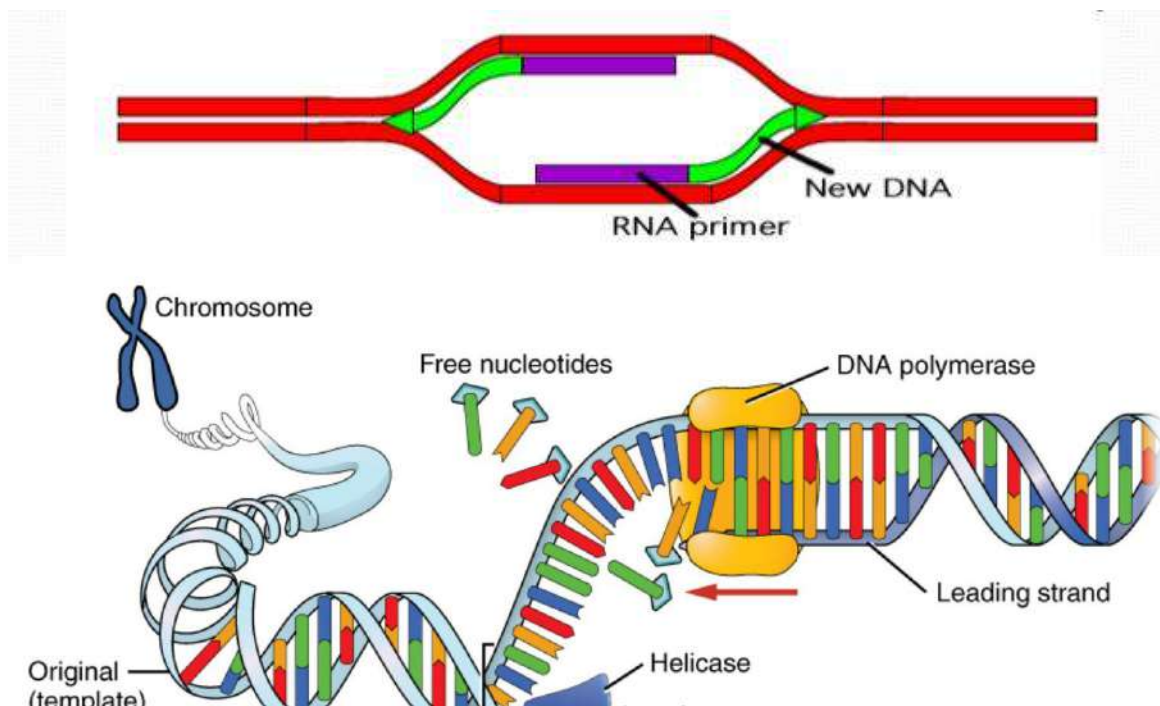
**Polymerase** is general name for an enzyme that joins the nucleotides together. There are **several types of DNA polymerases** that have different roles in both DNA replication and DNA repair.

## Properties of DNA polymerase:

1. DNA polymerase will only synthesized DNA from 5' to 3' end direction.
2. DNA polymerase lack the ability to initiates new strand of DNA and can only elongates pre-existing strands (*unlike DNA polymerases, RNA polymerases can start a new strand without a pre-existing strands*).
3. A special mechanism is needed to initiate new DNA strand. This involves synthesis of short **RNA primer** every time a new DNA strand is started.

# DNA Polymerase

4. Special RNA polymerase called **primase** make the RNA primers that are needed for strand initiation during DNA replication.
5. The leading strand in DNA replication needs only one RNA primer for strand initiation, while in the lagging strand, a new RNA primer must be created each time a new portion is made.
6. DNA polymerases possess the ability of proofreading. This means detecting nucleotide mistakes and make correction on the new DNA strand that is being made.





# Nucleotides The Precursor for DNA Synthesis

The precursor for DNA synthesis are the deoxyribonucleosides 5'tri-phosphate, also called **deoxy-NTPs** such as **dATP, dTTP, dGTP, dCTP**.

The DNA precursor, which contain deoxyribose, are made from the corresponding ribose-containing nucleotides.

Reduction of ribose to deoxyribose is catalyzed by the enzyme **ribonucleotide reductase**.

Upon polymerization, the high energy bond in the phosphate group is cleaved releasing energy to supply the DNA synthesis.



# KEY POINTS

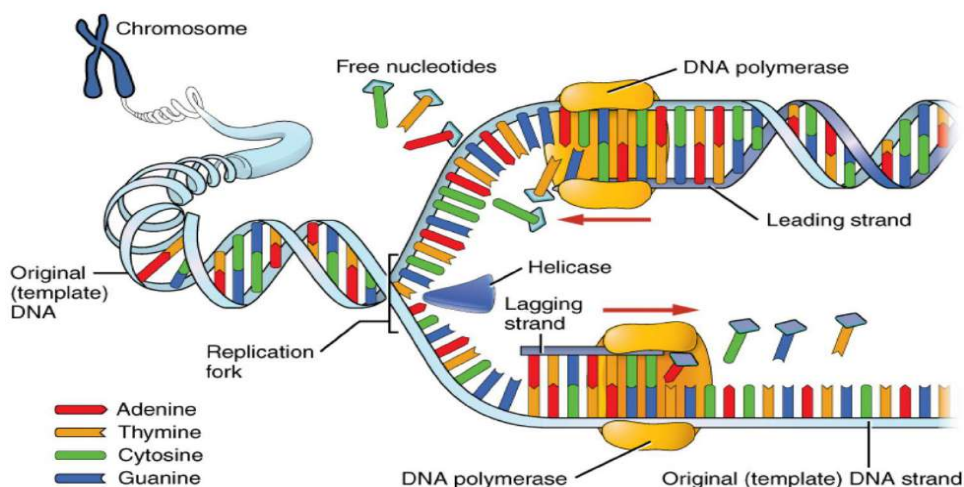
## Replication Concept

### Mitosis & Meiosis

**Horizontal Gene Transfer (HGT).** Side way DNA transfer without cell division.

**Vertical Gene Transmission.** Is when DNA or genes transmitted to the new generation from the previous generation.

**DNA Replication.** Is the process by which the DNA of the ancestral cell is duplicated prior to cell division. Upon cell division, each of descendant receives one complete copy of the DNA that is identical to its predecessor.



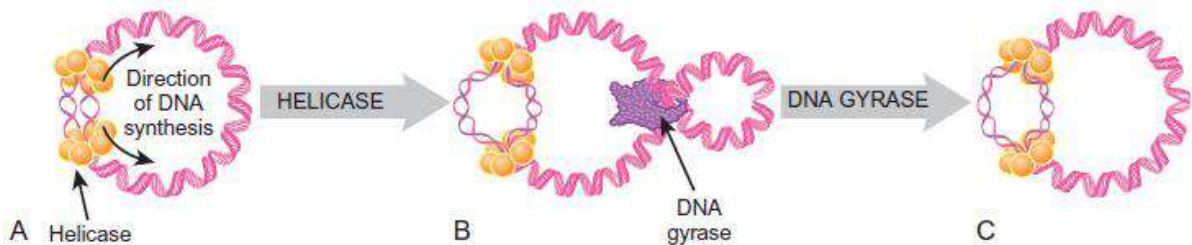


# KEY POINTS

**Leading strand** is the DNA synthesized strand that runs from 5' to 3' end.

**Lagging strand** is the discontinuous DNA synthesized strand that runs from 3' to 5' end.

## DNA Replication in Prokaryote: Bacteria



## DNA Polymerase

**Polymerase** is general name for an enzyme that joins the nucleotides together. There are **several types of DNA polymerases** that have different roles in both DNA replication and DNA repair.

## Nucleotides The Precursor for DNA Synthesis

The precursor for DNA synthesis are the deoxyribonucleosides 5'tri-phosphate, also called **deoxy-NTPs** such as **dATP, dTTP, dGTP, dCTP**.



## ***Lecture 3 Questions Practice***

### **Q1:- Define the followings:**

- a. Helicase.
- b. Replication fork.

### **Q2:- Enumerate the followings:**

- a. Enumerate two properties of DNA polymerase.
- b. Enumerate the precursors for DNA synthesis.

### **Q3:- Fill in the blanks:**

- a. ----- is the DNA synthesized strand that runs from 5' to 3' end.
- b. ----- is the discontinuous DNA synthesized strand that runs from 3' to 5' end.

### **Q4:- Mark the following sentences TRUE or FALSE:**

- a. DNA replication in eukaryotes is called bi-directional replication.
- b. A special mechanism is needed to initiate new DNA strand. This involves synthesis of short RNA primer every time a new DNA strand is started.

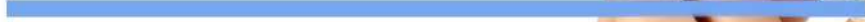


# Molecular Biology

4<sup>th</sup> lecture

DNA Replication

# OBJECTIVES



- Chromosome Replication.
- Replicating Linear DNA in Eukaryotes.
- Structure of Chromosome.



# Chromosome Replication

The **DNA replication** must be **synchronized** with **cell division**. So far, DNA replication mechanism has been discussed in the *last lecture*. This involves **starting** DNA Replication at a **specific location** on the chromosome and **stopping** when the chromosome has been successfully copied.

The DNA replication in **Prokaryotes** starts at a unique site on the chromosome known as the **origin of replication**, and continues in both directions around the circle.

The **origin of replication** contains **initiation complex** which contains five proteins: **DnaA, DnaB, DnaC, DNA gyrase and SSB** (Single-Stranded DNA-Binding protein). **DnaA protein**, is the main protein in chromosome initiation.



# Chromosome Replication

The **origin of replication** of the chromosome is called ***oriC***.

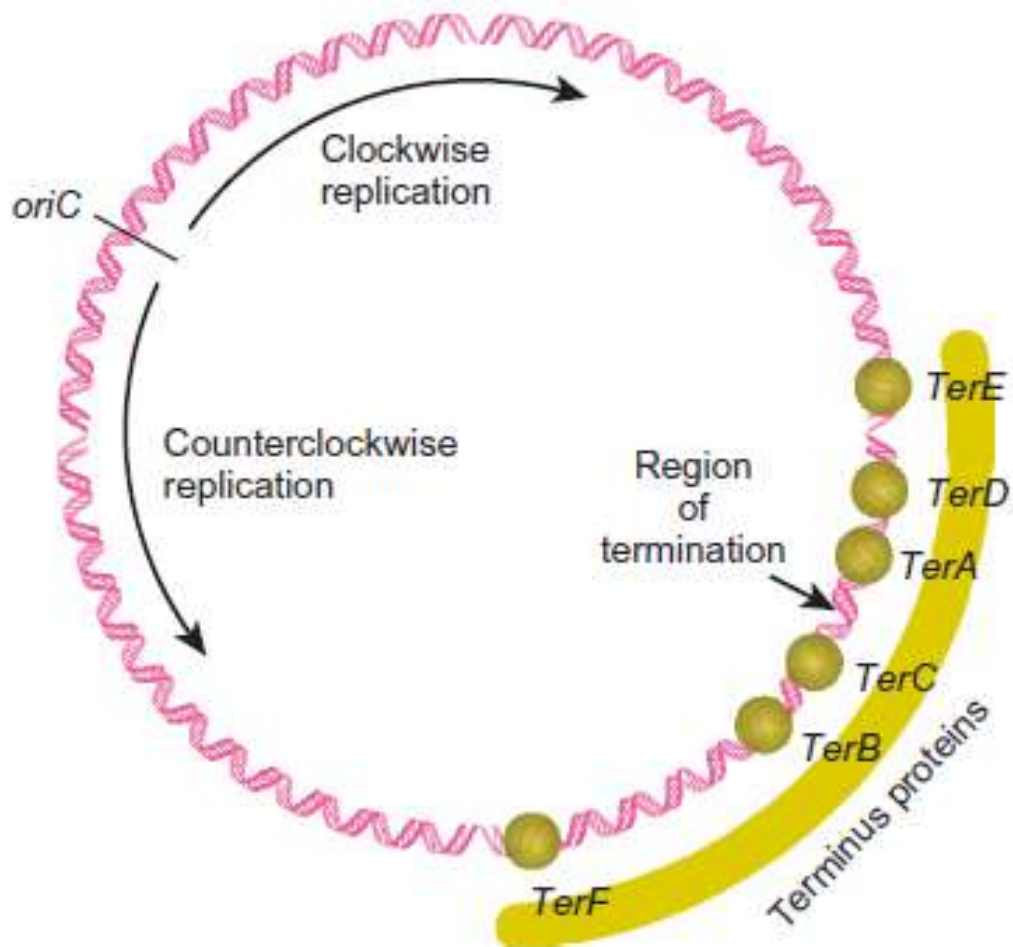
***oriC*** is a 245 base-pair region that is required for chromosome initiation and consists of two sub-regions.

The DNA replication in **Prokaryotes** ends at a site on the chromosome known as the **terminus**. The **terminus** of the chromosome is called ***terC***.

The ***terC*** contains several ***ter*** sites that prevent further movement of the replication fork. Types of ***ter*** are ***TerC, TerB, TerF, TerA, TerD, and TerE***. Since replication continues in two directions in prokaryotes, the ***Ter*** sites act **asymmetrically**.

***TerC, TerB, and TerF*** prevent **clockwise movement of forks** and ***TerA, TerD, and TerE*** prevent **counterclockwise movement**. The two sites ***TerA and TerC*** are most often used.

# Chromosome Replication





## Replicating Linear DNA in Eukaryotes

Replicating a linear molecule of DNA requires certain adaptations. Since DNA polymerases can **only elongate, not initiate**, a new strands of DNA must be initiated with **RNA primer**.

Since DNA synthesis always proceeds from **5' to 3' end**, one of these RNA primers must be located right at the 5' end of each new strand when replicating linear DNA.

When this terminal RNA primer is removed, it cannot be replaced with DNA, as there is no pre-existing OH group at 3' end for DNA polymerase to elongate.

If nothing were done about this problem, each round of replication the DNA molecule will grow shorter by the length of an average RNA primer. Circular prokaryotic DNA molecules **do not have ends** and so do not face this problem.



# Replicating Linear DNA in Eukaryotes

Eukaryotes have solved this problem of replicating linear DNA using structures known as **telomeres**, which are located at the ends of their chromosomes.

**Telomere is a region of repetitive nucleotide sequence at each end of a chromosome.**

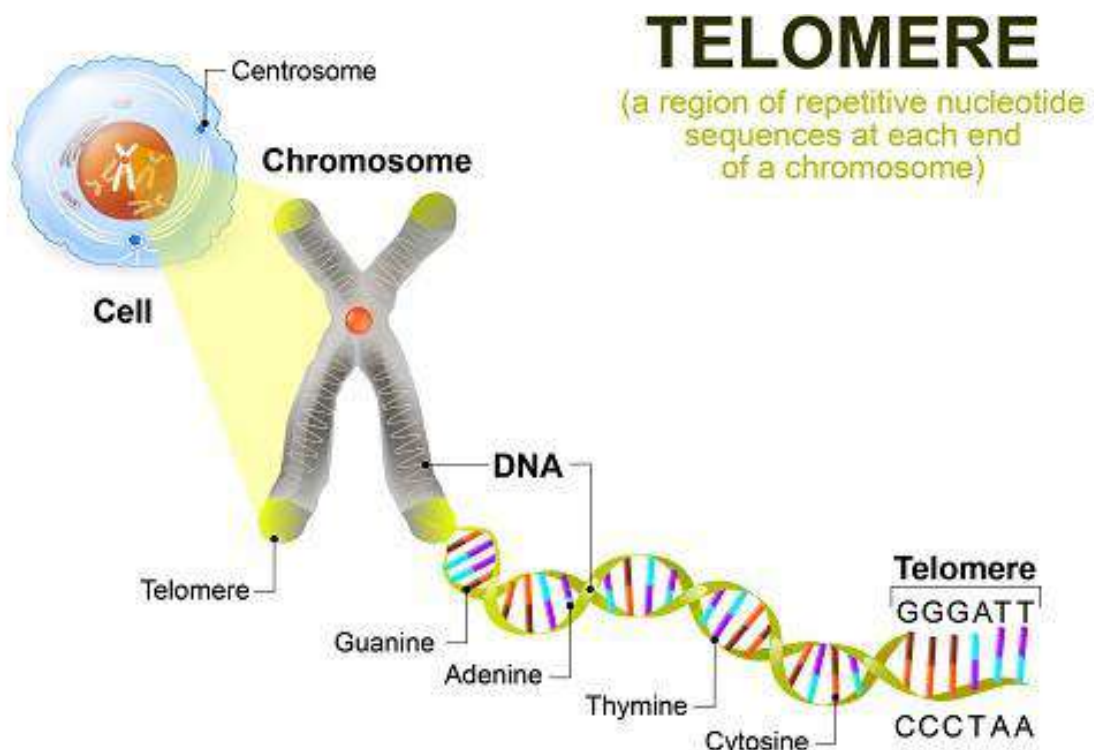
Telomeres consist of multiple **tandem repeats** (20 to several hundred) of a short sequence, usually of six bases (TTAGGG in vertebrates including humans).

During each replication cycle, the chromosomes are indeed shortened and several of the telomere repeats are lost. However, **no coding information is lost.**

# Replicating Linear DNA in Eukaryotes

In addition, in cells where the enzyme **telomerase** is present, the lost DNA is later replaced by adding several of the six bp (base pair; TTAGGG) units to the 3' end after each replication cycle.

The telomere structures also protect the ends of chromosomes against degradation by **exonucleases**.





# Replicating Linear DNA in Eukaryotes

Eukaryotic chromosomes are usually very long and have many replication origins distributed along each chromosome.

Replication is bidirectional as in Prokaryote (bacteria). A pair of replication forks starts at each origin of replication and the two forks then move in opposite directions. The bulges where the DNA is in the process of replication are called **replication bubbles**.

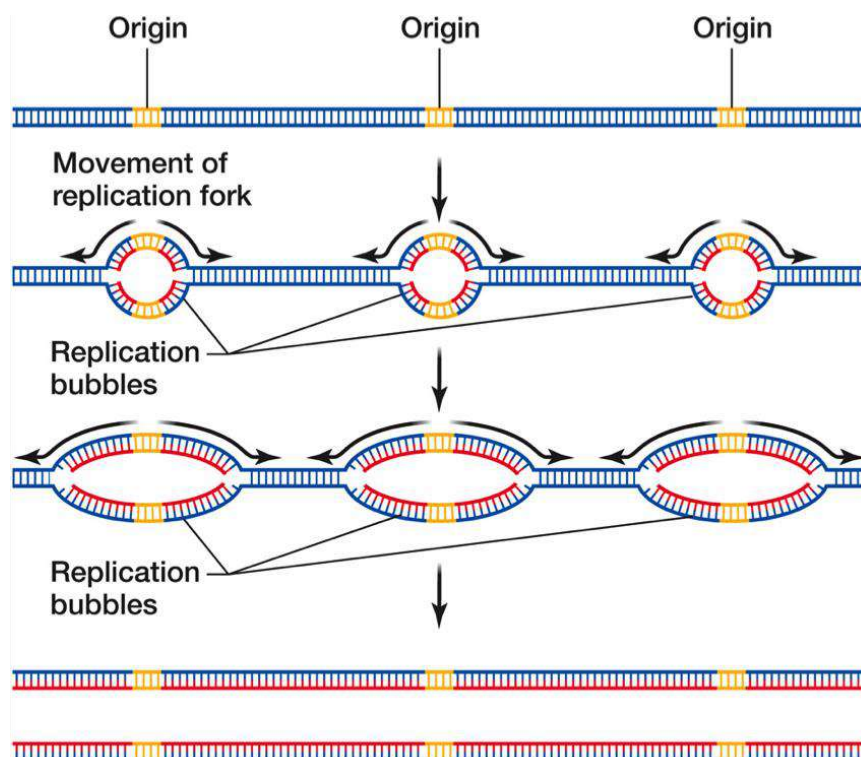
A huge number of replication origins function simultaneously during eukaryotic DNA replication (between 10,000 and 100,000 replication origins in a dividing human somatic cell).

# Replicating Linear DNA in Eukaryotes

This creates problems in synchronization.

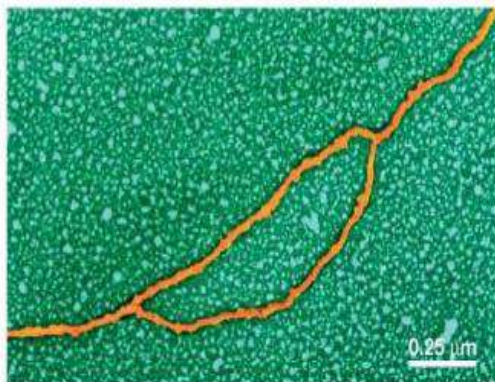
Synthesis at each replication origin must be coordinated to make sure that each chromosome is completely replicated.

Equally, each replication origin must initiate **once** and **once only** during each replication cycle in order to avoid duplication of DNA segments that have already been replicated.

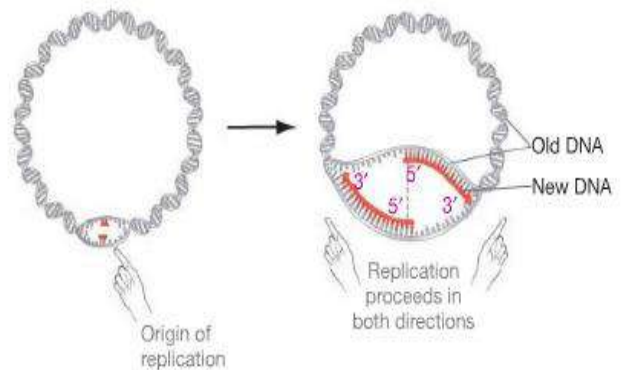


# Replicating Linear DNA in Eukaryotes

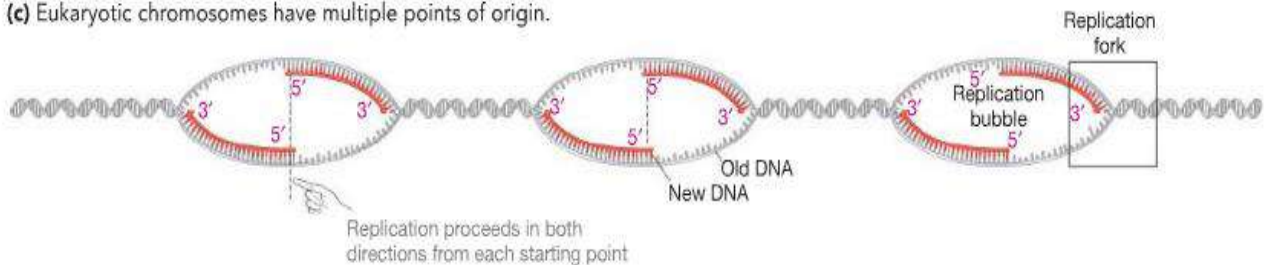
(a) A chromosome being replicated



(b) Bacterial chromosomes have a single point of origin.



(c) Eukaryotic chromosomes have multiple points of origin.





# Replicating Linear DNA in Eukaryotes

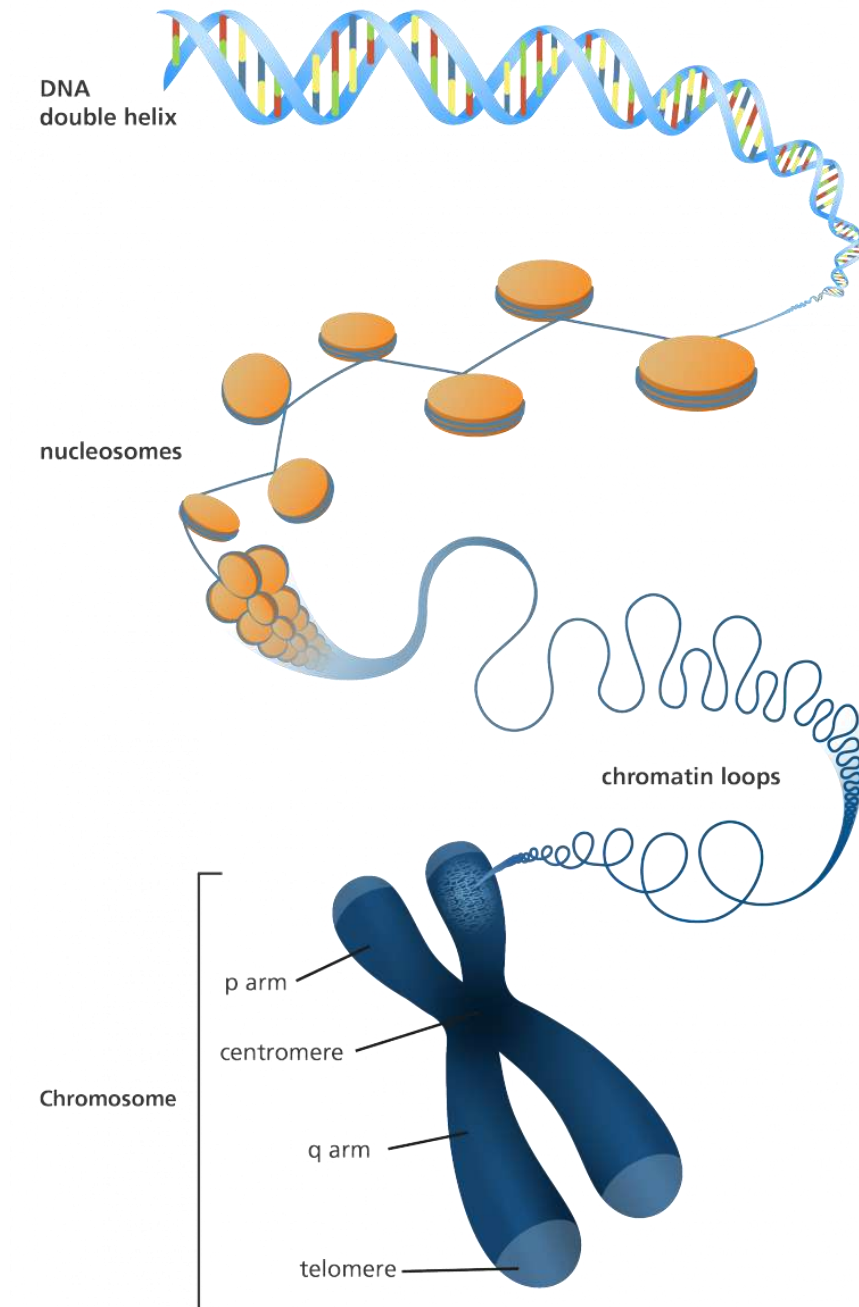
The DNA molecule in Eukaryote is tightly wound around **nucleosomes** (basic packing unit of DNA), consisting of **histones** (type of proteins), to condense the genome and maintain chromosomal integrity.

**Nucleosome** is a structural unit of an Eukaryotic chromosome that consisting of a length of DNA coiled around a core of histones.

**Histone** is a group of basic proteins found in chromatin, binds to DNA molecule and help control the activity of genes.

Histones are critical to gene expression and numerous modifications of these proteins are essential to proper cell function.

# Structure of Chromosome





# KEY POINTS

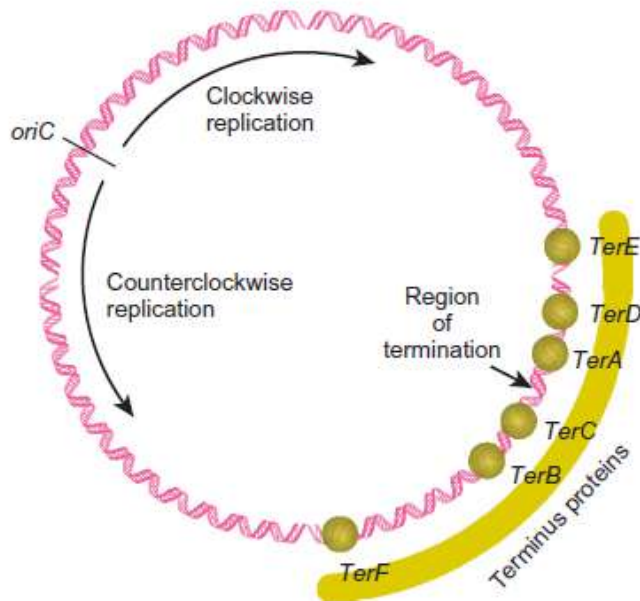
## Chromosome Replication

The **origin of replication** of the chromosome is called ***oriC***.

The DNA replication in **Prokaryotes** ends at a site on the chromosome known as the **terminus**. The **terminus** of the chromosome is called ***terC***.

Types of ***ter*** are ***TerC***, ***TerB***, ***TerF***, ***TerA***, ***TerD***, and ***TerE***. Since replication continues in two directions in prokaryotes, the ***Ter*** sites act **asymmetrically**.

***TerC***, ***TerB***, and ***TerF*** prevent **clockwise movement of forks** and ***TerA***, ***TerD***, and ***TerE*** prevent **counterclockwise movement**. The two sites ***TerA*** and ***TerC*** are most often used.

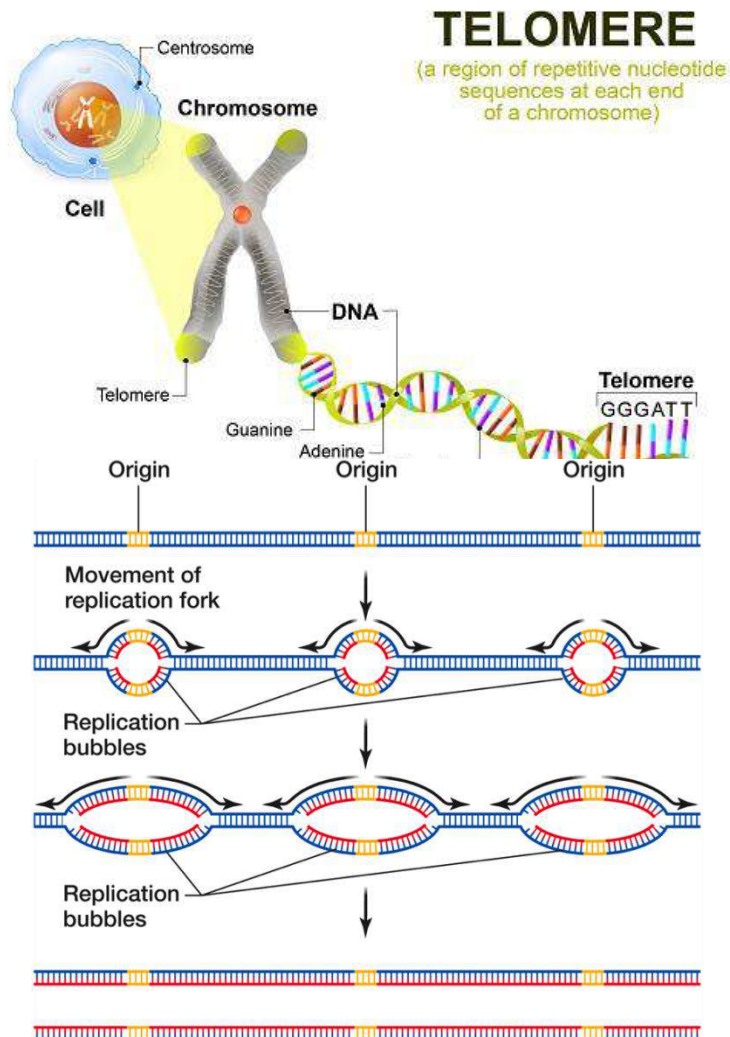




# KEY POINTS

## Replicating Linear DNA in Eukaryotes

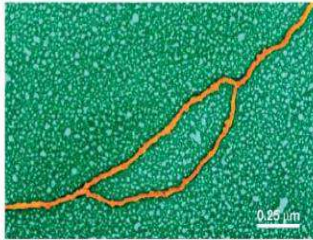
**Telomere** is a region of repetitive nucleotide sequence at each end of a chromosome.



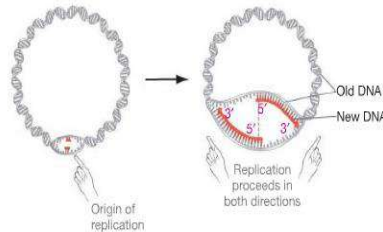


# KEY POINTS

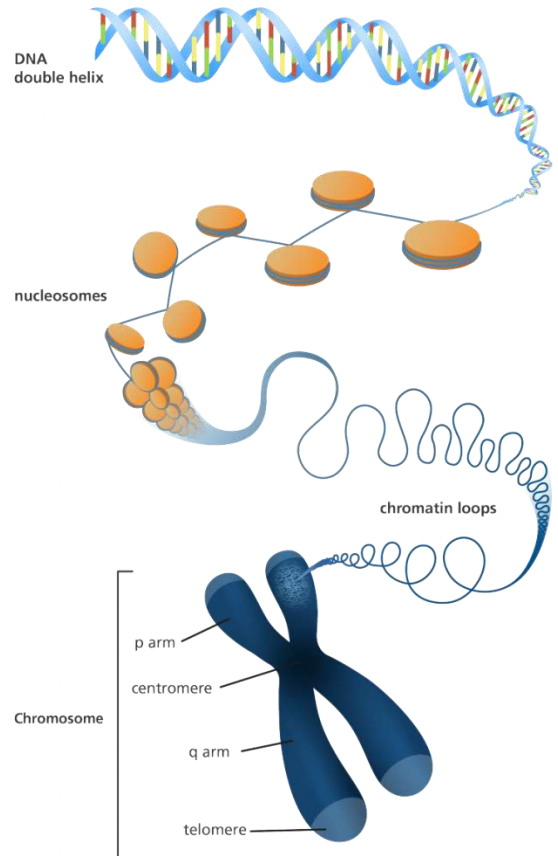
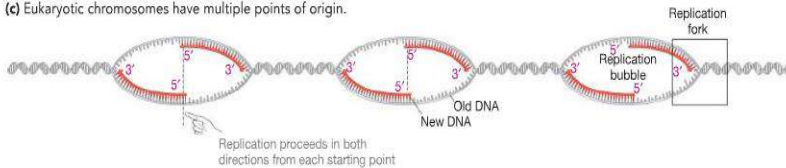
(a) A chromosome being replicated



(b) Bacterial chromosomes have a single point of origin.



(c) Eukaryotic chromosomes have multiple points of origin.



**Nucleosome** is a structural unit of an Eukaryotic chromosome that consisting of a length of DNA coiled around a core of histones.

**Histone** is a group of basic proteins found in chromatin, binds to DNA molecule and help control the activity of genes.



## ***Lecture 4 Questions Practice***

### **Q1:- Define the followings:**

- a. Telomere.
- b. Replication bubble.

### **Q2:- Enumerate the followings:**

- a. Initiation complex proteins.
- b. Numerate *ter* sites that prevent further movement of the replication fork.

### **Q3:- MCQ:**

- 1. The telomere structures also protect the ends of chromosomes against degradation by?
  - a. Endonucleases.
  - b. Telomerase.
  - c. Exonucleases.
  - d. Polymerases.
- 2. Each replication origin must initiate once and once only during each replication cycle in order to avoid duplication of?
  - a. DNA segments that have already been replicated.
  - b. mRNA template.
  - c. mRNA that have been synthesized.
  - d. Coding sequence template.



# Molecular Biology

## 5<sup>th</sup> lecture

### DNA Transcription

# OBJECTIVES



→ DNA Transcription.



# DNA Transcription

In order to use the genetic information encoded in DNA, **genes** must be **expressed**. The first step is to make an **RNA copy** of the **DNA sequence**.

**DNA transcription:** is the synthesis of an RNA copy of information encoded on DNA. This refers to **messenger RNA (mRNA)**.

In some cases, such as ribosomal RNA (rRNA) and transfer RNA (tRNA), the RNA is the final product of gene expression.

The mRNA serves as an **intermediate carrier** of **genetic code** which then used by the **ribosomes** to **translate** to **proteins**, the final gene products.



# DNA Transcription

A **genes** or a **clusters of genes**, are transcribed **separately**. Therefore, only small regions of the DNA are transcribed at a time.

This allows different genes to be expressed under **different conditions** and allows the organism to **adapt** to its **surroundings** (environment).

As a result, it is essential for a cell to know **where on the DNA to start transcription, where to stop transcription, and when to turn gene or a cluster of genes on and off**.

# DNA Transcription

## Genes are expressed via making RNA

The word expressed means that the gene products whether proteins or RNA molecules, must be made.

## Genes divided into two major groups:

1. Those whose final product is an RNA molecule (e.g. mRNA, tRNA, rRNA).
2. Those whose final product is protein.

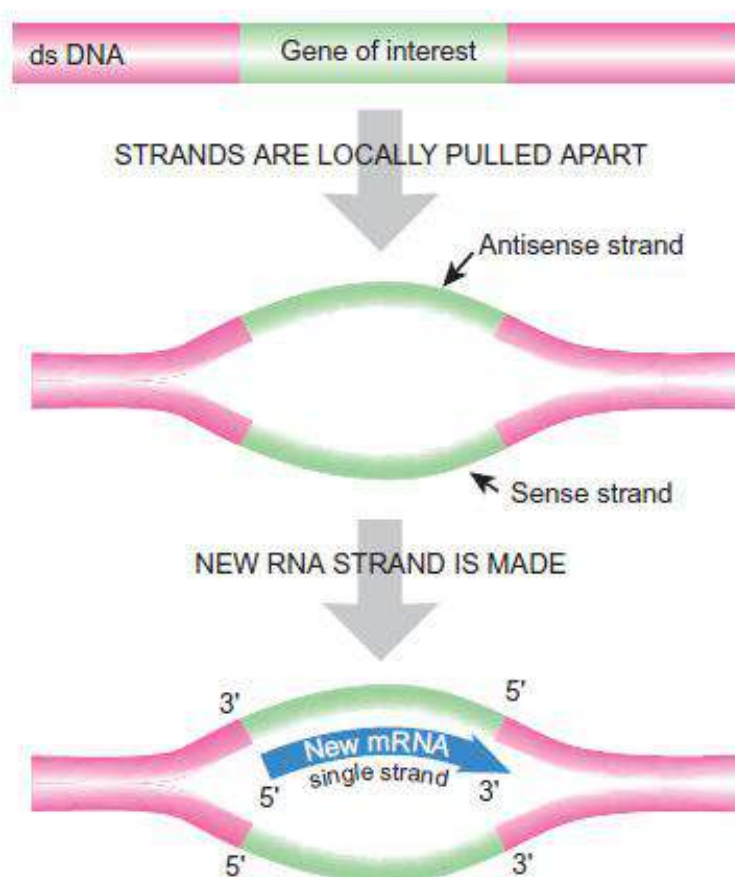
## Steps of DNA transcription

1. Double stranded DNA must be **pulled apart temporally**.
2. **RNA polymerase start synthesizing RNA using DNA sequence as a template.**
3. The sequence of the RNA message is complementary to the **antisense strand** of the DNA from which it is synthesized.

# DNA Transcription

**Antisense strand** is a strand of DNA that is complementary to the coding sequence and is used as a template for synthesizing mRNA.

**Sense strand** is a strand of DNA that is coding sequence.





# DNA Transcription

Although a chromosome carries hundreds or thousands of genes, only a fraction of these are in use at any given time.

In a typical single bacterial cell, about **1000 genes**, or about 25% of the total, are expressed under any particular set of growth conditions.

Man have around **22,000 protein-coding genes** whose expression **varies** under **different conditions** and in **different tissues**.

Some genes are required for the **fundamental operations** of the cell and are **expressed** under **most conditions**. These are known as **constitutive or housekeeping genes**.

Other genes **vary** in **expression** in response to **changes** in the **environment**.



# DNA Transcription

**Constitutive gene** is a gene that is expressed all the time.

**Housekeeping genes** are genes that are switched on all the time because they are needed for essential life functions.

In Prokaryote (bacteria), **citron** refer to **structural gene** in other words, a coding sequence or segment of DNA encoding a polypeptide or protein. An **Open reading frame (ORF)**, is any sequence of bases (in DNA or RNA) that encodes a protein.

In Eukaryote, the majority of genes are transcribed to give a separate mRNA, which encodes the information for only a single protein and is known as **monocistronic mRNA**.

In Prokaryote (bacteria) a clusters of related genes, known as **operons**, are often found next to each other on the chromosome and transcribed together to give a single mRNA called **polycistronic mRNA**.



# DNA Transcription

## Initiation and regulation of DNA transcription

How is the beginning of gene recognition starts?

In front of each gene is a region of regulatory DNA called **promoter** (is not transcribed), the sequence to which RNA polymerase binds together with other sequences involved in the control of gene expression.

The first base of the mRNA for a protein-encoding gene is not the first base of the protein-coding sequence. Between these two points (between the promoter and the protein-encoding gene) there is a short stretch known as the **5'-untranslated region (5'-UTR)**, meaning it will not be translated to form protein.

At the far end of the mRNA there is another short region, beyond the end of the protein-coding sequence that is not translated that called the **3'-untranslated region (3'-UTR)**.

# DNA Transcription

**Prokaryotic (Bacterial) RNA polymerase consists of two major components:**

1. **The core enzyme** is responsible for RNA synthesis.
2. **The sigma subunit** is responsible for recognizing the promoter.

## **The mRNA transcription (manufacturing the message)**

After the **sigma subunit** is bound to the **promoter**, the **RNA polymerase core enzyme opens up the DNA double helix locally to form the transcription bubble.**

Once the DNA helix has been opened, a **single strand of RNA** is **generated** using **one** of the **DNA strands** as a **template** . After the RNA polymerase has bound to the DNA and initiated a new strand of RNA, the **sigma subunit** is no longer needed and detaches from the DNA leaving behind the core enzyme. The core enzyme travels along the DNA, **elongating the mRNA** .

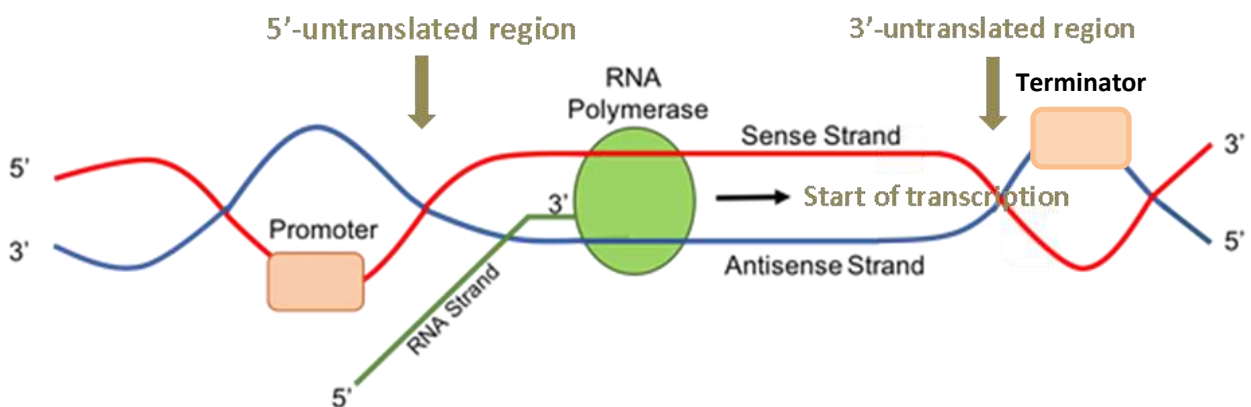
*Prokaryotic RNA polymerase is inhibited by the antibiotic Rifampicin, which is used to treat tuberculosis and leprosy, in combination with other antibiotics.*

# DNA Transcription

## The RNA polymerase knows where to stop (end of message manufacturing)

As there is a recognition site at the front of each gene, so there is a special **terminator sequence** at the end, which located in the template strand of DNA.

After the DNA and RNA have separated at the **terminator structure**, the RNA polymerase falls off and departs to find another gene to transcribed and repeat the process.





# DNA Transcription

## How does a gene turn on?

Some genes, known as housekeeping or constitutive genes are switched on all the Time and they are expressed constantly.

In Prokaryote (bacteria) these housekeeping or constitutive genes often have promoter sequences very close or identical and they are always recognized by the sigma subunit of RNA polymerase and are expressed under all conditions.

However, if only relatively low amounts of the gene product are needed, this is acceptable.



# DNA Transcription

Genes that are only needed under a specific conditions, sometimes have poor recognition sequences of their promoters. In these cases, the **promoter** is **not recognized** by sigma subunit unless another **accessory protein** is there to **help**.

These accessory proteins are known as **gene activator proteins** and are different for different genes. Each activator protein may stimulate the transcription of one or more genes. A group of genes that are all recognized by the same activator protein will be expressed together under similar conditions, even if the genes are at different places on the DNA.

Higher organisms (Eukaryote) have many genes that are **expressed to different extents** in **different tissues** and at **different stages**. Because of that, Eukaryotic genes are often controlled by **multiple activator proteins**, known as **transcription factors**



# DNA Transcription

## How does a gene turn off?

Genes may be controlled by **positive regulation (activator protein)** or **negative regulation**.

In negative regulation a **repressor protein** binds to the DNA and insures that the **gene is turned off**.

Only when the **repressor** is **removed** from the DNA, the **gene** can be **transcribed**.  
The site where a **repressor** binds is called the **operator sequence**.

The mechanism by which repressors prevent transcription is often **vague**. The repressor sometimes blocks the binding of RNA polymerase to the promoter, simply by getting in the way.



# DNA Transcription

## The DNA transcription in Eukaryote is more complex

Since Eukaryotic cells have more many genes than Prokaryote (bacteria), the whole process of DNA transcription and its regulation is more complex.

Eukaryotes have **three different types** of RNA polymerase in the nucleus that transcribed **different types** of **nuclear genes**.

Additionally, **mitochondria** and **chloroplasts** have their **own RNA polymerases** similar to those of bacteria (Prokaryote).



# KEY POINTS

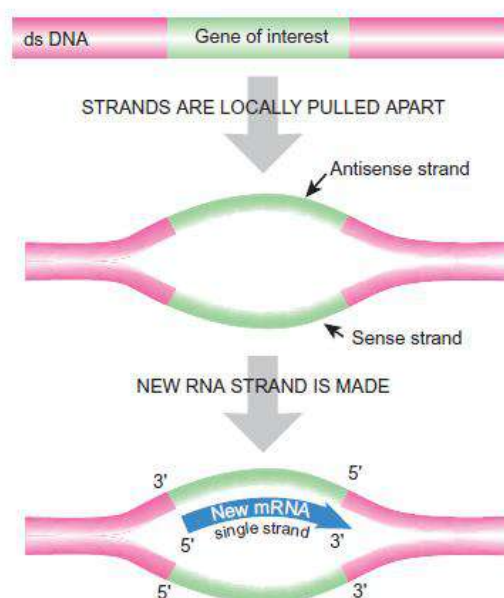
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**Genes divided into two major groups:**

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2. Those whose final product is protein.

**Steps of DNA transcription**

1. Double stranded DNA must be **pulled apart temporally**.
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3. The sequence of the RNA message is complementary to the **antisense strand** of the DNA from which it is synthesized.





# KEY POINTS

**Constitutive gene** is a gene that is expressed all the time.

**Housekeeping genes** are genes that are switched on all the time because they are needed for essential life functions.

In front of each gene is a region of regulatory DNA called **promoter** (is not transcribed), the sequence to which RNA polymerase binds together with other sequences involved in the control of gene expression.

**5'-untranslated region (5'-UTR)**

**3'-untranslated region (3'-UTR).**

**Prokaryotic (Bacterial) RNA polymerase consists of two major components:**

1. The **core enzyme** is responsible for RNA synthesis.
2. The **sigma subunit** is responsible for recognizing the promoter.

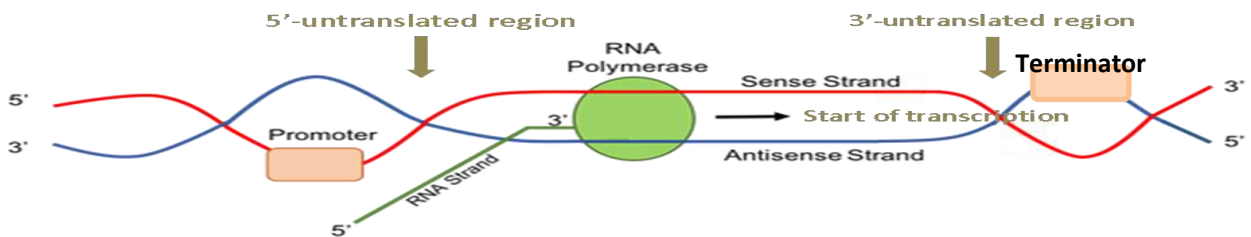
**The mRNA transcription (manufacturing the message)**

**The RNA polymerase knows where to stop (end of message manufacturing)**

As there is a recognition site at the front of each gene, so there is a special **terminator sequence** at the end, which located in the template strand of DNA.



# KEY POINTS



How does a gene turn on?

How does a gene turn off?

The DNA transcription in Eukaryote is more complex

Eukaryotes have **three different types** of RNA polymerase in the nucleus that transcribed **different types** of nuclear genes.

**Mitochondria** and **chloroplasts** have their **own RNA polymerases**.



## ***Lecture 5 Questions Practice***

### **Q1:- Define the followings:**

- a. Promoter.
- b. Antisense strand.
- c. Housekeeping gene.

### **Q2:- Enumerate the followings:**

- a. The two major groups of the genes.
- b. Enumerate the two major components of prokaryotic (Bacterial) RNA polymerase.

### **Q3:- MCQ:**

- 1. Genes are expressed via making?
  - a. DNA.
  - b. RNA.
  - c. Protein.
  - d. Expression factor.
- 2. Eukaryotes have three different types of RNA polymerase in the ----- that transcribes different types of nuclear genes.
  - a. Cytoplasm.
  - b. Ribosome.
  - c. Nucleus.
  - d. DNA.



# Molecular Biology

## 6<sup>th</sup> lecture

### Gene Expression and Regulation

# OBJECTIVES



- Gene Expression
- Gene Transcription
- Gene Translation
- Gene Regulation



# Gene Expression

Gene expression process is used by all known life, Eukaryote, prokaryote and viruses.

In genetics, the gene expression is the most fundamental level at which the **genotype** give rise to **phenotype**.

**Gene expression** is the process by which information from a gene (genetic code\ nucleotides sequence) is used in the synthesis of **functional** gene **product**.

**Protein –coding gene (structural gene)** is a gene when expressed, its final product is **protein** that produces **structure of the cell**.

**Non-protein-coding gene** is a gene when expressed, its final product is **functional RNA** such as **tRNA**, **rRNA** and **regulatory RNA**.



# Gene Expression

The process of gene expression involves **two main steps**:-

1. **Transcription:** the production of messenger RNA (**mRNA**) by the enzyme RNA polymerase, and the processing of the resulting mRNA molecule.
2. **Translation:** the use of **mRNA** to direct protein synthesis, and the subsequent post-translational processing of the protein molecule.

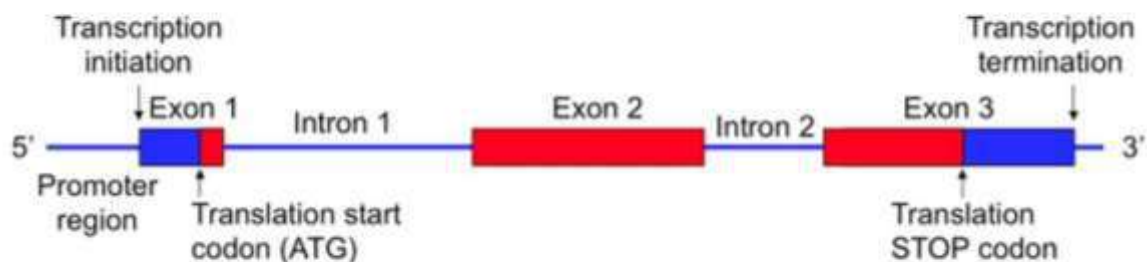
Some genes are responsible for the production of other forms of RNA that play a role in **translation**, including transfer RNA (**tRNA**) and ribosomal RNA (**rRNA**).

# Gene Expression

A **structural gene** involves a number of different **components**:-

**Exons:** they are code for amino acids and collectively **determine the amino acid sequence of the protein product**. It is these portions of the gene that are represented in final mature mRNA molecule.

**Introns:** They are portions of the gene that **do not code for amino acids**, and are removed (**spliced**) from the mRNA molecule before translation.





# Gene Expression

**Gene control regions are:-**

**Start site:** A start site for transcription.

**Promoter:** A region a few hundred nucleotides upstream (5' to 3' end) of the gene. It is not transcribed into mRNA, but plays a role in controlling the transcription of the gene.

**Transcription factors** bind to specific nucleotide sequences in the **promoter region** and **assist** in the **binding** of **RNA polymerase**.



# Gene Expression

**Enhancers:** Some **transcription factors (activators)** bind to regions called enhancers that **increase the rate of transcription**.

These sites may be thousands of nucleotides from the coding sequences or within an intron. Some enhancers are conditional and only work in the presence of other factors as well as transcription factors.

**Silencers:** Some **transcription factors (repressors)** bind to regions called silencers that **depress the rate of transcription**.



# Gene Transcription

**Transcription** is the process of RNA synthesis, controlled by the interaction of promoters and enhancers.

Several different types of RNA are produced, including **messenger RNA (mRNA)**, which specifies the sequence of amino acids in the protein product. In addition, **transfer RNA (tRNA)** and **ribosomal RNA (rRNA)**, which play a role in the translation process.

**Transcription involves four steps:-**

1. **Initiation:** The DNA molecule **unwinds** and separates to form a small **open complex**. RNA polymerase binds to the promoter of the **template strand**.



# Gene Transcription

- 2. Elongation:** The RNA polymerase moves along the template strand, synthesizing an mRNA molecule.

In prokaryotes RNA polymerase is a holoenzyme consisting of a number of subunits, including a **sigma factor** (transcription factor) that recognizes the promoter.

In eukaryotes there are three **RNA polymerases I**, **RNA polymerases II** and **RNA polymerases III**. The process includes a **proofreading mechanism**.



# Gene Transcription

- 3. Termination:** In prokaryotes there are two ways in which transcription is terminated:
- a) Rho-dependent termination,** a protein factor called **Rho** is responsible for disrupting the complex involving the template strand, RNA polymerase and RNA molecule.
  - b) Rho-independent termination,** a loop forms at the end of the RNA molecule, causing it to detach itself.

Termination in **eukaryotes** is more **complicated**, involving the addition of additional adenine nucleotides at the 3' end of the RNA transcript (a process referred to as **polyadenylation**).



# Gene Transcription

- 4. Post transcriptional processing.** After transcription the RNA molecule is processed in a number of ways:
- a) Introns are removed.
  - b) Exons are spliced.

Together forms a mature mRNA molecule consisting of a single protein-coding sequence. RNA synthesis involves the normal base pairing rules, but the base **thymine** is replaced with the base **uracil**.



# Gene Translation

In translation the mature mRNA molecule is used as a template to assemble a series of **amino acids** to produce a **polypeptide** with a specific amino acid sequence. The complex in the cytoplasm at which this occurs is called a **ribosome**.

Ribosomes are a mixture of ribosomal proteins and ribosomal RNA (rRNA) which consist of a large subunit and a small subunit.

## Translation involves four steps:-

1. **Initiation.** The **small subunit** of the ribosome binds at the 5' end of the mRNA molecule and moves in a 3' end direction until it meets a start codon. It then forms a complex with the large unit of the ribosome complex and an initiation tRNA molecule.



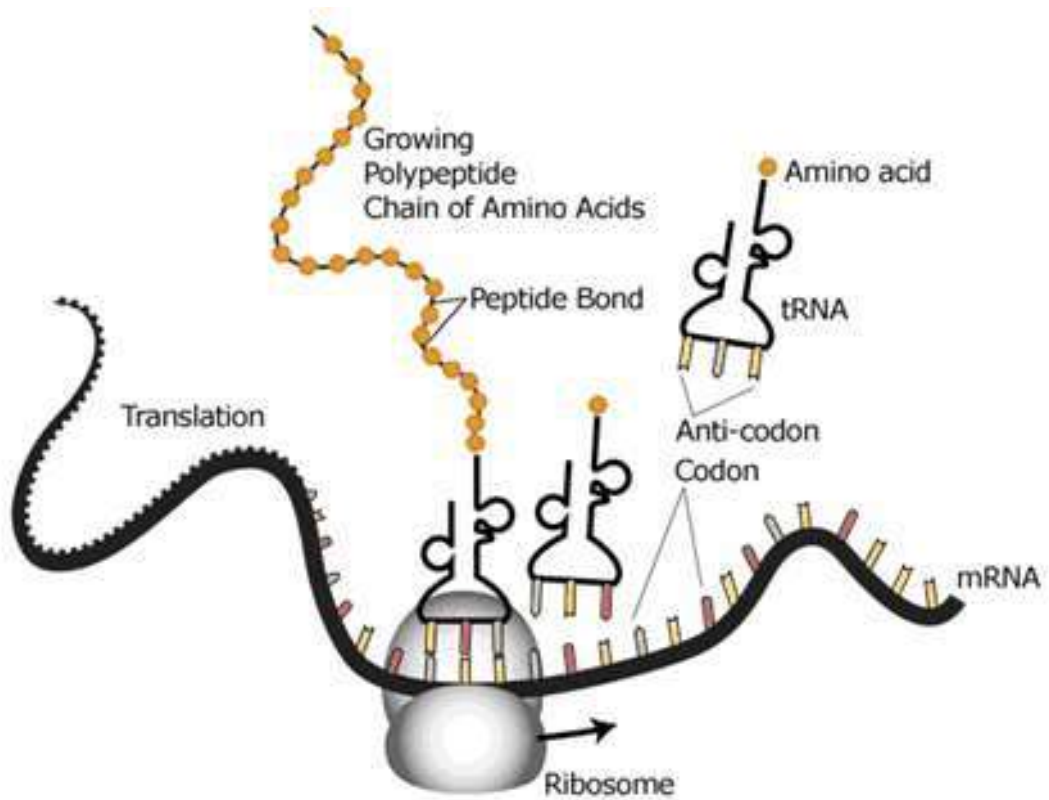
# Gene Translation

2. **Elongation.** Subsequent codons on the mRNA molecule determine which tRNA molecule linked to an amino acid binds to the mRNA. An enzyme **peptidyl transferase** links the amino acids together using **peptide bonds**. The process continues, producing a chain of amino acids as the ribosome moves along the mRNA molecule.
  
3. **Termination.** Translation is terminated when the ribosomal complex reached one or more **stop codons**.

The ribosomal complex in eukaryotes is larger and more complicated than in prokaryotes. In addition, the processes of transcription and translation are divided in eukaryotes between the **nucleus (transcription)** and the **cytoplasm (translation)**, which provides more opportunities for the regulation of gene expression.

## 4. Post translational processing

# Gene Translation





# Gene Regulation

Gene regulation is a label for the cellular processes that control the rate and manner of gene expression. A complex set of interactions between genes, RNA molecules, proteins (including transcription factors) and other components of the expression system determine **when and where specific genes are activated and the amount of protein or RNA product produced.**

Some genes are expressed **continuously**, as they produce proteins involved in basic metabolic functions. Some other genes are expressed as part of the process of cell differentiation. Moreover, some genes are expressed as a result of cell differentiation.



# Gene Regulation

## Mechanisms of gene regulation include:-

- Regulating the **rate of transcription**. This is the most economical method of regulation.
- Regulating the **processing of RNA molecules**. This including alternative splicing to produce more than one protein product from a single gene.
- Regulating the **stability of mRNA molecules**.
- Regulating the **rate of translation**.

**Transcription factors** are proteins that play a role in regulating the transcription of genes by binding to specific regulatory nucleotide sequences.



# KEY POINTS

**Gene expression** is the process by which information from a gene (genetic code\ nucleotides sequence) is used in the synthesis of **functional gene product**.

**Protein –coding gene (structural gene)**

**Non-protein-coding gene**

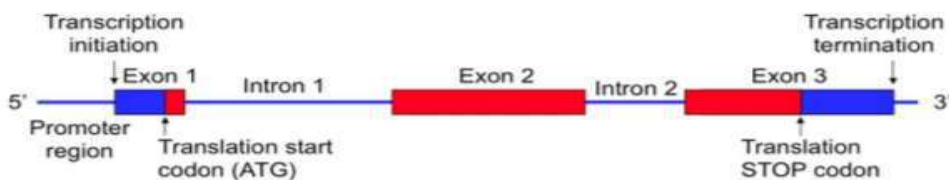
The process of gene expression involves **two main steps**:-

**Transcription → mRNA**

**Translation → protein synthesis**

**Exons:** they are code for amino acids and collectively **determine the amino acid sequence of the protein product**.

**Introns:** They are portions of the gene that **do not code for amino acids**, and are removed (**spliced**) from the mRNA molecule before translation.





**Gene control regions are:-**

**Start site:** A start site for transcription.

**Promoter:** A region a few hundred nucleotides upstream (5' to 3'end) of the gene. It is not transcribed into mRNA, but plays a role in controlling the transcription of the gene.

**Enhancers:** Some **transcription factors (activators)** bind to regions called enhancers that **increase the rate of transcription**.

**Silencers:** Some **transcription factors (repressors)** bind to regions called silencers that **depress the rate of transcription**.

**Transcription** is the process of RNA synthesis, controlled by the interaction of promoters and enhancers.

**Transcription involves four steps:-**

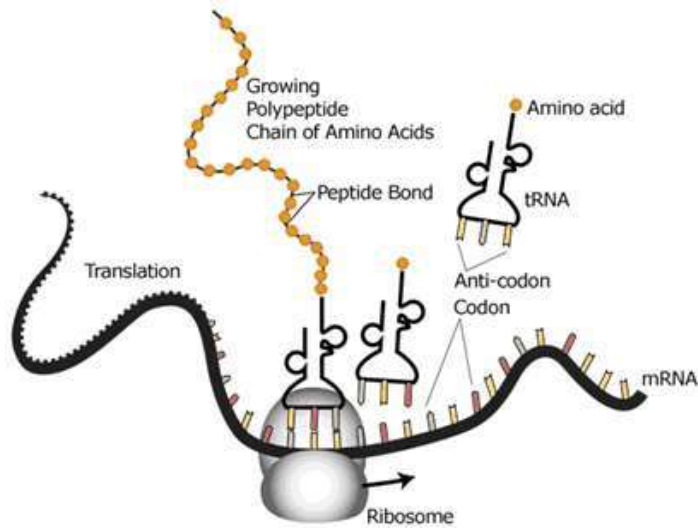
**Initiation → Elongation → Termination (Rho-dependent & Rho-independent termination) → Processing**

**Translation (protein synthesis) involves four steps:-**

**Initiation → Elongation → Termination → Post Transcriptional Processing**



# KEY POINTS



## Gene Regulation

**Mechanisms of gene regulation include:-**

- Regulating the **rate of transcription**.
- Regulating the **processing of RNA molecules**.
- Regulating the **stability of mRNA molecules**.
- Regulating the **rate of translation**.

**Transcription factors** are proteins that play a role in regulating the transcription of genes by binding to specific regulatory nucleotide sequences.



## ***Lecture 6 Questions Practice***

### **Q1:- Define the followings:**

- a. Transcription.
- b. Translation.
- c. Exons.

### **Q2:- Enumerate the followings:**

- a. Enumerate gene translation steps.
- b. Enumerate mechanisms of gene regulation.

### **Q3:- MCQ:**

1. Enhancers: Some transcription factors (activators) bind to regions called enhancers that increase:
  - a. The rate of transcription.
  - b. The mode of transcription.
  - c. The manner of transcription.
  - d. The type of transcription.
2. Silencers: Some transcription factors (repressors) bind to regions called silencers that depress:
  - a. The mode of transcription.
  - b. The manner of transcription.
  - c. The rate of transcription.
  - d. The type of transcription.



# Molecular Biology

## 7<sup>th</sup> lecture

### Post Transcriptional Modification

# OBJECTIVES



- Introduction
- RNA is Modified in Several Ways
- Processing of Ribosomal rRNA and Transfer tRNA
- Eukaryotic mRNA Contains a Cap and a Tail
- Transport of RNA Out of The Nucleus
- Degradation of mRNA



# Introduction

Proteins are the final gene products of most gene. Genes first transcribed to produce messenger RNA (mRNA) which then translated to give protein.

Sometimes, the **RNA** itself is the final gene product. In both cases, the RNA molecule that is the initial result of transcription, may undergo chemical modification before **fulfilling** its role.

This process is called RNA **post transcriptional modification** and may sometimes be very complex. The chemical nature of the modifications differs greatly.



# Introduction

**Post transcriptional modification:** is a set of biological processes common to most eukaryotic cells by which an RNA primary transcript is chemically modified following transcription from gene to produce a mature and functional RNA molecule that can then leave the nucleus and perform variety of different functions in the cell.

The main exception is bacterial mRNA, even though, a few of these mRNA molecules are processed and modified.

In eukaryotic cells, RNA modification is more complicated and mostly take place inside the nucleus before the RNA molecule is released into the cytoplasm. In some cases, the mRNA modification of RNA is mainly regulatory in effect.

In other cases, such as ribosomal RNA (rRNA) and transfer RNA (tRNA), the modifications enhance the performance of the RNA in its final role.



## RNA is Modified in Several Ways

The RNA molecule is made by RNA polymerase using a DNA template in the transcription process. Sometimes, the RNA molecule is ready to function immediately after it has been transcribed such as most bacterial mRNA.

However, in many cases, RNA molecule needs further processing and modification before it become functional.

The original RNA molecule before any processing and modification occurs, is known as the **primary transcript**. Sometimes, the primary transcript consider the **precursor** for other RNA types and called **pre-mRNA**, **pre-tRNA** and **pre-rRNA**.



## RNA is Modified in Several Ways

Certain RNA molecules such as **prokaryotic** and **eukaryotic rRNA** are modified by:-

### I. Cutting and\or joining. How?

1. The RNA is made as a longer precursor then **trimmed** to the correct length.
2. Several RNA molecules are included in the same primary transcript, which is then **cleaved** into several parts or **spliced**.

II. **Base modifications** occur primarily in **tRNA** and **rRNA** after the RNA is transcribed.

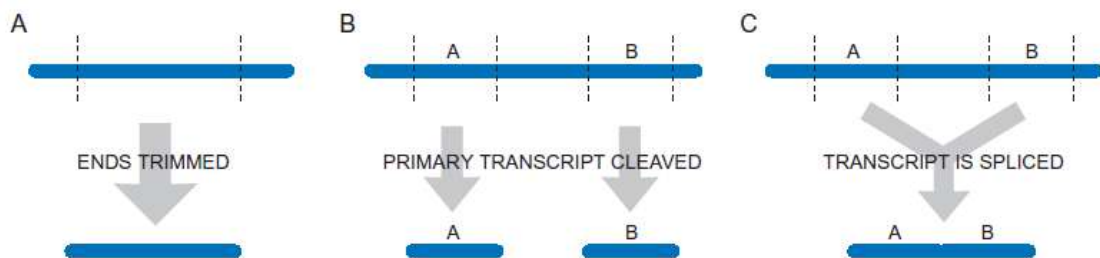
The abovementioned modifications are essential for their **proper function in protein translation**.

# RNA is Modified in Several Ways

All RNA classes are subjected to processing via:

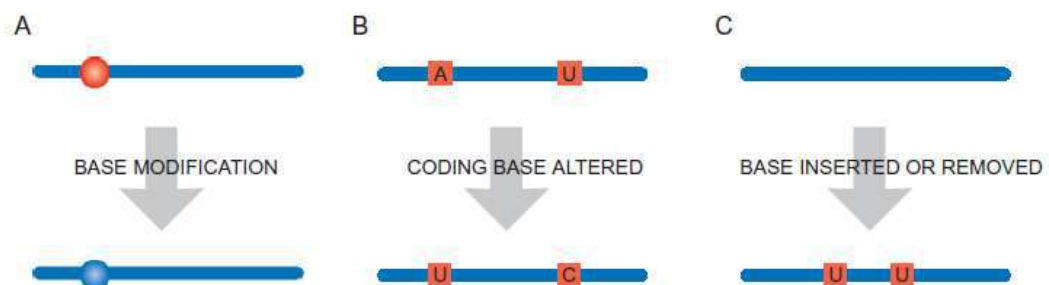
- I. **Cutting\ joining.** This process includes **ends trimming**, **cleaving the primary transcript** and **splicing of the primary transcript**.

## I CUTTING/JOINING



- II. **Base alteration.** This includes **base modification**, **altering the coding base** and **base insertion and removal**.

## II BASE ALTERATIONS





## RNA is Modified in Several Ways

In eukaryotes, the primary transcript for mRNA contains segments called **introns** or intervening sequences that are **not used to encode** the final protein product.

Splicing involves the **removal** of these **introns** and **rejoining** of the ends to create a **simplified linear mRNA** with an uninterrupted coding sequence that is translated into protein.



## Processing of Ribosomal rRNA and Transfer tRNA

The three rRNA molecules of prokaryotes (bacteria) are transcribed together to give a **single pre-rRNA**. This contains **16S rRNA**, **23S rRNA**, and **5S rRNA** joined by linking regions. In bacteria, this pre-rRNA transcript also includes some tRNAs.

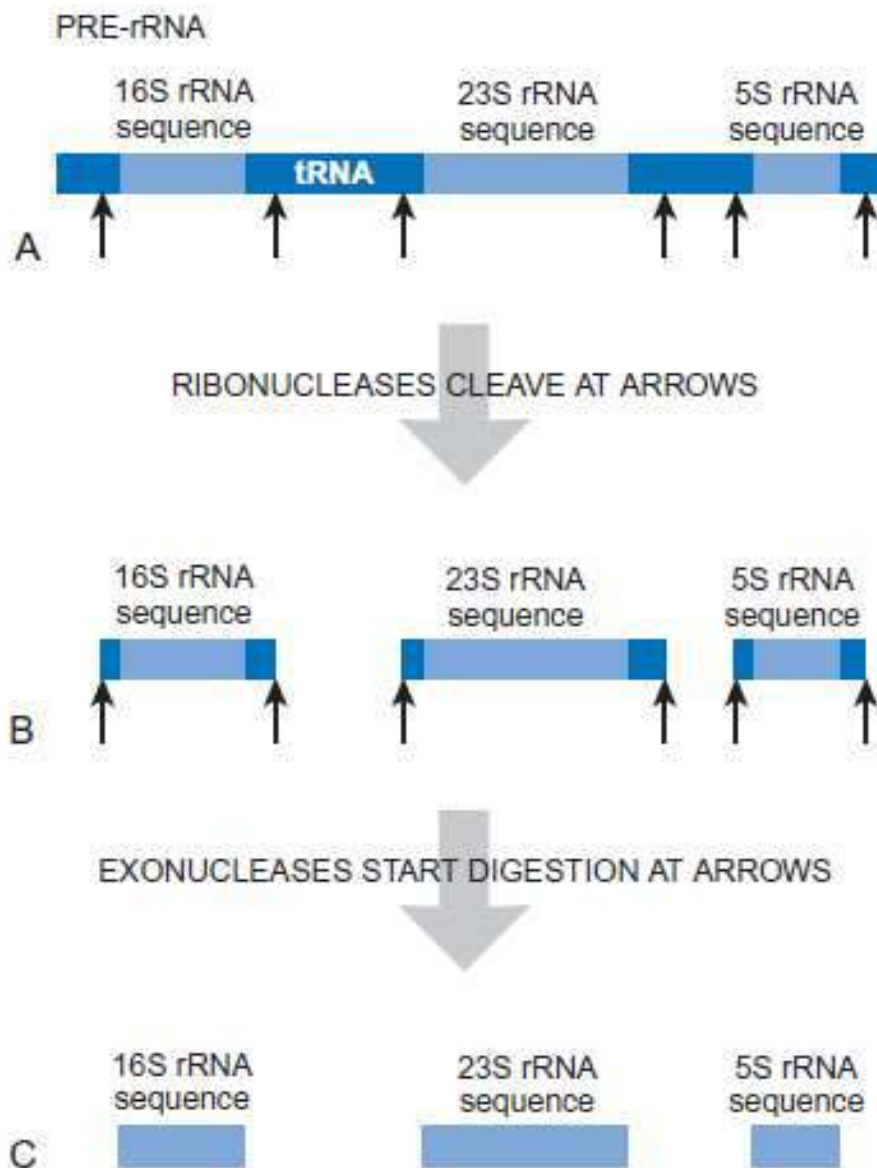
The three mature rRNAs are made by cleavage of the precursor (single pre-rRNA) by **ribonucleases**. This occurs in two stages:

**Stage I:** Internal cuts are made, separating the three rRNAs.

**Stage II:** The ends are trimmed by several exonucleases.

In eukaryotes, there are four types of rRNAs. The **5S rRNA** is made separately and does not need processing. The other three (**18S rRNA**, **28S rRNA**, and **5.8S rRNA**) are made as a **single pre-rRNA** and processed same as in prokaryotes (bacteria).

# Processing of Ribosomal rRNA and Transfer tRNA





## Processing of Ribosomal rRNA and Transfer tRNA

The **tRNAs** are transcribed as longer precursors (**single pre-rRNA**) that also need processing. Some of tRNAs are made individually, others are transcribed together.

In prokaryotes (bacteria) some are included in the pre-rRNA transcript. The 5' end of bacterial tRNA is trimmed by **ribonuclease P**. It consists of both an RNA molecule and a protein. The protein component merely modulates the activity of the RNA.

Recently, in eukaryotes, a protein only version of **ribonuclease P** has been discovered in animal and plants, but it totally absent from prokaryotes (bacteria).



## Eukaryotic mRNA Contains a Cap and a Tail

In eukaryotic cells, transcription of genes to give mRNA is much more complex than in prokaryotes. How?

**Firstly**, eukaryotic genes are inside the nucleus, not free in the cytoplasm.

**Secondly**, most eukaryotic genes are interrupted by segments of **noncoding DNA**, called the **introns**.

The RNA molecule resulting from transcription of a eukaryotic gene is known as the **primary transcript**. This is not yet mRNA because it still needs to be processed.

If the primary transcript were translated only, it would result in a huge, **dysfunctional protein** containing many extra random amino acids due to the **intron regions**. The primary transcript is trapped inside the nucleus until it is completely processed.



## Eukaryotic mRNA Contains a Cap and a Tail

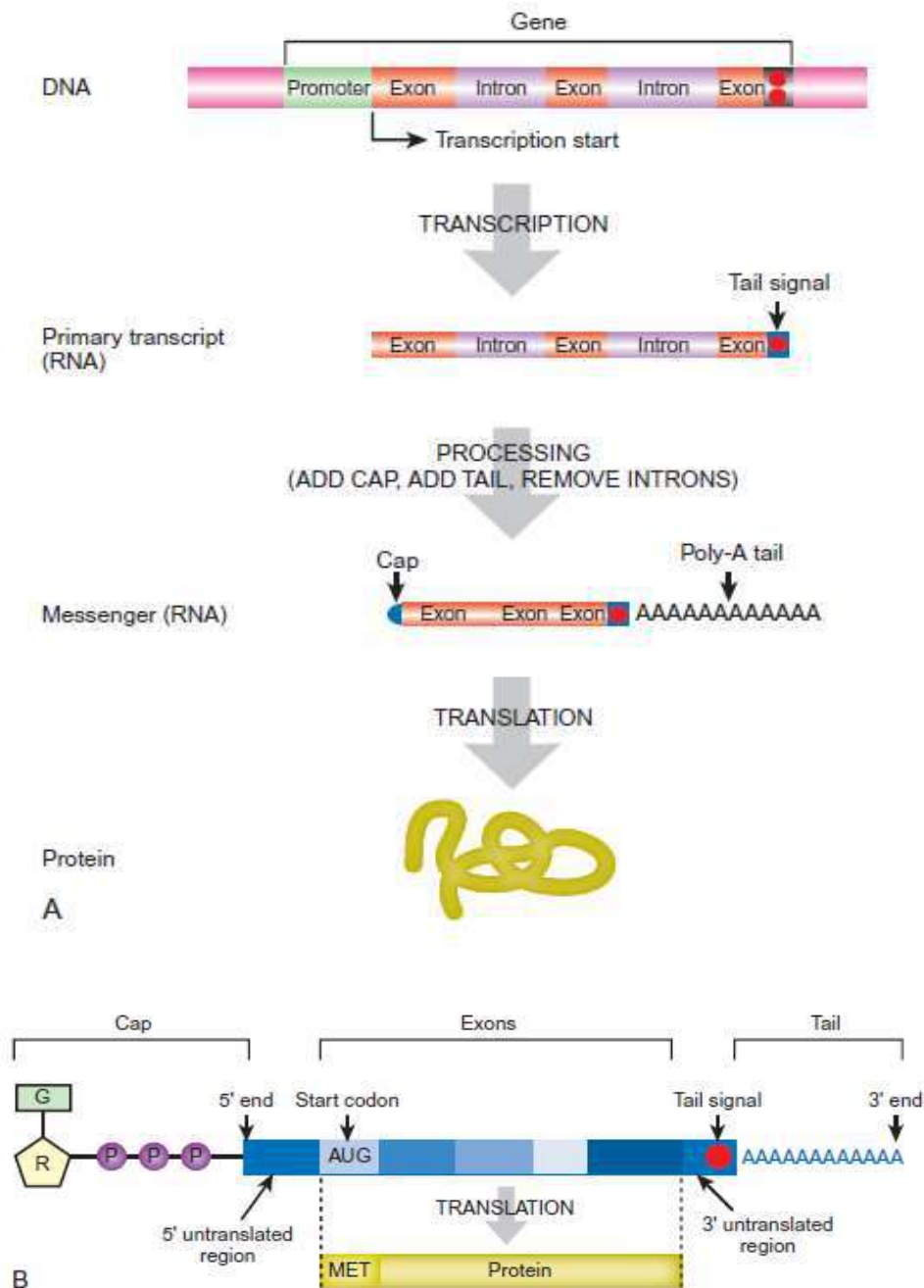
**The mRNA is processed inside the nucleus via:-**

**Firstly:** Adding a **cap** structure to the front and adding a **tail** of many adenines (AAAAAA) to the rear of the RNA molecule.

**Secondly:** Introns are removed by a process known as **splicing**. This involves **cutting out** the **introns** and **joining** the ends of the **exons** to generate an mRNA molecule that has only the exons.

After the processing is complete, the mRNA exits the nucleus to be translated by ribosomes.

# Eukaryotic mRNA Contains a Cap and a Tail





## Transport of RNA Out of The Nucleus

A double membrane that is crossed by pores surrounds the eukaryotic nucleus. Each nucleus has many pores that allow macromolecules in or out in a carefully controlled manner.

Small molecules may diffuse through the nuclear pore, but larger molecules require energy for transit. In the case of mRNA, the energy is derived by hydrolysis of **ATP** but for other forms of RNA, the energy is derived from **GTP** by hydrolysis too.

For mRNA to exit the nucleus, it must have received its **cap** and **tail** and had its **introns** spliced out.

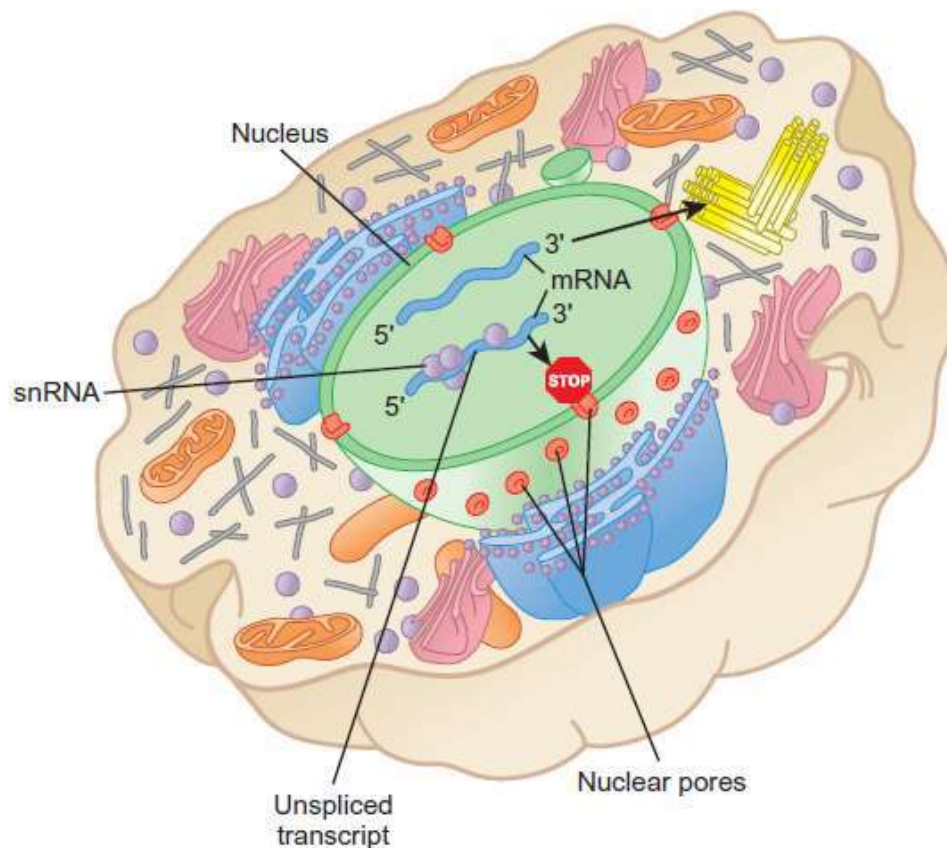
The exit of mRNA from the nucleus occurs in **three distinct stages**:

1. Docking onto the nuclear basket.
2. Crossing the central pore channel.
3. Being released by the cytoplasmic filaments.

Each stage is **highly regulated** and requires **specific protein** factors.

## Transport of RNA Out of The Nucleus

Only 25% - 35% of attempts at exit by mRNA are successful. The mRNA returns to the nucleus in the majority of cases and presumably tries again.





## Degradation of mRNA

In prokaryotes (bacteria), the mRNA molecules are short-lived, for few minutes only. Bacteria contain multiple **ribonucleases** that are involved both in **processing** the precursors to tRNA and rRNA and in the **degradation** of mRNA.

Bacterial mRNA is degraded in **two stages**:

**First stage:** An **endonuclease** cleaves regions that are unprotected by ribosomes.

**Second stage:** An **exonucleases** that move in a 3' to 5' end direction degrade the fragments.

Note that **overall degradation** moves in a 5' to 3' end direction due to the initial endonuclease following the ribosomes.

**Endonuclease:** A nuclease that cuts a nucleic acid in the middle.

**Exonuclease:** A nuclease that cuts a nucleic acid at the end.

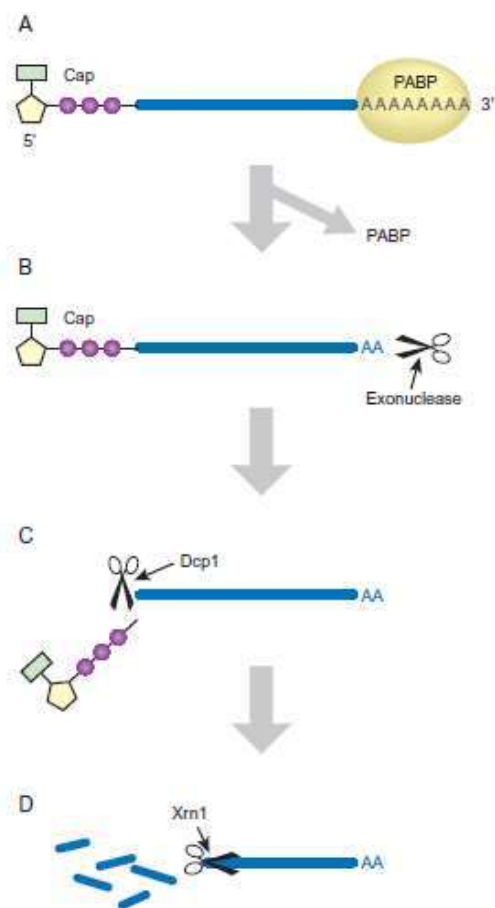
**Ribonuclease:** A nuclease that cuts RNA.

## Degradation of mRNA

In eukaryote, degradation of mRNA follows a different pathway.

**Firstly:** the poly (AAAAA) **tail** and then the **cap** must be **removed** before **nuclease** digestion of the body of the RNA and the **poly (A)-binding protein (PABP)** is being released.

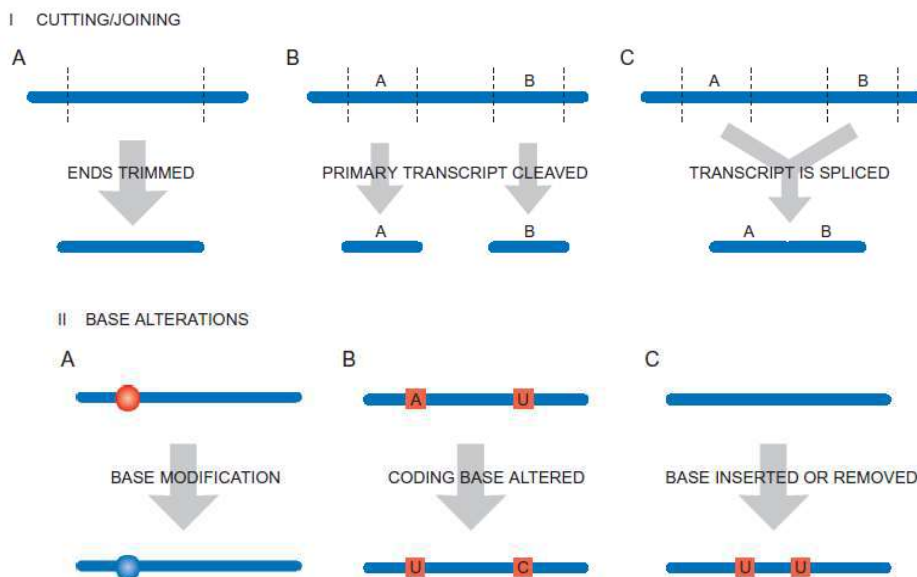
**Secondly:** An exonuclease (**Xrn1**) degrades the mRNA in the 5' to 3' end direction.





# KEY POINTS

**Post transcriptional modification:** is a set of biological processes common to most eukaryotic cells by which an RNA primary transcript is chemically modified following transcription from gene to produce a mature and functional RNA molecule that can then leave the nucleus and perform variety of different functions in the cell.



## Eukaryotic mRNA Contains a Cap and a Tail

In eukaryote, mRNA is much more complex than in prokaryotes. How?

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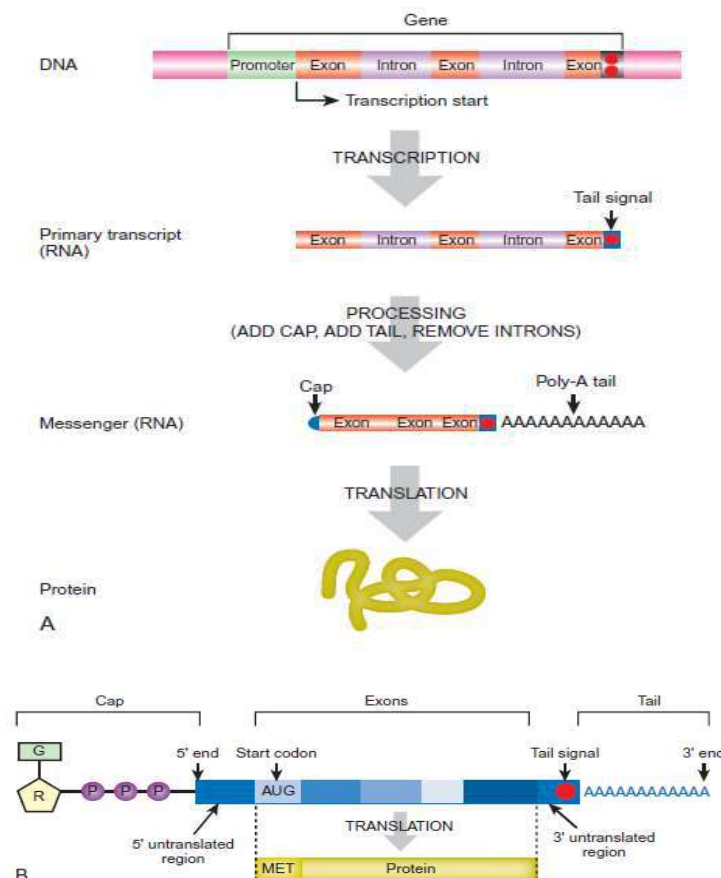


# KEY POINTS

**The mRNA is processed inside the nucleus via:-**

**Firstly:** Adding a **cap** structure to the front and adding a **tail** of many adenines (AAAAAA) to the rear of the RNA molecule.

**Secondly:** Introns are removed by a process known as **splicing**. This involves **cutting out** the **introns** and **joining** the ends of the **exons** to generate an mRNA molecule that has only the exons.





# KEY POINTS

## Transport of RNA Out of The Nucleus

The exit of mRNA from the nucleus occurs in **three distinct stages**:

1. Docking onto the nuclear basket.
2. Crossing the central pore channel.
3. Being released by the cytoplasmic filaments.

## Degradation of mRNA

Bacterial mRNA is degraded in **two stages**:

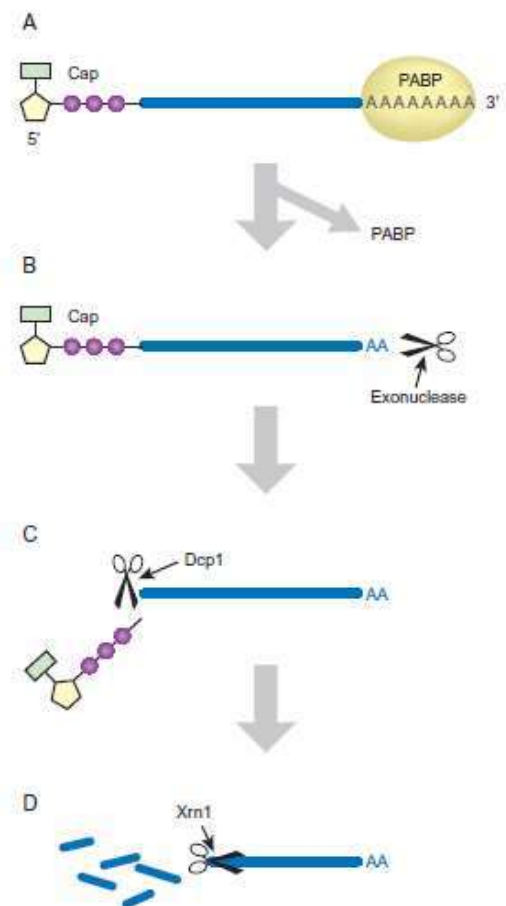
**First stage:** An **endonuclease** cleaves regions that are unprotected by ribosomes.

**Second stage:** An **exonuclease** that move in a 3' to 5' end direction degrade the fragments.

In eukaryote, degradation of mRNA follows a different pathway.

**Firstly:** the poly (AAAAA) **tail** and then the **cap** must be **removed** before **nuclease** Digestion of the body of the RNA and the **poly (A)-binding protein (PABP)** is being released.

**Secondly:** An exonuclease (**Xrn1**) degrades the mRNA in the 5' to 3' end direction.





## ***Lecture 7 Questions Practice***

### **Q1:- Define the followings:**

- a. Post transcriptional modification.
- b. Primary Transcript.

### **Q2:- Enumerate the followings:**

- a. Enumerate RNA processing ways.
- b. Enumerate the stages of mRNA exit from the nucleus.

### **Q3:- MCQ:**

- 1. The exonuclease (Xrn1) degrades the mRNA in the:
  - a. 3' to 3' end direction.
  - b. 5' to 3' end direction.
  - c. 3' to 5' end direction.
  - d. 5' to 5' end direction.
- 2. The first step in mRNA degradation is:
  - a. Cap and tail must be added.
  - b. Cap and tail must be fixed.
  - c. Cap and tail must be removed.
  - d. Cap and tail must be transferred.

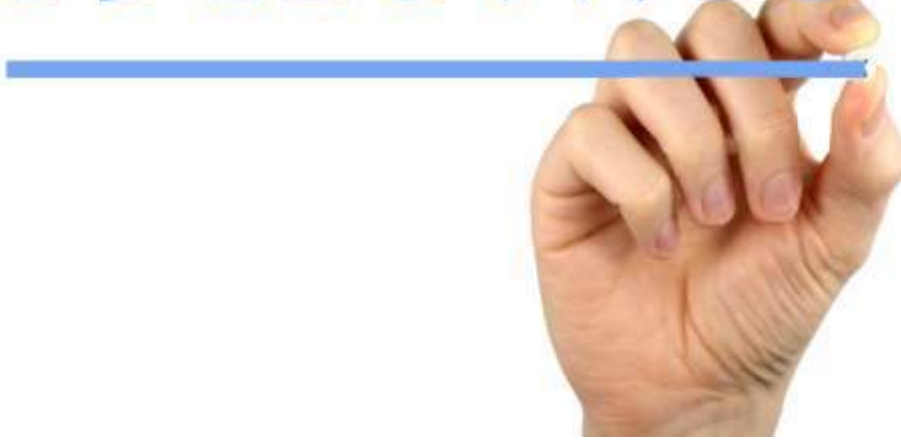


# Molecular Biology

## 8<sup>th</sup> lecture

### Translation and Protein Synthesis

# OBJECTIVES



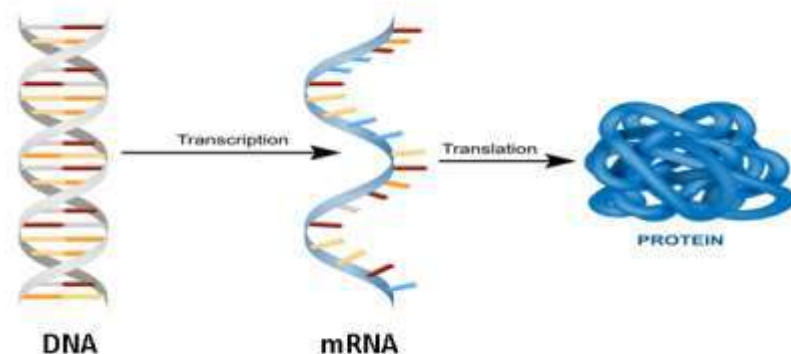
- Translation and Protein Synthesis
- Translation of mRNA
- The Ribosome
- Transfer RNAs (tRNAs)
- Protein Synthesis: Initiation, Elongation, Termination
- Protein Targeting, Folding and Modification

# Translation and Protein Synthesis

The process of **translation** or **protein synthesis** is the second step of gene expression which involves the decoding of mRNA message by the ribosome. The end product is polypeptide (protein).

Proteins perform almost every function of a cell serving as both functional protein (enzymes) and structural protein (cell structure).

The genetic code (found on DNA) is transcribed (copied) to mRNA which then translated to proteins.





## Translation of mRNA

Translation of mRNA template converts genetic information (nucleotide sequence) into **amino acids** to create protein products.

A **protein sequence** consists of 20 commonly occurring **amino acids**. Each amino acid is defined within the **mRNA** by a **triple nucleotides** called **codon**. The **relationship** between an **mRNA codon** and its **corresponding amino acid** is called the **genetic code**.

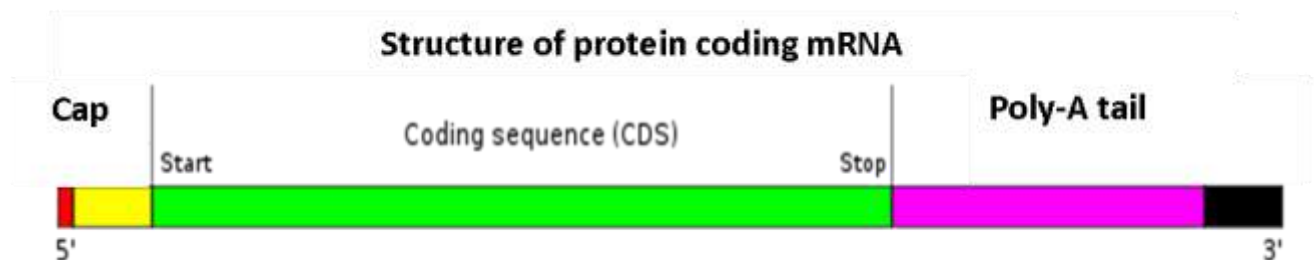
There are codons **do not code for any amino acids** which their **function** is to **terminate protein synthesis** and **release** the polypeptide chain from the translation site in the ribosome. These are called **stop codons** or **nonsense codons** (**UAA, UAG, UGA**).

The **codon AUG** has a special function, specifying the amino acid **methionine**, it serves as the **start codon** to **initiate translation**.

## Translation of mRNA

The **start codon (AUG)** is set at the **beginning** of mRNA message near the 5' end and the **stop codons** are placed at the **end** of mRNA message near the 3' end.

Each set of three nucleotides found between the start codon and the stop codon is called **coding sequence (CDS)** which will be translated to a protein.





# The Ribosome

Translation requires the involvement of an **mRNA**, **ribosomes**, **tRNA**, and **various enzymatic factors**.

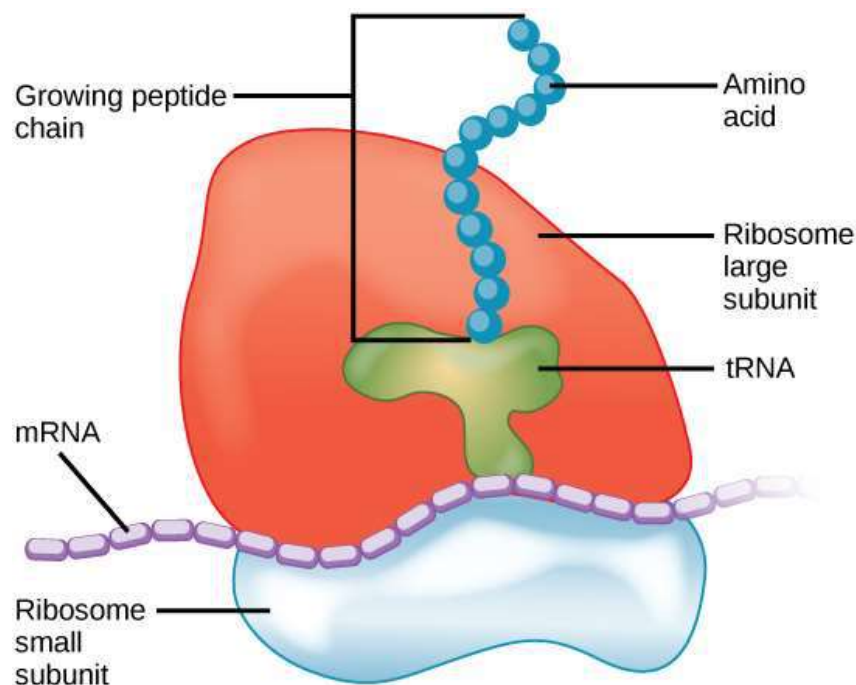
**Ribosome** is a complex macromolecule composed of **catalytic rRNA** called **ribozymes**, and **structural rRNA**.

Ribosomes dissociate into **large** and **small** subunits when they are not synthesizing proteins and re-associate during the **initiation of translation**. The small subunit is responsible for **binding the mRNA template**, whereas the large subunit **binds tRNA**.

# The Ribosome

Prokaryotes have **70S ribosomes**. The **small subunit** is **30S** and the **large subunit** is **50S**.

Eukaryotes have **80S ribosomes** in the cytoplasm and rough endoplasmic reticulum and **70S ribosomes** in **mitochondria** and **chloroplasts**. The **small subunit** is **40S** and a **large subunit** is **60S** for a total of **80S**.





# The Ribosome

Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5' to 3' end and synthesizing the polypeptide from the **N terminus (NH<sub>2</sub>)** to the **C terminus (COOH)**.

The complete structure containing an mRNA with multiple associated ribosomes is called a **polyribosome** (or **polysome**).

In prokaryotes, both **transcription and translation** can occur simultaneously, because both of these processes occur in the cytoplasm of the cell.

In eukaryotes, simultaneous transcription and translation is **not possible** until RNA synthesis is complete and has been modified and transported out of the nucleus.



## Transfer RNAs (tRNAs)

The **transfer RNAs (tRNAs)** are structural RNA molecules of many different types depending on the species. The tRNAs are exist in the cytoplasm.

Prokaryotes, typically have between **60** to **90** types. Each **tRNA** type binds to a specific codon on the mRNA template and adds the corresponding amino acid to the polypeptide chain.

The tRNA molecule interacts with three factors, **aminoacyl tRNA synthetases**, **ribosomes** and **mRNA**.



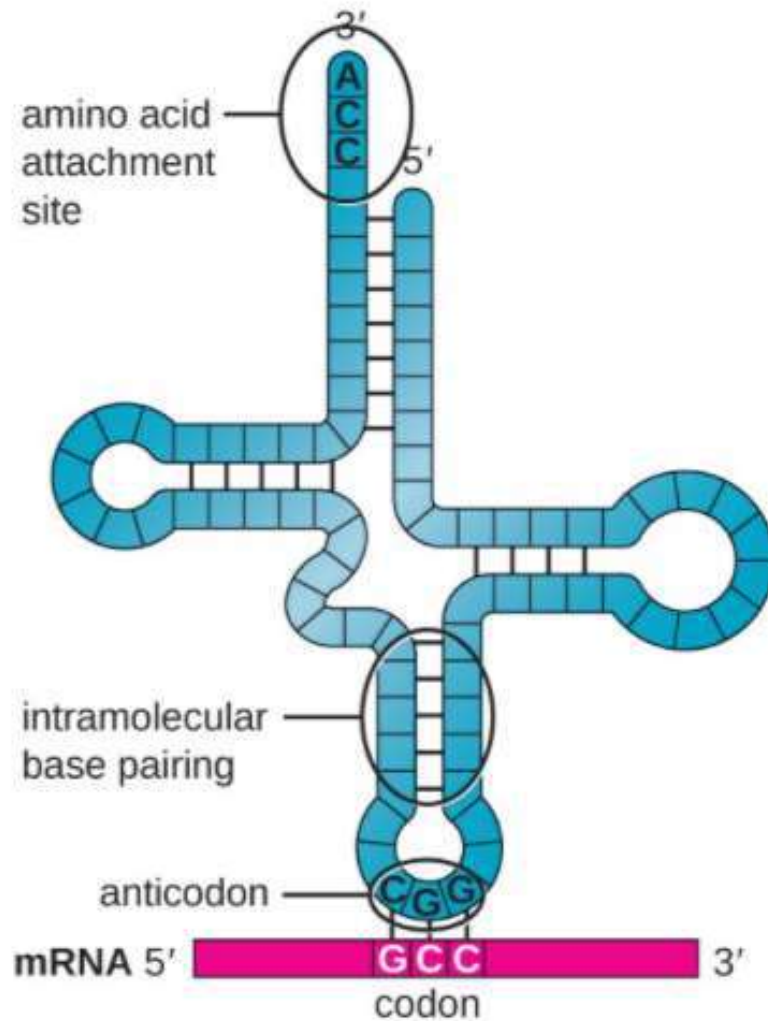
## The Transfer RNA (tRNA)

The mature tRNAs take on a 3D (three-dimensional) structure when complementary bases are exposed in the single stranded RNA molecule and hydrogen bond with each other.

This shape positions the amino acid binding site that called the **CCA amino acid binding end**, which is a **cytosine-cytosine-adenine** sequence at the 3' end of the tRNA, and the **anticodon** at the other end.

The **anticodon** is a three nucleotide sequence that bonds with an mRNA codon through complementary base pairing.

## The Transfer RNA (tRNA)





# Protein Synthesis: Initiation

The process of protein synthesis is similar in prokaryotes and eukaryotes. However, we will study protein synthesis in prokaryote (bacteria) and specify any differences between prokaryotes and eukaryotes.

Protein synthesis process occurs in three consecutive steps as follows:

## 1. Initiation.

The **initiation of protein synthesis** begins with the formation of an **initiation complex** which consists of:-

- a. The small **30S** ribosome.
- b. The **mRNA template**.
- c. Three **initiation factors** that help the ribosome assemble correctly.
- d. **Guanosine triphosphate (GTP)** as an energy source.
- e. **Initiator tRNA** carrying **N-formyl-methionine (fMet-tRNA<sup>fMet</sup>)** that interacts with the start codon AUG of the mRNA and carries a formylated methionine.



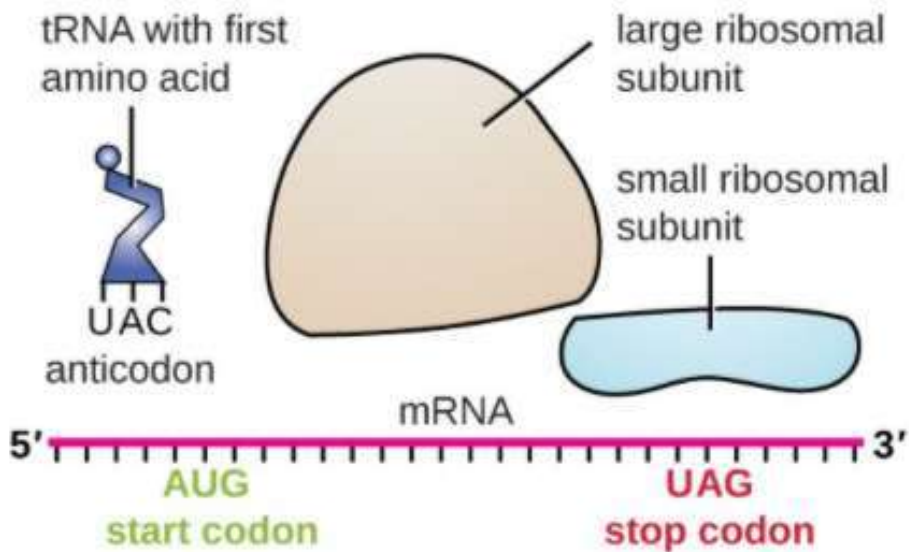
## Protein Synthesis: Initiation

After the initiator tRNA being interacted with the start codon, it anchors the **30S ribosomal subunit** at the correct location on the mRNA template. Then, the **50S ribosomal subunit** binds to the initiation complex forming an **intact ribosome**.

**In eukaryotes, initiation complex formation is similar with some differences:**

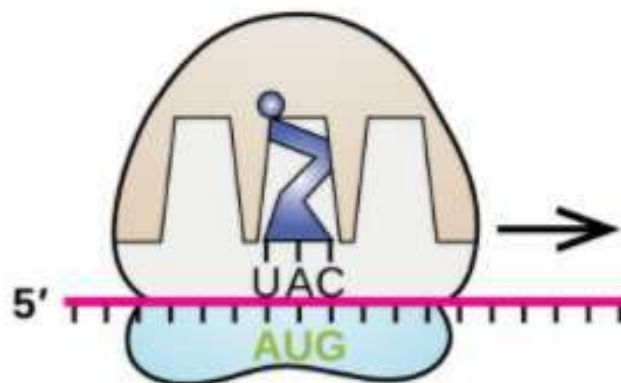
- i. The **initiator tRNA** is a different specialized tRNA carrying **methionine** that called **Met-tRNA<sub>i</sub>**.
- ii. The eukaryotic initiation complex recognizes the 5' end cap of the eukaryotic mRNA, then tracks along the mRNA in the 5' to 3' direction until the **AUG start codon** is recognized. At this point, the **60S subunit** binds to the complex of **Met-tRNA<sub>i</sub>**, **mRNA**, and the **40S subunit**.

# Protein Synthesis: Initiation



## INITIATION

Transitional complex forms, and tRNA brings first amino acid in polypeptide chain to bind to start codon on mRNA.





# Protein Synthesis: Elongation

## 2. Elongation.

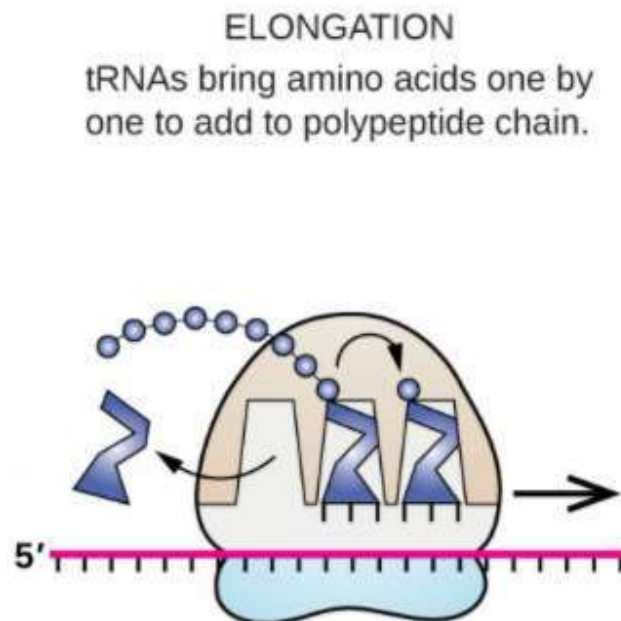
The basics of **elongation** phase is the same in prokaryotes and eukaryotes. The binding of the **50S ribosomal subunit** to produce the intact ribosome, it forms three functionally important ribosomal site as follows:

- a. The **A site (aminoacyl)** that binds incoming charged aminoacyl tRNAs.
- b. The **P site (peptidyl)** that binds charged tRNAs and carrying amino acids that have formed peptide bonds with the growing polypeptide chain.
- c. The **E site (exit)** that releases dissociated tRNAs so that they can be recharged with free amino acids.

## Protein Synthesis: Elongation

Elongation proceeds with single-codon movements of the ribosome each called a **translocation event**. During each translocation event, the charged tRNAs enter at the **A site**, then shift to the **P site**, and then finally to the **E site** for removal.

**Peptide bonds** form between the amino acids and it is catalyzed by **peptidyl transferase** enzyme. Elongation require **energy** derived from **GTP** (Guanosine triphosphate) hydrolysis.





# Protein Synthesis: Termination

## 3. Termination.

The termination of translation occurs when a **stop codon** (UAA, UAG, UGA) is encountered for which there is no complementary tRNA.

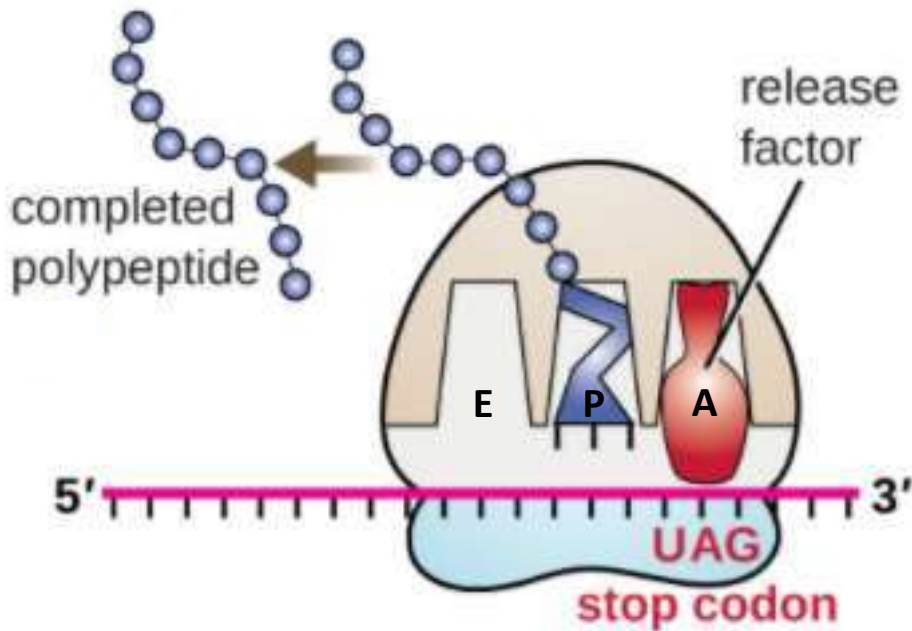
The **stop codons** are recognized by release factors in prokaryotes and eukaryotes that result in the **P site** amino acid **detaching** from its tRNA and releasing the newly made polypeptide.

The small and large ribosomal subunits dissociate from the mRNA and from each other, then recruited immediately into another translation initiation complex.

## Protein Synthesis: Termination

### TERMINATION

Release factor recognizes stop codon, translational complex dissociates, and completed polypeptide is released.





# Protein Targeting, Folding and Modification

During and after translation, polypeptides may need to be modified before they are biologically active.

**Post-translational modifications include:**

- 1. Removal of translated signal sequence.**
- 2. Proper folding of the polypeptide and association of multiple polypeptide subunits.**
- 3. Proteolytic processing** of an inactive polypeptide to release an active protein component.
- 4. Various chemical modifications** such as **phosphorylation, methylation, or glycosylation** of individual amino acids.

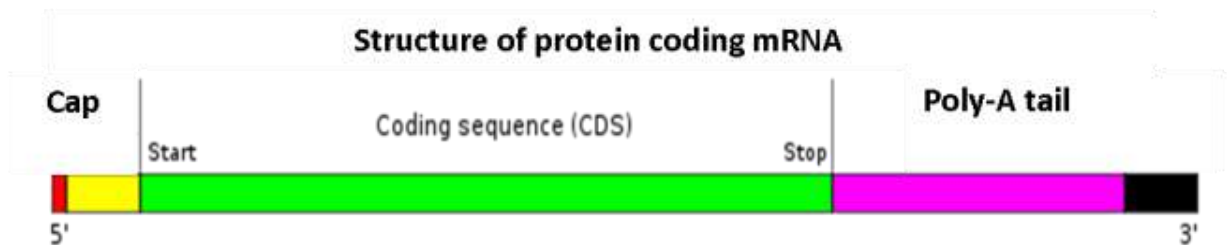


# KEY POINTS

The process of **translation** or **protein synthesis** is the second step of gene expression.

Translation of mRNA template converts genetic information (nucleotide sequence) into **amino acids** to create protein products.

The **codon AUG** has a special function, specifying the amino acid **methionine**, it serves as the **start codon** to **initiate translation**. These are called **stop codons** or **nonsense codons** (UAA, UAG, UGA).



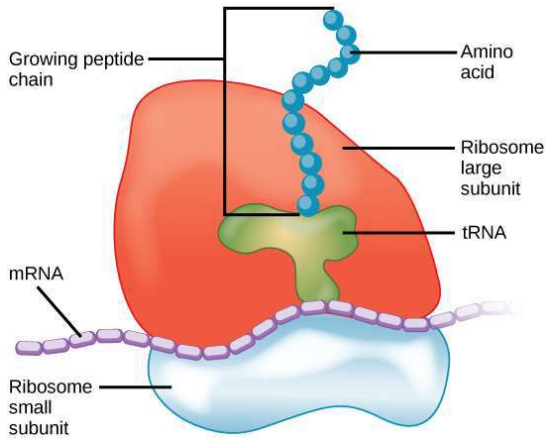
**Ribosome** is a complex macromolecule composed of **catalytic rRNA** called **ribozymes**, and **structural rRNA**.

Prokaryotes have **70S ribosomes**. The **small subunit** is **30S** and the **large subunit** is **50S**.

Eukaryotes have **80S ribosomes**. The **small subunit** is **40S** and a **large subunit** is **60S** for a total of **80S**.



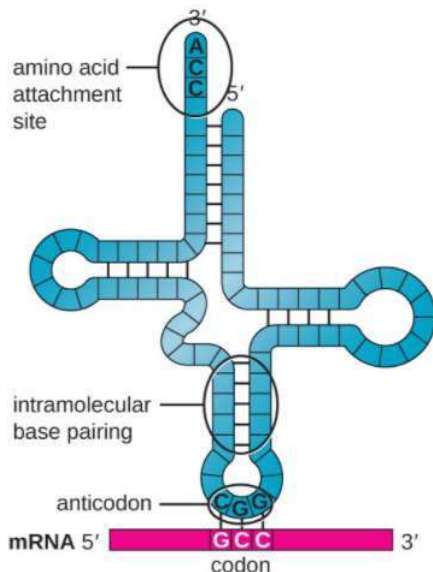
# KEY POINTS



The **transfer RNAs (tRNAs)** are structural RNA molecules of many different types depending on the species. The tRNAs exist in the cytoplasm.

The tRNA molecule interacts with three factors, **aminoacyl tRNA synthetases**, **ribosomes** and **mRNA**.

The amino acid binding site that called the **CCA amino acid binding end** at the 3' end of the tRNA, and the **anticodon** at the other end, which is a three nucleotide sequence that bonds with an mRNA codon through complementary base pairing.



Protein synthesis process occurs in three consecutive steps as follows:

**1. Initiation**, begins with the formation of an **initiation complex**:

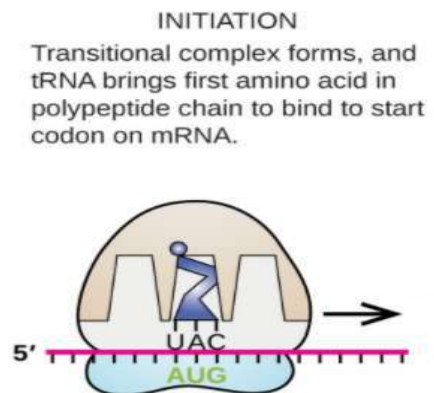
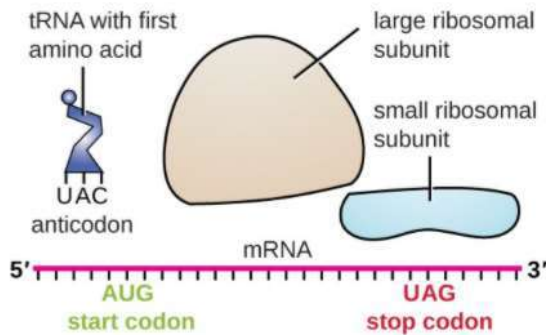
- The small **30S** ribosome.
- The **mRNA template**.
- Three **initiation factors**.
- Guanosine triphosphate (GTP)**.
- Initiator tRNA** carrying **N-formyl-methionine (fMet-tRNA<sup>fMet</sup>)**.



# KEY POINTS

In eukaryotes, initiation complex formation is similar with some differences:

- The **initiator tRNA** is a different → **Met-tRNA<sub>i</sub>**.
- The **60S subunit** binds to the complex of **Met-tRNA<sub>i</sub>**, **mRNA**, and the **40S subunit**.



## 2. Elongation.

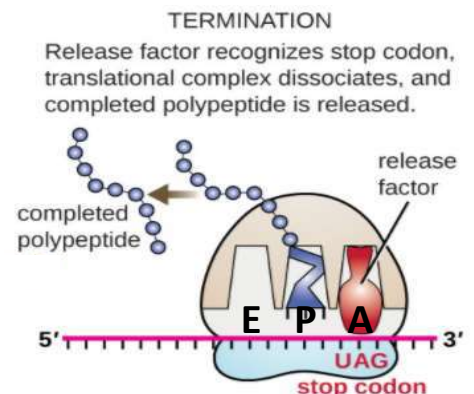
The **elongation** phase is the same in prokaryotes and eukaryotes. The binding of the **50S ribosomal subunit** to produce the intact ribosome.

- The **A site (aminoacyl)** that binds incoming charged aminoacyl tRNAs.
- The **P site (peptidyl)** that binds charged tRNAs.
- The **E site (exit)** that releases dissociated tRNAs.

## 3. Termination.

The termination of translation occurs when a **stop codon (UAA, UAG, UGA)** is encountered for which there is no complementary tRNA.

## Protein Targeting, Folding and Modification





## ***Lecture 8 Questions Practice***

### **Q1:- Define the followings:**

- a. Ribosome.

### **Q2:- Enumerate the followings:**

- a. Enumerate the functional ribosomal sites that involved in the elongation phase of protein synthesis.
- b. Enumerate only the protein post translational modifications.

### **Q3:- MCQ:**

1. Stop codons in protein translation process are:
  - a. UAA.
  - b. UAG.
  - c. UGA.
  - d. All the above.
2. Start codon in protein translation process is:
  - a. UAA.
  - b. UAG.
  - c. AUG.
  - d. UGA.



# Molecular Biology

## 9<sup>th</sup> lecture

### Post Translation Modifications and Inhibitors of Translation

# OBJECTIVES



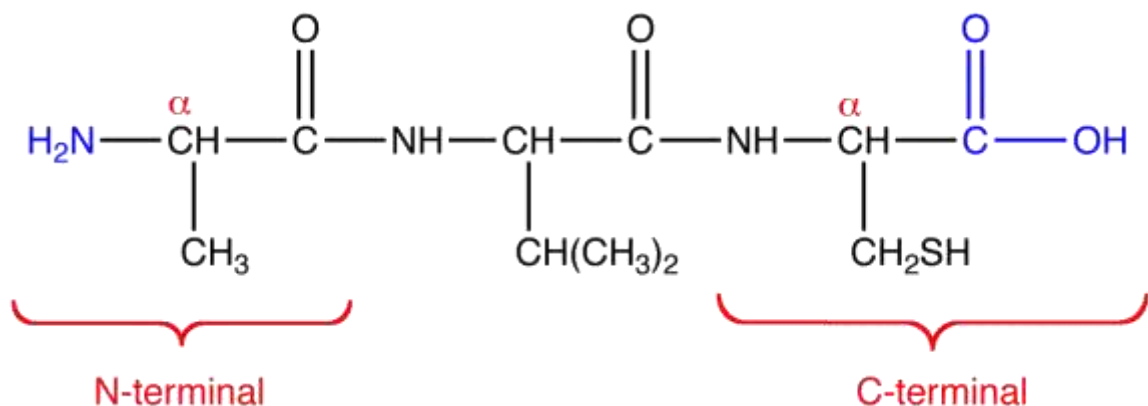
- **Post Translational Modifications**
- **Post Translational Modifications Location**
- **Types of PTMs**
- **Functions of PTMs**
- **Inhibitors of Translation**
- **Inhibitors of Translation Significance**

# Post Translational Modifications

**Post translational modifications (PTMs)** refer to any alteration in the **amino acid** sequence of the protein after its synthesis.

Post translational modifications may involve the modification of the amino acid side chain, terminal **amino (N)** or **carboxyl (C)** group by means of covalent or enzymatic means following protein synthesis.

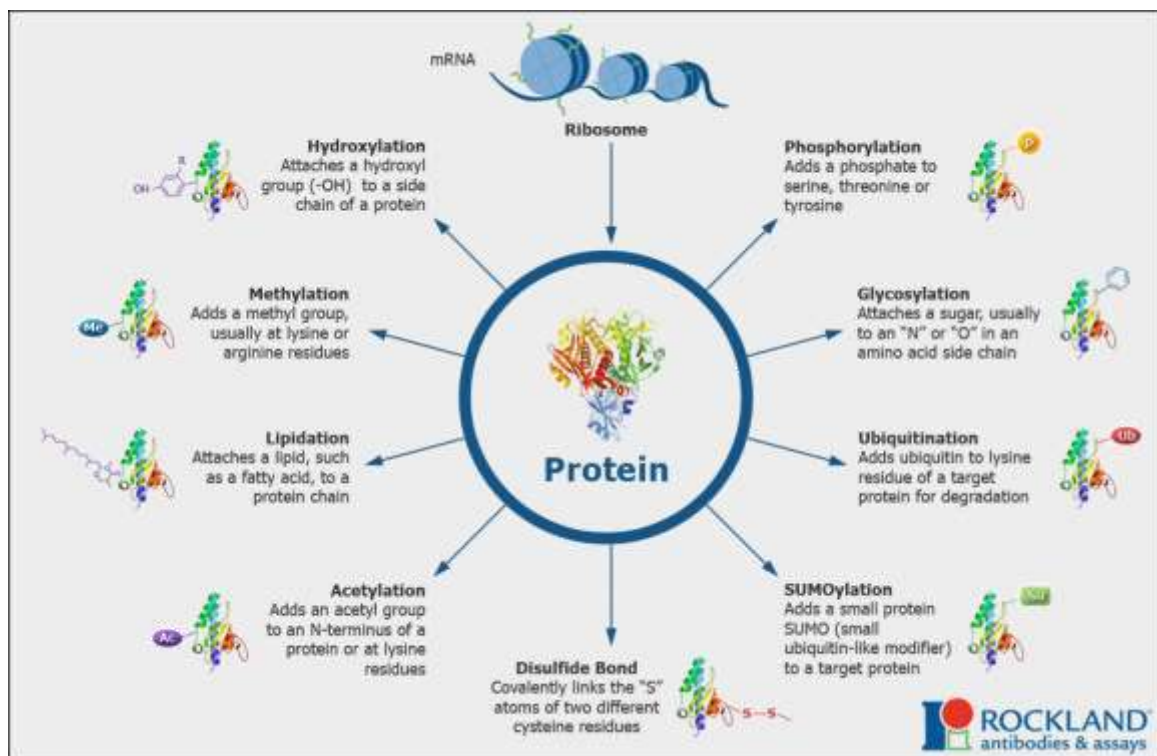
Generally, these modifications influence the **structure, stability, activity, cellular localization** or **substrate specificity** of the protein.



# Post Translational Modifications Location

Post translational modifications (PTMs) mainly occur in the **Endoplasmic Reticulum** of the cell but sometimes continue in the **Golgi apparatus** as well.

After protein synthesis is completed, it can be modified by various methods such as **Proteolysis**, **Phosphorylation**, **Glycosylation**, **Sulfation**, **Methylation**, **Hydroxylation** and addition of other groups.



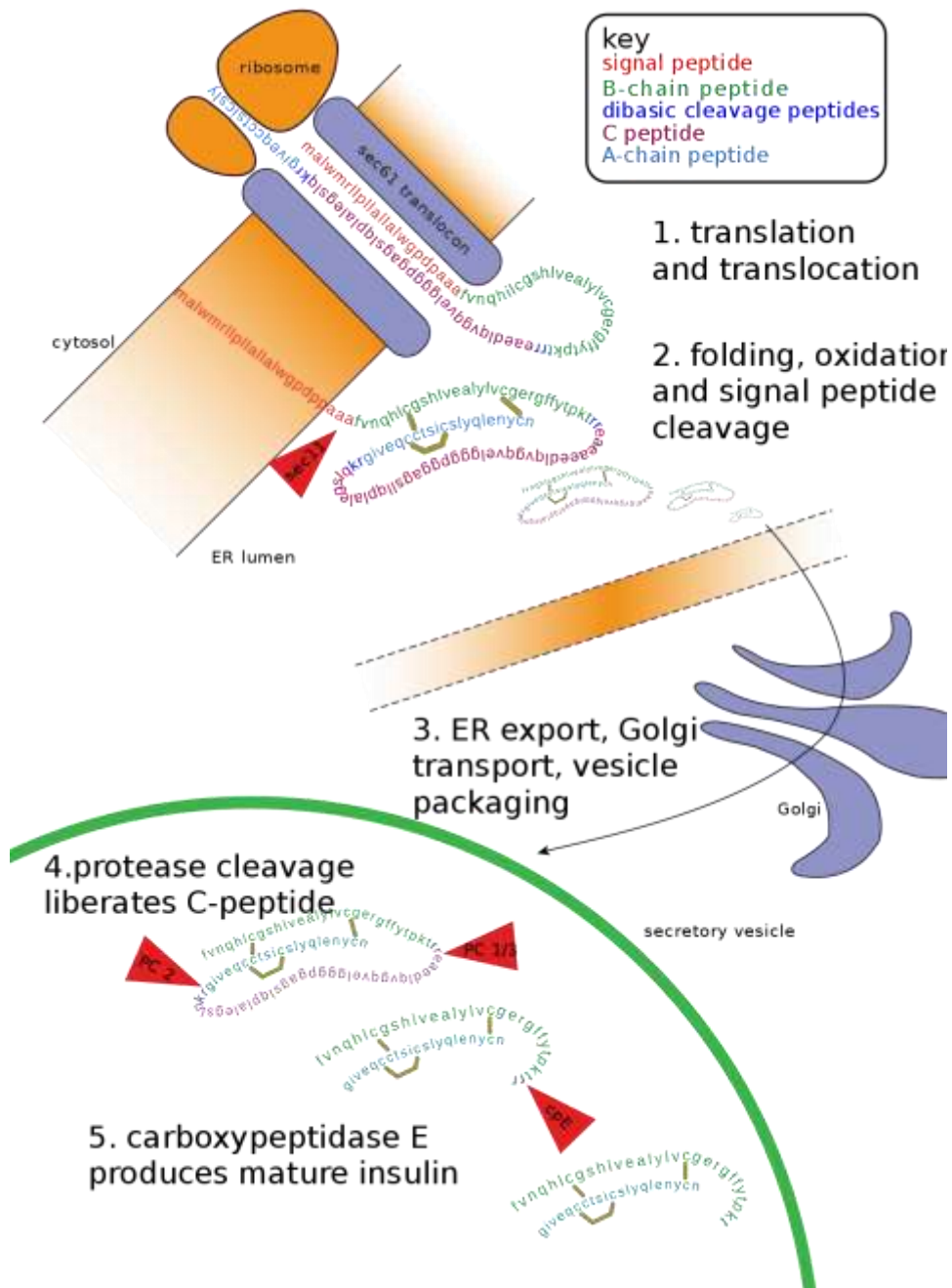


# 1. Proteolysis

The newly synthesized protein is released into the lumen of the Endoplasmic Reticulum, which signal the enzyme **peptidases** to cleave the peptides sequence of the protein resulting in the final sequence.

For example, Insulin is synthesized in the cells in its **inactive form** which cannot perform its function. Post translational modifications of the Insulin ensure proper function which involves the removal of some part of protein to convert it into a three dimensional active form.

# 1. Proteolysis





## 2. Phosphorylation

Phosphorylation is the addition of one or more **phosphate groups** to the protein. Post Translational Phosphorylation is one of the **most common** protein modifications that occur in animal cells.

Majority of phosphorylation occurs as **a mechanism to regulate the biological activity** of a protein.

In animal cells Serine, Tyrosine and Threonine are the amino acids that subjected to the phosphorylation.



### 3. Glycosylation

Glycosylation is the addition of **carbohydrate molecules** to the polypeptide chain and modifying it into **glycoproteins**.

Many of the proteins that are destined to become a part of **plasma membrane** or to be **secreted from the cell**, have carbohydrate chains attached to the nitrogen of Asparagine (N-linked) or the hydroxyl groups of Serine, Threonine (O-linked).

The **N-glycosylation** occurs in **Endoplasmic Reticulum** and **O-glycosylation** occurs in the **Golgi apparatus**.



## 4. Sulfation

Sulfate modification takes place by the addition of Sulphate molecules and these modifications of proteins occurs at Tyrosine residues.

Tyrosine Sulfation accomplished via the activity of **tyrosylprotein sulfotransferase (TPST)** which take place in Golgi apparatus.

There are two known types of TPSTs which are **TPST-1** and **TPST-2**. The **universal sulfate donor** is 3'-phosphoadenosine-5'-phosphosulphate (PAPS).



## 5. Methylation

The transfer of **one-carbon methyl groups** to **nitrogen or oxygen** of amino acid side chains increases the **hydrophobicity** of the protein and can neutralize a negative amino acid charge when bound to carboxylic acids.

Methylation is mediated by **methyltransferases** and the **S-adenosyl methionine (SAM)** is the **primary methyl group donor**.



## 6. Hydroxylation

The biological process of adding **hydroxyl group** to a protein amino acid is called hydroxylation.

Protein hydroxylation is one type of PTMs that involves the conversion of  $-CH$  group into  $-COH$  group and these hydroxylated amino acids are involved in the regulation of some transcription factors.

Among the 20 amino acids, the two amino acids regulated by this method are **Proline** and **Lysine**.



## 7. Other PTMs

### a. SUMOylation.

**SUMO** (small ubiquitin related modifier), are 100 amino acid residue proteins which bind to the target protein in the same way as ubiquitin. They also have transcription regulatory activity of the protein and help in the transport of the target protein from cytosol to the nucleus.

### b. Disulfide bond formation.

It stabilizes protein structure and involved in redox processes.

### c. Lipidylation, Acetylation, Prenylation.



# Functions of PTMs

**The PTMs have significant biological functions which include:-**

1. Aids in proper protein folding.
2. Provides stability to the protein by **increasing protein half life**.
3. Protein sorting or translocation.
4. Regulates protein activity and function.
5. Protects the protein against cleavage by **proteolytic enzyme** via blocking the cleavage sites.



# Inhibitors of Translation

**Translational (protein synthesis) inhibitors** are substances that disrupt the processes that lead directly to the generation of new proteins in cells.

**Inhibition of translation occurs at different levels as below:-**

1. Protein synthesis inhibitors usually act at the **ribosome level**, taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures.
2. Protein synthesis inhibitors work at different stages of **prokaryotic mRNA** translation into proteins like initiation, elongation and termination.
3. Protein synthesis inhibitors targeting different stages of the mRNA translation.



# Inhibitors of Translation Significance

Since the inhibitors of translation act on different levels of protein synthesis. Therefore, antimicrobial drugs can be used to **treat bacterial infection in human**.

Also antimicrobial drugs can be changed if **resistance develops**.

## Antimicrobial mechanism of action :

1. Inhibits an enzyme involved in cell wall synthesis, protein synthesis or nucleic acid synthesis such as RNA polymerase and peptidyl transferase.
2. Disrupt cell membrane permeability. These antimicrobial or antibiotics tend to bind to cell membrane phospholipids or forms monovalent ion channels in the cell wall allows free movement of  $\text{Na}^+$  and  $\text{K}^+$  ions across the cell wall.
3. Interfere with cell wall synthesis, especially peptidoglycan synthesis.
4. Interfere with DNA synthesis via inhibiting the enzyme DNA gyrase or RNA polymerases.
5. Interfere with protein synthesis by blocking the ribosomal complex assemblage (30S and 50S subunits).



**Post translational modifications (PTMs)** refer to any alteration in the **amino acid** sequence of the protein after its synthesis.

PTMs → modification of the amino acid side chain, terminal **amino (N)** or **carboxyl (C)** group by means of covalent or enzymatic means.

PTMs → influence the **structure, stability, activity, cellular localization** or **substrate specificity** of the protein.

PTMs → occur in the **Endoplasmic Reticulum** of the cell but sometimes continue in the **Golgi apparatus** as well.

**Types of PTMs** → **Proteolysis, Phosphorylation, Glycosylation, Sulfation, Methylation, Hydroxylation** and addition of other groups.

### **Functions of PTMs**

1. Aids in proper protein folding.
2. Provides stability to the protein by **increasing protein half life**.
3. Protein sorting or translocation.
4. Regulates protein activity and function.
5. Protects the protein against cleavage by **proteolytic enzyme** via blocking the cleavage sites.



# KEY POINTS

**Translational (protein synthesis) inhibitors** are substances that disrupt the processes that lead directly to the generation of new proteins in cells.

1. Protein synthesis inhibitors usually act at the **ribosome level**, taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures.
2. Protein synthesis inhibitors work at different stages of **prokaryotic mRNA** translation into proteins like initiation, elongation and termination.
3. Protein synthesis inhibitors targeting different stages of the mRNA translation.

## **Translation inhibitors significance**

Since the inhibitors of translation act on different levels of protein synthesis. Therefore, antimicrobial drugs can be used to **treat bacterial infection in human**.

Also antimicrobial drugs can be changed if **resistance develops**.



## ***Lecture 9 Questions Practice***

### **Q1:- Define the followings:**

- a. Post translational modifications (PTMs).
- b. Translational (protein synthesis) inhibitors.

### **Q2:- Enumerate the followings:**

- a. Enumerate the functions of post translational modifications of a protein.
- b. Enumerate the different levels at which the protein translation inhibitors occur.

### **Q3:- MCQ:**

1. Glycosylation is the addition of carbohydrate molecules to the polypeptide chain and modifying it into:
  - a. Hemoglobin.
  - b. Glycoproteins.
  - c. Myoglobin.
  - d. Lipoproteins.
2. The universal Sulfate donor in protein Sulfation process is:
  - a. Tyrosylprotein sulfotransferase (TPST).
  - b. 3'-phosphoadenosine-5'-phosphosulphate (PAPS).
  - c. Tyrosylprotein sulfotransferase- 1 (TPST-1).
  - d. Tyrosylprotein sulfotransferase-2 (TPST-2).



# Molecular Biology

10<sup>th</sup> lecture

Repair of DNA,  
Types of DNA Damage and Repair

# OBJECTIVES



- Introduction
- Types of DNA Damage
- Repair Mechanism



# Introduction

The DNA is a very stable molecule. However, sometimes it can be damaged. Equally, DNA is able to maintain the integrity of information contained in it.

The continuation of genetic material from generation to generation depends on keeping the rates of mutation at low level. Additionally, DNA has many elaborate mechanisms to repair any damage or distortion.

The most frequent sources of damage to DNA are the **mistakes** in DNA replication and **chemical changes** in DNA. Malfunction of the process of replication can lead to **incorporation** of **wrong bases**, which are mismatched with the complementary strand.



# Introduction

The damage causing chemicals, **break** the **backbone of the strand** and **chemically alter the bases**. Alkylation, oxidation and methylation cause **damage to the bases**. X-rays and gamma radiations cause **single** or **double stranded breaks in DNA**.

A change in the sequence of bases if replicated and passed on to the next generation becomes **permanent** and leads to **mutation**.

However, mutations are also necessary to provide raw material for evolution. Without evolution, the new species, even human beings would not have risen. Therefore, a **balance** between **mutation** and **repair** is necessary.



# Types of DNA Damage

Damage to DNA includes any deviation from the usual double helix structure.

The damage types of DNA are:

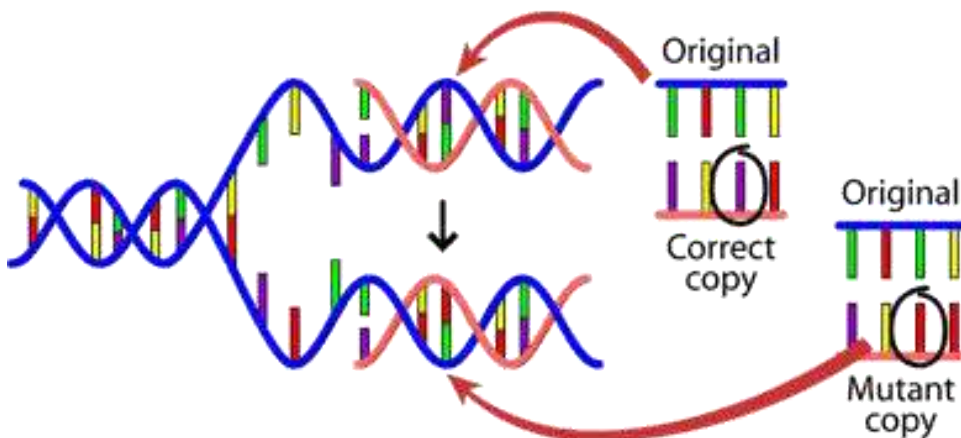
1. Simple Mutations.
2. Deamination.
3. Missing Bases.
4. Chemical Modification of Bases.
5. Formation of Pyrimidine Dimers (Thymine Dimers).
6. Strand Breaks.

# 1. Simple Mutations

Simplest mutations are **switching** of **one base** for another **base**. This includes:-

- a. **Substitution**, one pyrimidine is substituted by another pyrimidine and purine with another purine.
- b. **Trans-version**, involves substitution of a pyrimidine by a purine and purine by a pyrimidine such as T by G or A and A by C or T.
- c. **Deletion and insertion** of a single nucleotide or a small number of nucleotides.

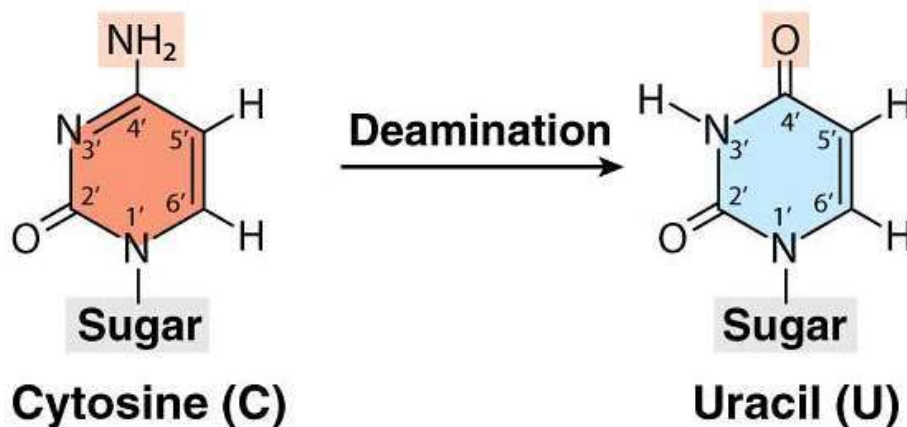
**Note:** Mutations that change a **single nucleotide** are called **point mutations**.



## 2. Deamination

The common alteration of DNA damage includes deamination of **cytosine (C)** to form **uracil (U)** which base pairs with adenine (A) in next replication instead of guanine (G) with which the original cytosine would have paired.

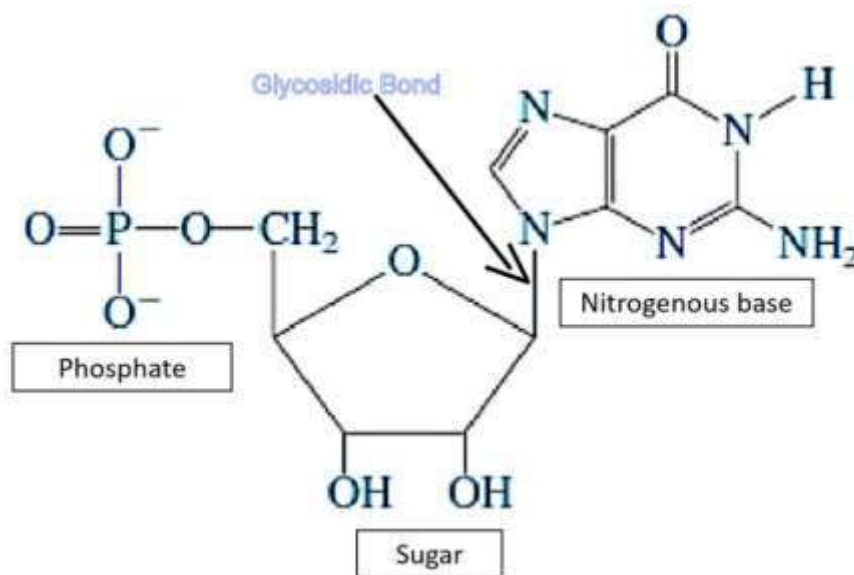
As uracil is not present in DNA, adenine (A) base pairs with thymine (T). Therefore C-G pair is replaced by A-T in next replication cycle.



### 3. Missing Bases

Cleavage of **N-glycosidic** bond between **purine** and **sugar** causes loss of purine base from DNA.

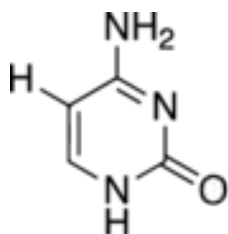
This is called **de-purination**. This **apurinic** site becomes **non-coding lesion**.



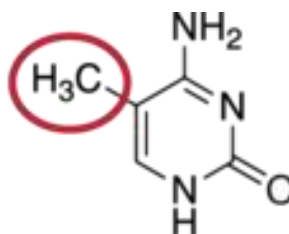
## 4. Chemical Modification of Bases

Chemical modification of any of the four bases of DNA leads to modified bases. Methyl groups ( $\text{CH}_3$ ) are added to various bases.

Guanine ( $\text{G}+\text{CH}_3$ ) forms 7-methylguanine, 3-methylguanine. Adenine ( $\text{A}+\text{CH}_3$ ) forms 3-methyladenine. Cytosine ( $\text{C}+\text{CH}_3$ ) forms 5- Methylcytosine.



**Cytosine**



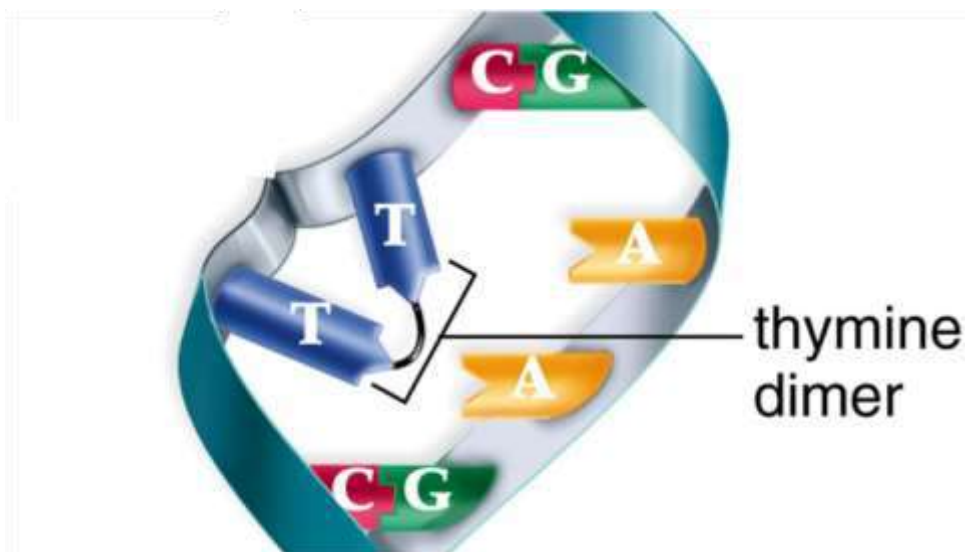
**methylated Cytosine**

Replacement of amino group by a ketone group ( $\text{R}_2\text{C}=\text{O}$ ) converts 5-methylcytosine to thymine.

## 5. Formation of Pyrimidine Dimer (Thymine Dimer)

Formation of thymine dimers is very common in which a covalent bond (**cyclobutyl ring**) is formed between adjacent thymine bases.

This leads to loss of base pairing with opposite strand. A bacteria may have thousands of dimers immediately after exposure to **Ultraviolet Radiations (UV)**.

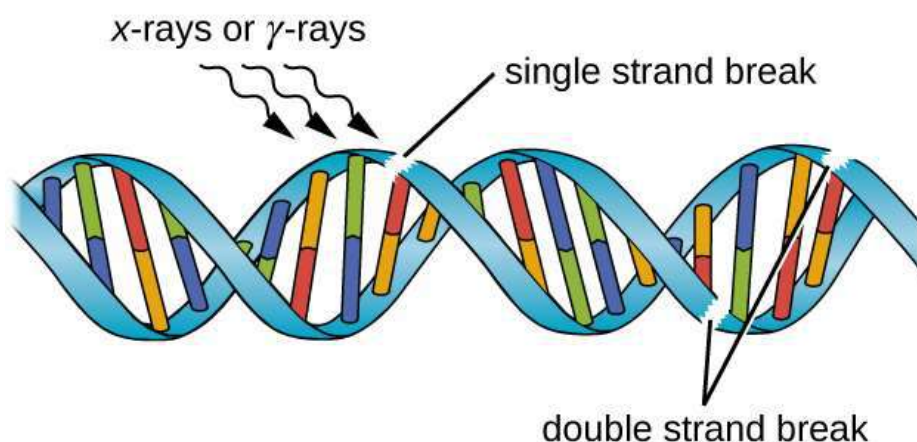


## 6. Strand Breaks

Sometimes **phosphodiester bonds** break in one strand of DNA helix. This is caused by various **chemicals** like **peroxides**, **enzymes** like **DNase** and **by various physical materials** like **radiation**. This leads to breaks in DNA backbone. Single strand breaks are more common than double strand breaks.

Sometimes **X-rays**, **electronic beams** and **gamma radiations** may cause **phosphodiester bonds** breaks in both strands which may not be directly opposite to each other. This leads to double strand breaks.

Some sites on DNA are more susceptible to damage, these are called **hot-spots**.





# Repair Mechanism

Most kinds of damage create **impairments** to **replication** or **transcription**. Altered bases cause **miss-pairing** and can cause **permanent alteration to DNA** sequence after replication.

In order to maintain the integrity of information contained in DNA; the DNA has various repair mechanisms.

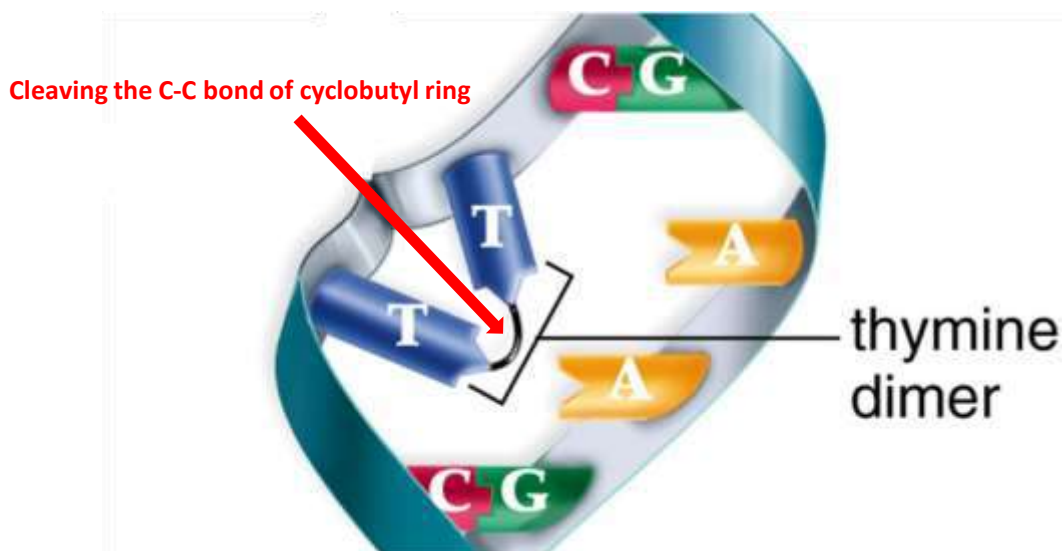
**The repair mechanisms include the following:-**

- 1. Direct repair.**
- 2. Excision repair.**
- 3. Mismatch base repair.**
- 4. Recombination Repair (Retrieval System).**
- 5. SOS Repair Mechanism.**

# 1. Direct Repair

The damage is reversed by a **repair mechanism** which is called **photo reactivation**. This mechanism involves a **light dependent enzyme** called **DNA photolyase**.

The enzyme is present in almost all cells of prokaryotes and eukaryotes. It uses energy from the **visible light** to cleave the **C-C bond of cyclobutyl ring** of the thymine dimers and the damage is repaired.





## 2. Excision Repair

It includes **base excision repair** and **nucleotide excision repair**:-

- a. **Base excision repair system**, involves **N-glycosylase enzyme** which recognizes the **abnormal base** and hydrolyses **glycosidic bond** between the base and the sugar.

**The base excision repair system includes three steps:-**

1. Incision is done by **endonuclease** cleaves the DNA backbone at the 5' end of the abnormal base.
2. Excision is done by **exonuclease** removes the abnormal base.
1. Synthesis is done by **DNA polymerase** replaces it with normal base and **DNA ligase** forms phosphodiester bonds which glue the ends of the newly added base.



## 2. Excision Repair

**b. Nucleotide repair system**, this repair system includes three steps:-

1. Incision is done by **endonuclease** precisely on both side of the damaged patch of the strand.
2. Excision is done by **exonuclease** to remove the damaged strand.
3. Synthesis is done by **DNA polymerase** using complementary strand as a template. Finally, **DNA ligase** forms phosphodiester bonds which glue the ends of the newly synthesized strand to the main DNA strand.



### 3. Mismatch Base Repair

Sometimes **wrong bases** are **incorporated** during replication process, **G-T** or **C-A** pairs are formed. The **wrong base** is always **incorporated** in the **newly synthesized strand only**.

Therefore, in order to distinguish the two strands for the purpose of repair, the **adenine bases** of the template strand are **labeled** by **methyl group**. In this way the newly replicated DNA helix is **hemi-methylated**. The excision of wrong bases occur in the non-methylated strand.



## 4. Recombination Repair (Retrieval System)

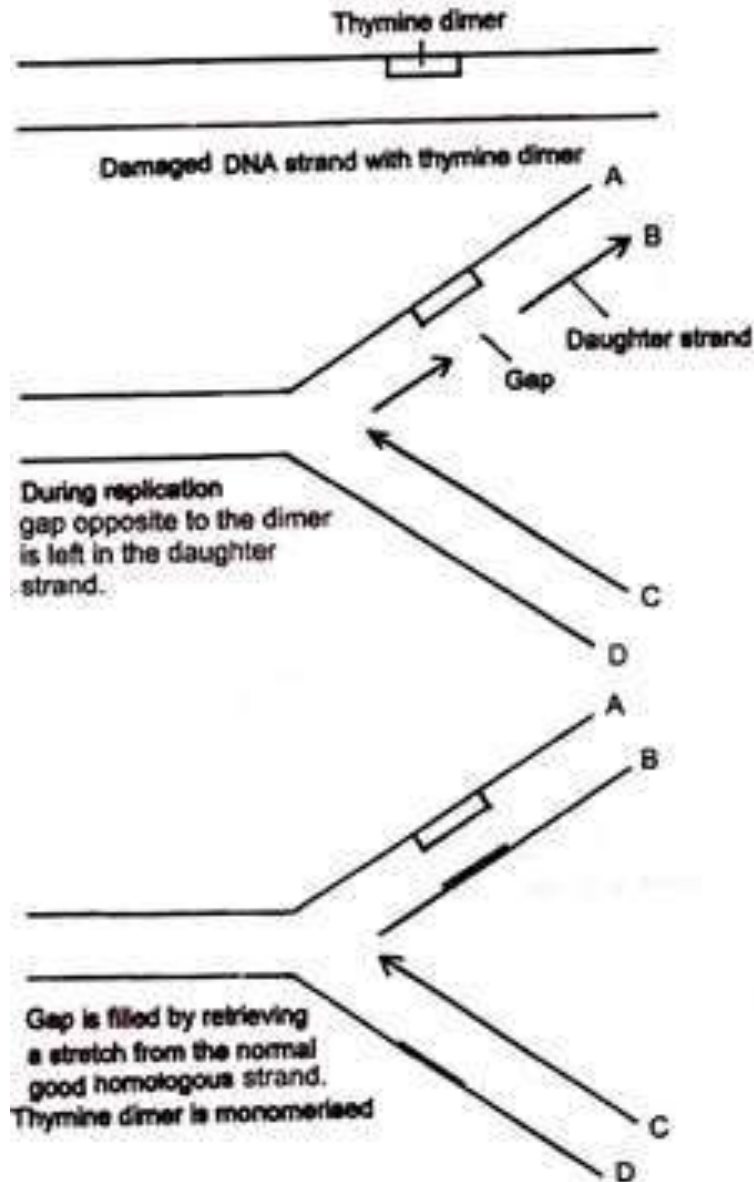
In thymine dimer or other type of damage, DNA replication cannot proceed properly. A **gap opposite to thymine dimer** is left in the newly synthesized strand. The **gap is repaired by recombination mechanism** or **retrieval system** that also called **sister strand exchange**.

During replication of DNA two identical copies are produced. Replicating DNA molecule has four strands **A, B, C** and **D**. Strands A and C have same DNA, sequence. Strands B and D also have same sequence as they are identical.

Thymine dimer is present in strand A. The replication fork passes the dimer as it cannot form hydrogen bonds with incoming adenine bases, thus creating a gap in the newly synthesized strand B.

In recombination repair system a **short identical segment of DNA** is retrieved from strand D and is inserted into the gap of strand B. But this creates another gap in strand D which is easily filled up by DNA polymerase using normal strand C as a template.

## 4. Recombination Repair (Retrieval System)





## 5. SOS Repair Mechanism

Sometimes the replicating machinery is unable to repair the damaged portion and bypasses the damaged site. This is known as **bypass system** or **trans-lesion synthesis** which is an **emergency repair system**.

This mechanism is catalyzed by a special class of DNA polymerases called **Y-family of DNA polymerases** which synthesized DNA directly across the damaged portion.



# KEY POINTS

**The damage types of DNA are:**

- 1. Simple Mutations.**
- 2. Deamination.**
- 3. Missing Bases.**
- 4. Chemical Modification of Bases.**
- 5. Formation of Pyrimidine Dimers (Thymine Dimers).**
- 6. Strand Breaks.**

**The repair mechanisms include the following:-**

- 1. Direct repair.**
- 2. Excision repair.**
- 3. Mismatch base repair.**
- 4. Recombination Repair (Retrieval System).**
- 5. SOS Repair Mechanism.**



## ***Lecture 10 Questions Practice***

### **Q1:- Define the followings:**

- a. Point mutation.

### **Q2:- Enumerate the followings:**

- a. Enumerate only the damage types of DNA.
- b. Enumerate the DNA repair mechanisms.

### **Q3:- MCQ:**

1. Photo reactivation repair mechanism involves a light dependent enzyme called:
  - a. DNA polymerase.
  - b. DNA photolyase.
  - c. RNA polymerase.
  - d. DNA ligase.
2. In SOS repair mechanism, DNA synthesized directly across the damaged portion via a special class of DNA polymerases called:
  - a. X-family of DNA polymerases.
  - b. Y-family of DNA polymerases.
  - c. Z-family of DNA polymerases.
  - d. None of the answers.



# Molecular Biology

11<sup>th</sup> lecture

## Gene Mutation And Chromosomal Aberrations

# OBJECTIVES



- **Gene Mutation**
- **Types of Gene Mutation**
- **Chromosomal Aberrations**
- **Types of Chromosomal Aberrations**
- **Differences Between Gene Mutation and Chromosomal Aberrations**



# Gene Mutation

**Gene mutation:** is a permanent alteration of the DNA sequence of the gene.

**Point mutation:** is a single base pair substitution that can change the genetic code of the gene, which can ultimately produce a different protein. Point mutations are the most common type of mutation.

**There are three types of point mutations as follows:-**

1. Silent mutation.
2. Missense mutation.
3. Nonsense mutation.

Gene mutations can also occur due to **insertion** or **deletion** of base pairs from the original gene sequence.

These mutations are vital due to their ability to change the template DNA and shift the reading frame, which decides the amino acid sequence of the protein.



## Types of Gene Mutation

**Heredity mutations:** are inherited from parent to offspring. These mutations are located in **gametes** of the parents such as ova cells and sperms. Hence they are referred to as **germ line mutations**. When gametes fertilize, zygote receives the gene mutations and passes to every cell of the offspring body.

**Acquired mutations:** occur in certain cells or certain period during the person's life. They are mainly caused due to environmental factors such as UV radiation or toxic chemicals, and happen mostly in somatic cells. Hence, acquired mutations are not passed to the next generation.



# Chromosomal Aberrations

Chromosomes are structures made up of long DNA strands tightly wound around histones and packed into chromatin. In human cell, there are 46 chromosomes in 23 pairs.

A chromosome contains thousands of genes, each gene is a specific region of a chromosome or specific DNA fragment which bears a genetic code to synthesize a protein.

Chromosomes and genes decide the genetic information of an organism. It is very important to keep them intact and precise. However, due to many reasons, chromosomes and genes can be mutated which can lead to various disease conditions.



# Chromosomal Aberrations

Chromosomal aberration is an **abnormal condition of the chromosome number and structure** that causes complications.

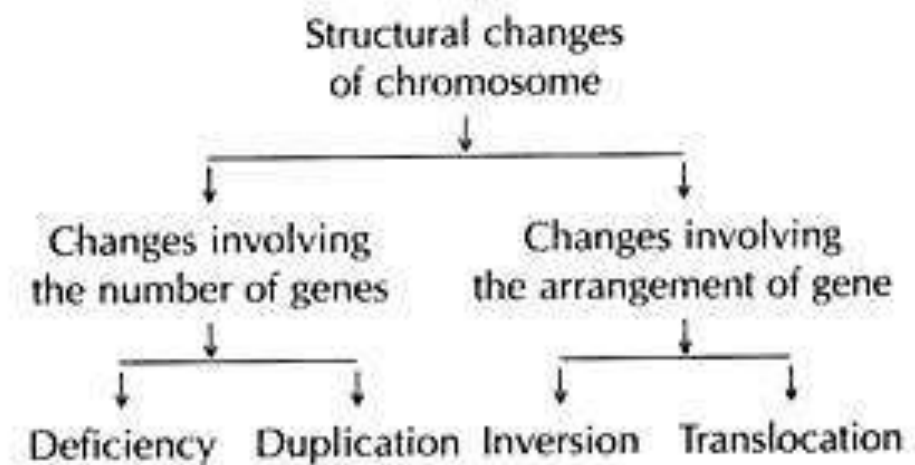
Gene mutation is a **permanent alteration** of the DNA sequence of the gene. The key difference between chromosomal aberration and gene mutation is that chromosomal aberration refers to a **change in a chromosome number or structure** while **gene mutation is an alteration of the sequence of the gene** which can cause changes in genetic code.

A chromosomal aberration always refers to a change in a **large segment** of a chromosome, containing more than one gene region.

# Types of Chromosomal Aberrations

**Chromosomal aberrations** also known as **chromosomal anomaly, chromosomal abnormality or chromosome mutation.**

It can occur due to a **loss, gain or rearrangement** of a **portion** of a chromosome (affects chromosome structure) or due to a **missing** (X0) or **extra** complete chromosomes (XXY) affects the chromosome number. These chromosomal abnormalities are also passed to the offspring (next generation).

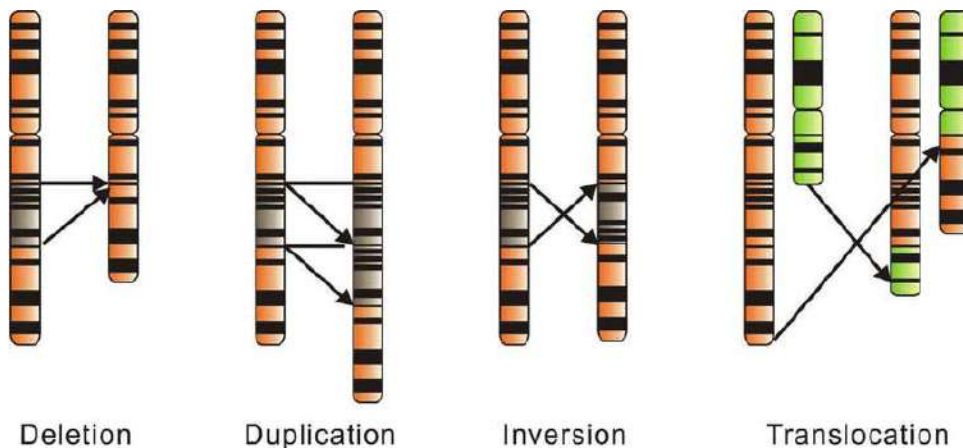


# Types of Chromosomal Aberrations

There are four main types of structural chromosomal aberrations:-

1. **Deletion**, when a big segment of chromosome is lost.
2. **Duplication**, when a segment of chromosome is repeated twice.
3. **Translocation**, when one segment of a chromosome is transferred into a non-homologous chromosome.
4. **Inversion**, when a segment of chromosome is changed at 180° rotation.

All these changes are responsible for the changes in the intact chromosome structure and overall genetic balance of the organism.





# Types of Chromosomal Aberrations

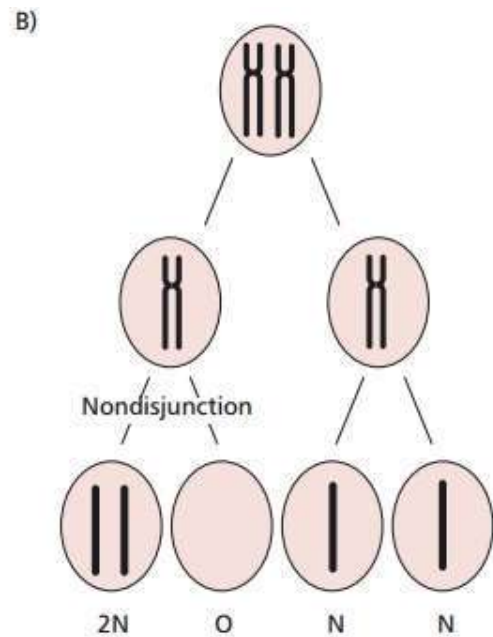
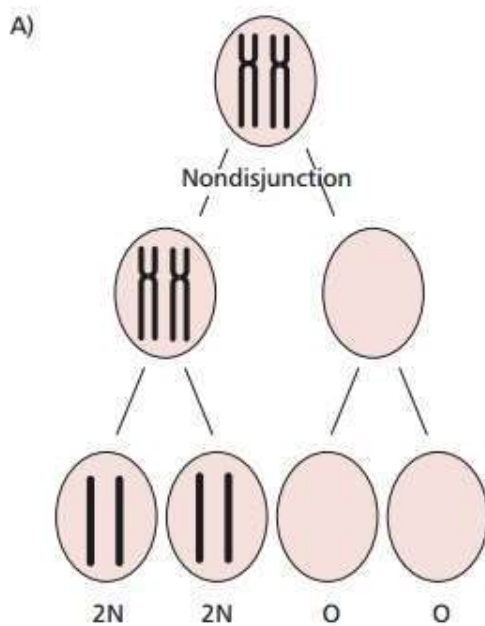
**Numerical chromosomal aberrations** mostly occur due to errors of cell division following meiosis or mitosis.

**Chromosomal nondisjunction** is the main reason for the abnormal number of chromosomes in gametes and in offspring. This situation is known as **aneuploidy** (presence of an abnormal number of chromosomes).

Some gametes are produced with missing chromosomes while some gametes have extra chromosomes. Both cases create offspring with an unusual number of chromosomes.

If the changes occur in **ova cells** or **sperms**, those anomalies are transmitted to every cell of the body. Chromosome anomalies can happen **randomly** or can be **inherited** from parents. The origin of these anomalies can be detected by performing chromosomal studies on both babies and parents.

# Types of Chromosomal Aberrations





# Differences Between Gene Mutation and Chromosomal Aberrations

## Gene Mutation

1. Gene mutation is an alteration that occurs in the DNA base sequence of a gene.
2. Gene mutation does not cause changes to the total number of chromosomes in an organism.
3. Gene mutation commonly refers to a single gene alteration.
4. Nucleotide damage is small in scale compared to chromosomal aberration. However, it can cause serious health problems.

## Chromosomal Aberration

1. Chromosomal aberration is any change in the number and structure of chromosomes in an organism.
2. Chromosomal aberration can change the total number of chromosomes in an organism.
3. Chromosomal aberration can include many gene alterations.
4. Damages due to chromosomal aberrations are large scale compared to gene mutation.



**Gene mutation:** is a permanent alteration of the DNA sequence of the gene.

**Point mutation:** is a single base pair substitution that can change the genetic code of the gene, which can ultimately produce a different protein. Point mutations are the most common type of mutation.

**There are three types of point mutations as follows:-**

1. Silent mutation.
2. Missense mutation.
3. Nonsense mutation.

### **Types of Gene Mutation**

**Heredity mutations or Acquired mutations.**

Chromosomal aberration is an **abnormal condition of the chromosome number and structure** that causes complications.

**Structural chromosomal aberrations or Numerical chromosomal aberrations.**



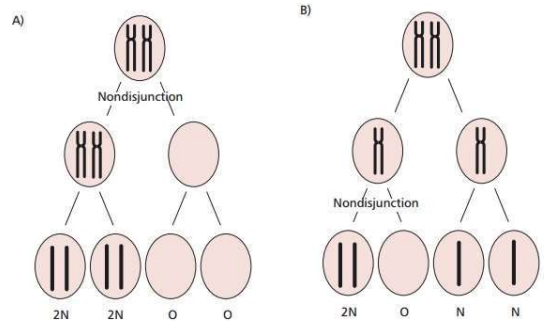
# KEY POINTS

There are four main types of structural chromosomal aberrations:-

1. Deletion.
2. Duplication.
3. Translocation.
4. Inversion.

## Numerical chromosomal aberrations

mostly occur due to errors of cell division following meiosis or mitosis.





# KEY POINTS

## Gene Mutation

1. Gene mutation is an alteration that occurs in the DNA base sequence of a gene.
2. Gene mutation does not cause changes to the total number of chromosomes in an organism.
3. Gene mutation commonly refers to a single gene alteration.
4. Nucleotide damage is small in scale compared to chromosomal aberration. However, it can cause serious health problems.

## Chromosomal Aberration

1. Chromosomal aberration is any change in the number and structure of chromosomes in an organism.
2. Chromosomal aberration can change the total number of chromosomes in an organism.
3. Chromosomal aberration can include many gene alterations.
4. Damages due to chromosomal aberrations are large scale compared to gene mutation.



## ***Lecture 11 Questions Practice***

### **Q1:- Define the followings:**

- a. Acquired mutation.
- b. Hereditary mutation.

### **Q2:- Enumerate the followings:**

- a. Enumerate the differences between gene mutation and chromosomal aberration.

### **Q3:- MCQ:**

- 1. Chromosomal ----- is the main reason for the abnormal number of chromosomes in gametes and in offspring.
  - a. None of the answers.
  - b. Nondisjunction.
  - c. Disjunction.
  - d. Junction.



# Molecular Biology

## 12<sup>th</sup> lecture

### Cause of Mutation- Chemical and Physical Agents

# OBJECTIVES

A hand is shown holding a blue horizontal line that extends across the width of the word 'OBJECTIVES'.

1. Learning about the chemical agents that can cause mutations.
2. Learning about the physical agents that can cause mutations.



# Introduction

Mutation is a process that produces a gene or chromosome that differs from the **wild type**.

It is most commonly defined as a **spontaneous permanent change** in a **gene** or **chromosome** which usually produces a **detectable effect** in the organism and is **transmitted** to the **offspring's**.

Mutated gene or chromosome results from a **mutational process** and the organism or cell whose changed phenotype is attributed to a mutation is said to be a **mutant**.

The mutation may result due to changes either on the gene or the chromosome itself. Therefore, generally mutation maybe:

1. **Gene mutation** where the allele of a gene changes.
2. **Chromosome mutation** where segments of chromosomes, whole chromosomes, or entire sets of chromosomes change.



# Causes and Mechanisms of Mutations

1. Errors in DNA replication.
2. Errors in DNA repair.
3. Environmental mutagen causes DNA damage that is not repaired correctly.
4. Transposons and insertion sequences (a mobile DNA elements that can move from one location in the chromosome to another or the DNA element may jump into a gene, thus, mutating it).
5. External Causes are mutagenic agents that damage DNA such as **chemical mutagens**, **physical mutagens** or biological mutagens.



# Agents of Mutations

1. Substances or agents which induce **artificial mutations** are called **mutagens** or **mutagenic agents**.
2. Mutagens may be of **physical, chemical** or biological origin.
3. Mutagens may **act directly on the DNA**, causing direct damage to the DNA, and most often result in replication error.
4. Some mutagens **may act on the replication mechanism** and chromosomal partition.
5. Many mutagens are **not mutagenic by nature**, but can form **mutagenic metabolites** through cellular processes.



# Physical Mutagens

## 1. Radiation.

- a. **Ionizing radiations** such as **X-rays, gamma rays** and **alpha particles** cause DNA breakage. Ionizing radiations cause breaks in **pentose-sugar-phosphate backbone** of DNA causing chromosomal mutations such as break, deletion, insertion, inversion and translocation.
- b. **Ultraviolet radiations** with wavelength above 260nm are absorbed readily producing **pyrimidine dimers**, which can cause **error in replication**.

## 2. Temperature.

The rate of all chemical reactions are influenced by **temperature**. It is reported that the **rate of mutation is increased** due to **increase in temperature**.

Temperature probably affects both **thermal stability** of DNA and the **rate of reaction of other substances** with DNA.



## Chemical Mutagens

1. **Reactive oxygen species (ROS)**, such as superoxide, hydroxyl radicals and hydrogen peroxide and many more of these highly reactive species which are generated by **normal cellular processes**.
2. **Metal ions**, such as Nickel, Cadmium, Chromium, Cobalt, Arsenic and Iron can cause mutations. They work via **producing ROS**, hindering the DNA repair mechanism causing DNA hyper-methylation or may directly damages the DNA.
3. **Deamination agents**, such as nitrous acid which can cause transition mutations via converting **cytosine** to **uracil**.



## Chemical Mutagens Continues,,

4. **Polycyclic aromatic hydrocarbon (PAH)**, these materials can bind to DNA and form **adducts** (direct addition of two or more molecule producing single reaction product) .
5. **Nitrosamines**, are an important group of mutagens found in **tobacco**, and may also be formed in **smoked meats and fish** via the interaction of amines in food with nitrites added as preservatives.
6. **Alkaloids** from plants, such as those from Vinca species, may be converted by **metabolic processes** into the **active mutagen** or **carcinogen**.



## Chemical Mutagens Continues,,

7. **Benzene**, an industrial solvent and precursor in the production of drugs, plastics, synthetic rubber and dyes.
8. **Alkylating agents**, such as Dimethyl sulphate (DMS), Ethyl methane sulphonate (EMS) and Ethyl ethane sulphonate (EES).
9. **Intercalating Agents**, there are certain dyes such as Acridine orange, Proflavine and Acriflavin which are three ringed molecules of similar structure as those of **purine** and **pyrimidine** pairs.

In aqueous solution these dyes can **insert themselves in DNA** (intercalate the DNA) between the bases in adjacent pairs by a process called **intercalation**. Therefore, these dyes distort the DNA and results in deletion or insertion after replication of DNA molecule.



## Significance of Mutations

1. Variants in genes (which are caused by mutations) are needed to study the transmission of traits.
2. Mutations can tell the researcher about the function of a gene product in a biological system.
3. Mutations are the basis for cancer and other genetic diseases.
4. Gene mutations serve as the source for most alleles in a population and is therefore the origin of genetic variation within a population.
5. Mutations drive evolution. Mutations are the raw material via which natural selection acts.



# KEY POINTS

- Gene mutation .
- Chromosome mutation.

## **Causes of Mutations**

- Errors in DNA replication.
- Errors in repair mechanism.
- Environmental mutagens.
- External causes → chemical agents & physical agents.

## **Agents of mutations**

- Artificial mutagens.
- Physical or chemical mutagens.
- Mutagens that act directly on DNA.
- Mutagens that act indirectly on replication mechanism.



# KEY POINTS

## **Physical agents**

- Radiation such as X-Ray, Gamma Ray, UV.
- Temperature.

## **Chemical agents**

- ROS, Metal ions, Deamination agents, PAH, Nitrosamines, Alkaloids, Benzene, Alkylating agents, Intercalating agents.

## **Significance of mutation**

- Variants in gene.
- Discover function of a gene.
- Basis for Cancer & genetic diseases.
- Source of most alleles.
- Drives evolution.



## ***Lecture 12 Questions Practice***

### **Q1:- Enumerate the followings:**

- a. Enumerate briefly the agents of mutation.
- b. Enumerate briefly the physical mutagens.

### **Q2:- MCQ:**

1. Alkaloids from plants, such as those from Vinca species, may be converted by metabolic processes into the active?
  - a. Mutagen.
  - b. Carcinogen.
  - c. Mutagens and carcinogens.
  - d. All the answers.
2. Alkylating agents that can cause mutation are?
  - a. Dimethyl sulphate (DMS).
  - b. Ethyl methane sulphonate (EMS).
  - c. Ethyl ethane sulphonate (EES).
  - d. All the answers.



# Molecular Biology

13<sup>th</sup> & 14<sup>th</sup> lecture

Recombinant DNA Technology

# OBJECTIVES



- Recombinant DNA technology.
- Steps of recombinant DNA technology.
- Applications of recombinant DNA technology.
- General idea about Gene Therapy.
- Advantages of recombinant DNA technology.



# Introduction

In almost all living organisms, the DNA is the most important molecule, it carries genetic information that serves as the basis for **development, growth, and reproduction** among other **important functions**.

Information contained in this molecule is first copied, via **transcription**, into **mRNA** before being **translated** to produce **proteins**.

Using this knowledge, scientists developed a new technology called, **Recombinant DNA technology** to **combine DNA molecules** from **two or more organisms** in order to **create a new molecule of DNA** that **consists of new genetic combinations**.



# Introduction

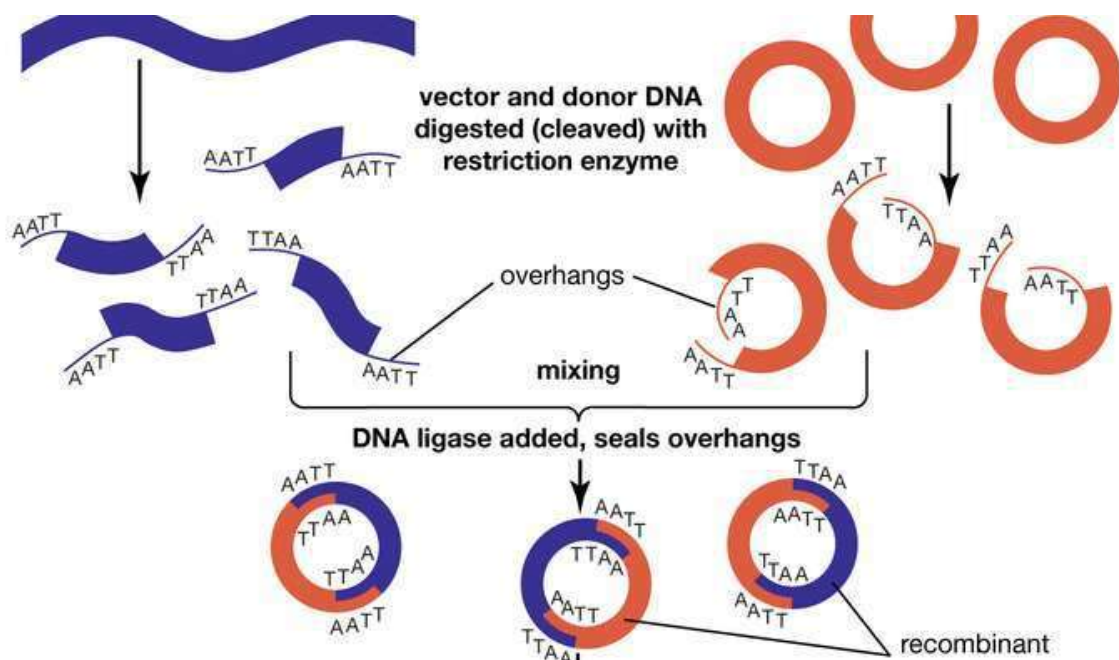
In **Recombinant DNA technology**, scientists isolate a **gene of interest** from **one organism** and **insert it** into a **vector** thereby forming a **recombinant DNA molecule**.

**Definition:** Recombinant DNA (rDNA) are molecules of DNA from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.

## Steps of Recombinant DNA Technology

Generally, recombinant DNA technology is a process that involves several important steps as follows:-

1. Isolation of the gene of interest (DNA sequence).
2. Insertion of the isolated gene into a vector.
3. Transformation- introduction of the modified vector into a host.
4. Selection of transformed host cells.
5. Expression of the gene introduced into the host.





# 1. Isolation of the Gene of Interest (DNA Sequence)

The main goal of recombinant DNA technology is to reproduce a gene (DNA sequence) that carries **genetic information of value** in **medicine, agriculture, and industries**.

In medical science, researchers are interested in **reproducing insulin** for patients with **Diabetes mellitus**.

Here, researchers have to identify the gene (**INS gene**) responsible for the production of **insulin** in **man** and isolate it in its **pure** form. In order to isolate the desired genes or a given sequence of the DNA molecule, it's first important to **obtain** DNA of the organism and **purify** the DNA from some of the other macromolecules like **lipids** and **proteins**.

Basically, this means that it's necessary to **break** the **cell open** in order to obtain the DNA. Depending on the cell type, different enzymes can be used.



# 1. Isolation of the Gene of Interest (DNA Sequence)

While **lysozyme** is normally used to **breakdown** the **cell wall** of a **bacterium**, **cellulase** is used to **breakdown** the **cell wall** of **plants** whereas **proteases** aid in the **removal** of **proteins** that are associated with the DNA. In addition, a variety of treatment methods are also used in the process of DNA purification.

Cells that do not have a cell wall as in most eukaryotic cells, **denaturing detergents** are used to lyse the cells in order to free the cell contents.

Detergents like **sodium dodecyl sulfate** is surface acting agents that disrupt the cell membrane allowing cell components to be released.

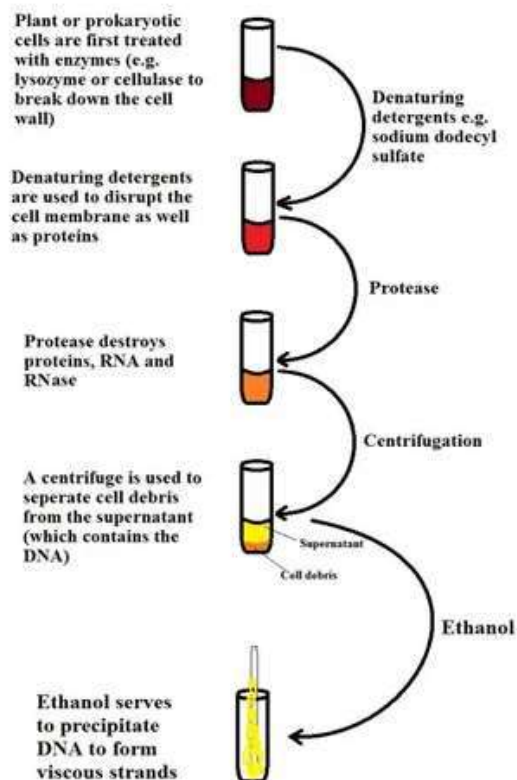
Once cell components released, they are treated with the enzyme **protease** which acts on and destroys **proteins**, **RNA** and **RNAs**. This is important as it **aids** in the **purification** of the **DNA**.

# 1. Isolation of the Gene of Interest (DNA Sequence)

Then a centrifuge is used to **precipitate cell debris** so that they settle at the bottom of the tube while the **supernatant (containing the DNA)** can be extracted.

Lastly, DNA is recovered through precipitation using ethanol. The enzyme **RNase** is also used during DNA purification to **degrade RNA**.

The figure below is a general representation of DNA isolation and purification:





# 1. Isolation of the Gene of Interest (DNA Sequence)

Once DNA is purified, restriction enzymes are used to isolate the gene of interest.

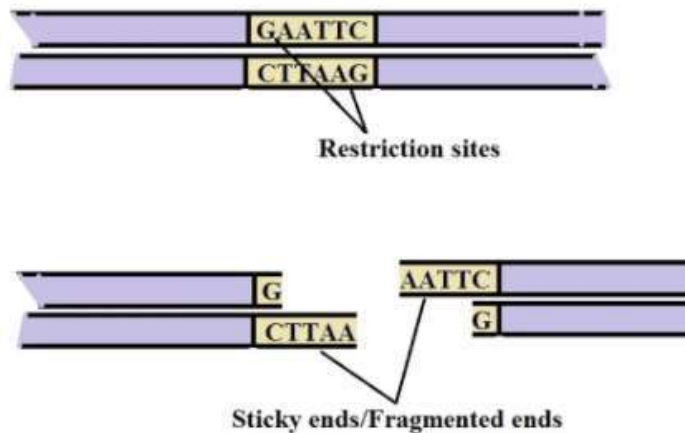
**Restriction enzyme:** is a type of enzyme that identifies a given sequence and cuts the DNA strand only at that particular site (the site with a specific nucleotide sequence e.g. INS gene).

This is achieved via the **incubation** of **purified DNA** with a given **restriction enzyme**, cutting the DNA molecule at specific points, at favorable conditions that promote enzymatic activities.

Restriction enzyme digestion may produce **DNA fragments** with **sticky ends** where a single strand tail extends at both ends of the fragment. These ends are also known as **cohesive ends** and consist of **base pairs** that ultimately pair-up with **complementary base pairs** of the **vector**.

# 1. Isolation of the Gene of Interest (DNA Sequence)

The figure below is a diagrammatic representation of the sticky ends:



The cohesive ends allow two fragments of DNA to be joined if they were produced by the same restriction enzyme. If the number of the desired gene is less than the number required for cloning, **polymerase chain reaction (PCR)** is used to produce more copies of the gene.

Apart from isolating genes of interest in the laboratory, they can also be obtained from **Genomic library** or **cDNA library**.



## 2. Insertion of the Isolated Gene into a Vector

The second step of recombinant DNA technology involves **inserting** the **genes isolated** in the first step into a suitable **vector**.

**Vector:** is a carrier that can carry the gene of interest into a given cell where it is replicated as the cell divides.

It's worth noting that the gene of interest isolated from the DNA molecule cannot be directly introduced into a cell. This is because it may be perceived as a **foreign material** and **destroyed**.

Therefore, a vector (e.g. plasmid or bacteriophage lambda) plays an important role in carrying the gene into the cell so that it can be replicated during normal cell division.



## 2. Insertion of the Isolated Gene into a Vector

**A vector to be used in recombinant DNA technology, it must have the following characteristics:-**

1. Able to self-replicating multiple copies.
2. Possess a promoter region, the region on the DNA that facilitates transcription of the target DNA.
3. Possess a cloning site, this refers to the restriction endonuclease cleavage site where foreign DNA fragment is inserted.
4. Have low molecular weight.
5. Easily isolates and purifies.



## 2. Insertion of the Isolated Gene into a Vector

In order to insert the DNA sequence/gene of interest into the vector, it's important that the ideal vector is selected and prepared. To prepare the vector and the gene of interest for insertion, two important enzymes are used, these are:-

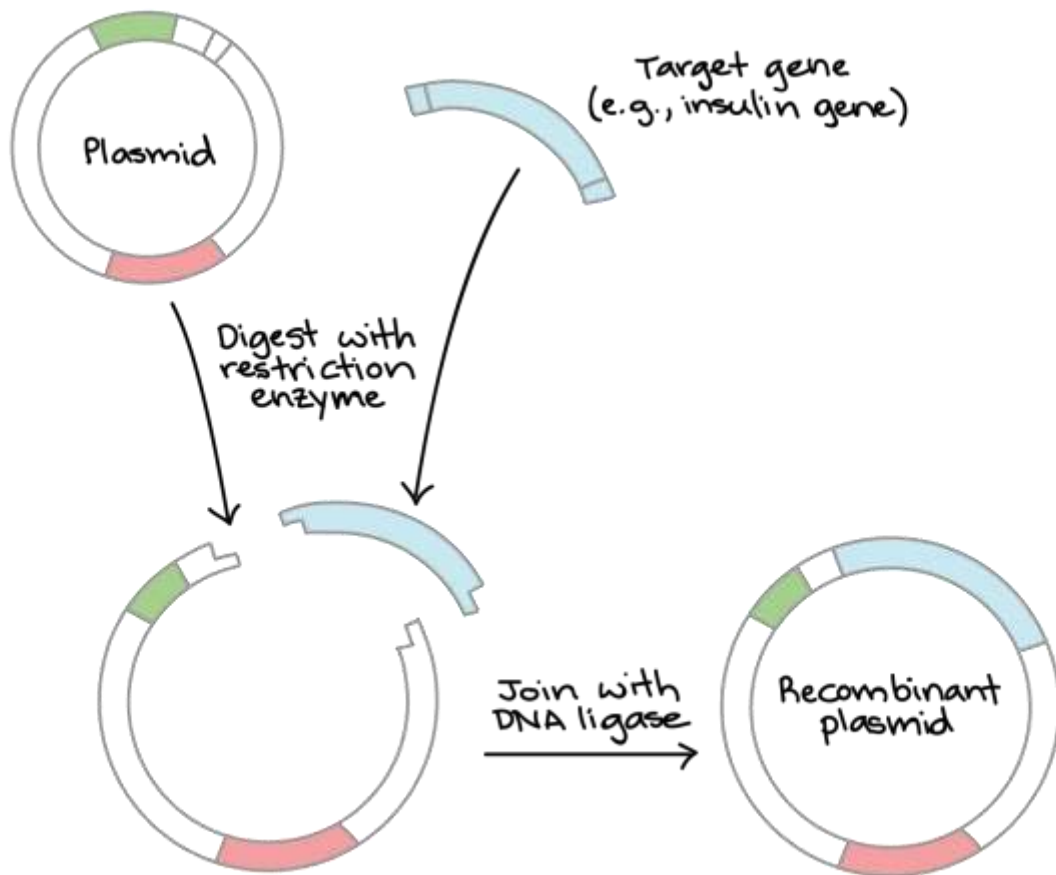
- a. **Restriction enzymes** that serve to cleave DNA at given sites having identified specific sequences of the DNA. One of the most common restriction enzymes is EcoRI.

In order for a given **vector** to join with **DNA fragment**, the two (**both the vector and the DNA fragment**) must be cleaved in a manner that produces complementary ends. This allows for complementary ends to be generated which ultimately join by pairing.

- b. **DNA ligase**, is a DNA joining enzyme. However, the two can only be joined if they have matching complementary ends.

## 2. Insertion of the Isolated Gene into a Vector

The figure below is a diagrammatic representation of restriction digestion and ligation process:





### 3. Transformation- Introduction of the Modified Vector into a Host

Transformation is the third step of recombinant DNA technology and involves the introduction of the recombinant DNA (modified plasmid) into the host cell (e.g. bacterium). The **primary aim** of this step is to **recover large amounts** of the DNA molecule.

One of the most commonly used hosts is the bacterium *E. coli*. This is because they are **inexpensive** to use and have **very fast growth**.

Although bacterial cells like *E. coli* are easy to use, they do not easily accept foreign genetic material. Therefore, these cells have to be treated in order to successfully introduce the new DNA.

### 3. Transformation- Introduction of the Modified Vector into a Host

One of the methods that was used to introduce the modified plasmid into the cell involved **cooling the cells and quickly heating them up**. This **created little pores** on the **cell membrane** through which the plasmids could enter the cell. However, over time, these pores will start closing up.

Currently, a number of new methods are being used to introduce vectors into the cells, these include:-

- a. **Microinjection.** This method involves the use of a glass needle (micro-capillary pipette) to directly introduce the new DNA material into the cell. Here, a micromanipulator (for precision) is used to direct the movement of the needle.
- b. **Electroporation.** In this method, an electrical field is applied in order to increase permeability of the cell membrane. This makes it easier for the modified plasmid to be inserted into the cell.
- c. **Ultrasound-mediated gene transfer.**
- d. **Silicon Carbide fiber mediated gene transfer.**
- e. **Calcium Chloride mediated transfer.**



## 4. Selection of Transformed Host Cells

The next step is selection of the host cells that contain the modified recombinant vector (modified plasmid).

Some of the cells take up plasmids that underwent self-ligation while others take up plasmids with unwanted genes whereas some do not take in any of the plasmids. In order to achieve the desired results, it's important to only **select cells that contain plasmids with the gene of interest.**

**Gel electrophoresis** is often used to separate pieces of DNA which in turn makes it possible to determine which plasmids took up the gene of interest.



## 5. Expression of the Gene Introduced into the Host

The last step of recombinant DNA technology is aimed at **increasing** the **production** of the **desired product**.

Generally, recombinant DNA technology is used to increase copies of a **given gene** in order to increase the production of a **given product**. Therefore, the host cells act as **bio-factories** in which the product is produced.

Under optimal conditions, the host cell continues dividing which not only allows for multiplication of the recombinant DNA but also increased production through gene expression. In this step, **bioreactors** are used for large scale production.



# Recombinant DNA Technology Applications

Recombinant DNA technology is used in a number of fields including:

- 1. Agriculture.** The recombinant DNA technology is used in agriculture to modify crops. This has proven beneficial in a number of ways including increasing crop yield, enhancing resistance to pests, and promoting the growth and development of given plants in areas where they would otherwise not grow.
- 2. Medicine.** In medicine, recombinant DNA technology is used for the production of various antibiotics, hormones, interferon, and vaccines. For instance, using *E. coli* bacteria as host cells, insulin is one of the most commonly produced hormones through recombinant DNA technology.
- 3. Industry.** In various industries, recombinant DNA technology is used for the purposes of producing different types of chemicals. Organic acids like citric acid are produced by microorganisms.



# Gene Therapy

**Gene therapy** involves the introduction of **certain genes** into the **genome** of an **individual** in order to **repair mutations**.

**Viruses** are intracellular parasites and so they have been used to introduce genes into the cell for gene therapy. In this case, the **disease causing part** of the **virus** is removed in order to prevent the development of disease and introduce the **therapeutic gene**.

Generally, the steps mentioned above are used to prepare the virus and use it to introduce the therapeutic gene into the cell. In the cell, the gene plays an important role in producing proteins or substances that are involved in the treatment.

**Primary goals** of gene therapy include **replacing mutated genes**, **repairing mutated genes** and **promote the destruction of diseased cells by the immune system**.



# Advantages of Recombinant DNA Technology

In recombinant DNA technology, genes with **better traits** are used for the purposes of **overcoming various limitations**.

1. **Plant pests resistance**, by introducing certain genes to plants, they become more resistant to certain pests and crop destruction by these pests is significantly reduced.
2. **Cancer treatment**, nowadays, cancer is one of the leading causes of death. Using recombinant DNA technology make it possible to treat cancer using effective genes.
3. **Development of vaccines**, recombinant DNA technology allow for the development of better vaccines that can be used to prevent a wide range of diseases affecting the global population.
4. **Food security**, recombinant DNA technology can be used to increase yields. This might be one of the most effective ways of ensuring food security across the world, and particularly in third world countries affected by food shortages and famines.



# KEY POINTS

**Definition:** Recombinant DNA (rDNA) are molecules of DNA from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.

## **Steps of recombinant DNA technology**

1. Isolation of the gene of interest (DNA sequence).
2. Insertion of the isolated gene into a vector.
3. Transformation- introduction of the modified vector into a host.
4. Selection of transformed host cells.
5. Expression of the gene introduced into the host.



# KEY POINTS

## 1. Isolation of the gene of interest (DNA sequence).

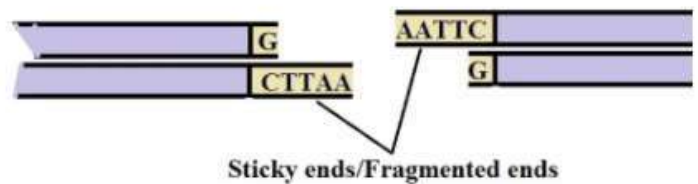
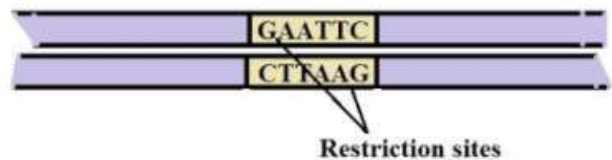
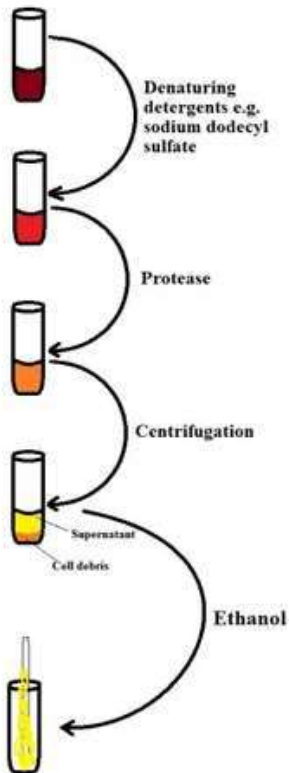
Plant or prokaryotic cells are first treated with enzymes (e.g. lysozyme or cellulase to break down the cell wall)

Denaturing detergents are used to disrupt the cell membrane as well as proteins

Protease destroys proteins, RNA and RNase

A centrifuge is used to separate cell debris from the supernatant (which contains the DNA)

Ethanol serves to precipitate DNA to form viscous strands



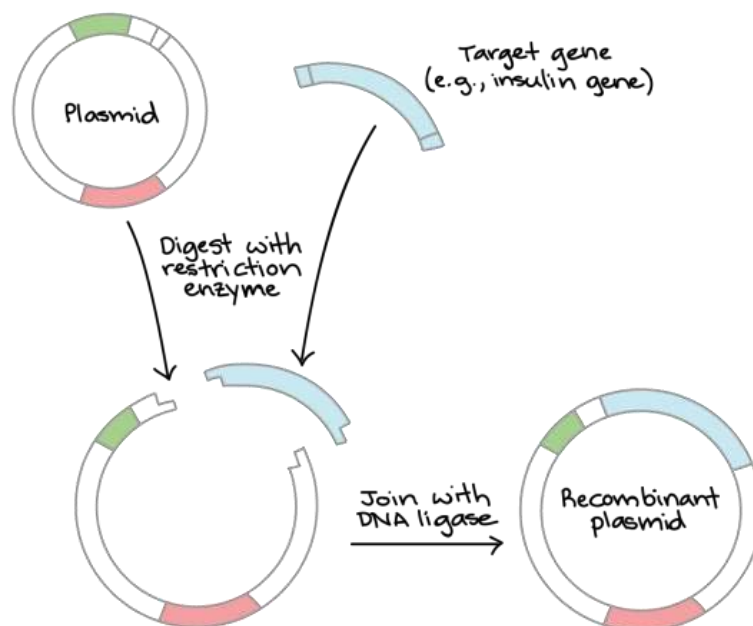


# KEY POINTS

## 2. Insertion of the isolated gene into a vector.

Vector characteristics:-

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2. Possess a promoter region.
3. Possess a cloning site, this refers to the restriction endonuclease cleavage site where foreign DNA fragment is inserted.
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# POINTS

### 3. Transformation- introduction of the modified vector into a host.

Methods used to introduce vectors into the cells:-

- a. **Microinjection.** This method involves the use of a glass needle (micro-capillary pipette) to directly introduce the new DNA material into the cell.
- b. **Electroporation.** In this method, an electrical field is applied in order to increase permeability of the cell membrane.
- c. **Ultrasound-mediated gene transfer.**
- d. **Silicon Carbide fiber mediated gene transfer.**
- e. **Calcium Chloride mediated transfer.**



# POINTS

## 4. Selection of transformed host cells.

It's important to only **select cells that contain plasmids with the gene of interest** via **gel electrophoresis**.

## 5. Expression of the gene introduced into the host.

- The host cells act as **bio-factories**.
- Bioreactors** are used for large scale production.

## Recombinant DNA technology applications

- Agriculture -Medicine -Industry

## Gene therapy

- replacing mutated genes
- repairing mutated genes
- promote the destruction of diseased cells by the immune system.



# KEY POINTS

## **Advantages of Recombinant DNA technology**

- Plant pests resistance.
- Cancer treatment.
- Development of vaccines.
- Food security.



## ***Lecture 13 & 14 Questions Practice***

### **Q1:- Define the followings:-**

- a. Gene therapy.
- b. Vector.

### **Enumerate the followings:**

- a. Enumerate briefly the recombinant DNA technology applications.
- b. Enumerate only the steps of recombinant DNA technology.

### **Q2:- MCQ:**

1. The second step of recombinant DNA technology involves inserting the genes isolated in the ----- into a suitable vector.
  - a. 1<sup>st</sup> step.
  - b. 2<sup>nd</sup> step.
  - c. 3<sup>rd</sup> step.
  - d. 4<sup>th</sup> step.
  - e. 5<sup>th</sup> step.
  - f. All the steps.
2. A type of microorganisms are intracellular parasites and so they have been used to introduce genes into the cell for gene therapy?
  - a. Bacteria.
  - b. Parasites.
  - c. Viruses.
  - d. Arthropods.



# Molecular Biology

## 15<sup>th</sup> lecture

### Role of Restriction Endonucleases, Plasmid and Cosmid Cloning Vectors

# OBJECTIVES



- Introduction of Restriction Endonucleases.
- Types of Restriction Endonucleases.
- Target Sites for Endonucleases.
- Nature of Cut Ends.
- Uses of Restriction Endonucleases.
- Cloning Vector Characteristics.
- Types of Cloning Vectors.



# Introduction

Restriction endonuclease enzymes occur naturally in bacteria as a **chemical weapon** against the invading **viruses**. They cut both strands of DNA when certain foreign nucleotides are introduced in the cell. Endonucleases break strands of DNA at internal positions in random manner.

**Restriction endonucleases:** are enzymes that recognizes a specific DNA base sequence (recognition sequence, recognition site, restriction sequence, restriction site) and cleaves both strands of DNA at or near that site.

The endonuclease cut the DNA, generating **restriction fragments** with **blunt** ends or **sticky** ends.



# Types of Restriction Endonucleases

## Restriction Endonucleases type I

These enzymes interact with an **unmodified recognition sequence** in double-stranded DNA and then attach to long DNA molecule. After travelling for distance between 1000 to 5000 nucleotides the enzymes cleaves only one strand of the DNA at an apparently random site, and creates a gap of about 75 nucleotides in length.

Type I restriction endonucleases are not useful for genetic engineering, because its **cleavage sites** are **non-specific**.

The cofactors for the enzyme are **Mg<sup>2+</sup> ions, ATP** and **S-adenosyl- methionine**.



# Types of Restriction Endonucleases

## Restriction Endonucleases type II

These enzymes recognize a **particular target sequence** in a double-stranded DNA molecule. They cleave the polynucleotide chain **within or near that sequence** to give rise to distinct DNA fragments of **defined length and sequence**. They require **Mg<sup>2+</sup> ions** for the action. Type II endonucleases are **used** for **gene manipulation** studies.

## Restriction Endonucleases type III

These enzymes cleave double-stranded DNA at **well-defined sites**. They require **ATP, Mg<sup>2+</sup> ions** and have very partial requirement for **S-adenosyl-methionine** for restriction. They have **intermediate properties** between Type I and Type II Restriction endonucleases.



## Target Sites for Endonucleases

A restriction endonuclease enzyme recognizes a specific recognition site (base sequence) on the DNA and makes a **cut** at this site **only**. These **target sites are four to six (4-6) nucleotides long**.

They exhibit **palindromic symmetry**, which means the nucleotide pair sequences are same reading forward or backward from a central axis of symmetry, like the phrase “madam I’m Adam” or the number 2002.

**The term palindromic has also been applied to sequences such as:**

5'-AGCCGA—

3'-TCGGCT—

both of which are palindromic strands.

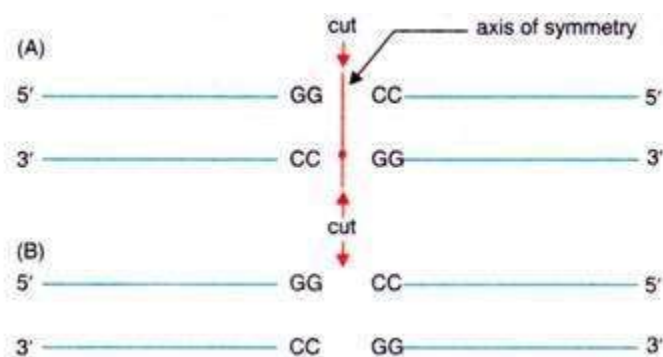
## Nature of Cut Ends

Two types of cut ends of DNA, **blunt** ends and **sticky** ends, are produced by the restriction endonucleases. The nature of these cut ends generated by the restriction endonucleases are very important in designing the gene cloning experiments.

### 1. Blunt cut ends:

In case of the blunt cut end, the enzyme makes a **simple double-stranded cut in the middle of the recognition sequence**. Thus the blunt ends are formed.

The **advantage** of generation of blunt end cuts during the joining of DNA fragments is that any pair of ends may be joined together irrespective of sequence. This is especially useful to join two defined sequences without introducing any additional material between them.

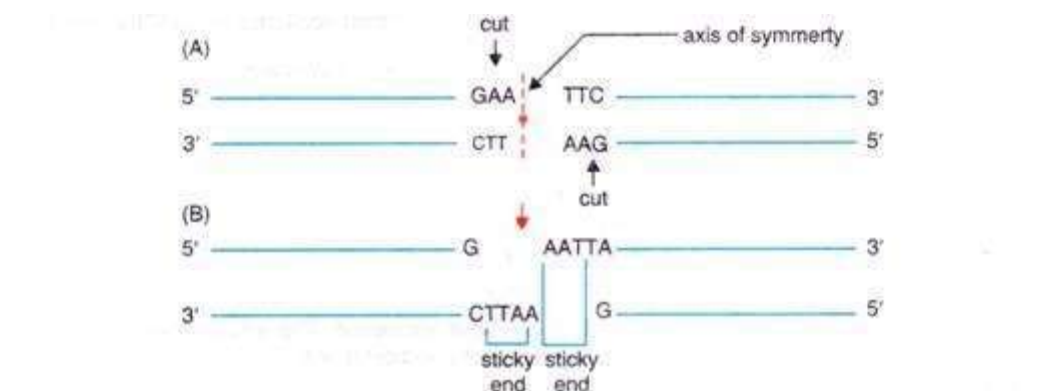


# Nature of Cut Ends

## 2. Sticky cut ends:

Many restriction enzymes make **staggered**, single-stranded cuts, producing **short single-stranded projections** at each end of the cleaved DNA, called sticky ends.

Since the restriction sites are symmetrical, so that both strands have the same sequence when read in the 5' to 3' direction. Thus, such staggered cuts will generate identical single-stranded projections on the either site of the cut.





# Uses of Restriction Endonucleases

In gene cloning experiments, DNA molecules have to be cut in a **very precise and reproducible manner**. Restriction endonuclease enzymes play an important role in cutting the **desired gene** as well as **cleaving** the **vector**.

## 1. Cutting the gene:

The required DNA fragment from a large DNA molecule should be cleaved in a precise manner for further genetic manipulations. A particular restriction endonuclease enzyme can recognize and bind to specific base sequence of the DNA and then will cleave it. It is highly reproducible and can be programmed according to DNA sequences of required gene and particular endonuclease enzymes identifying and cleaving it.

## 2. Cutting the vectors:

The function of a vector DNA molecule is to carry a gene of interest to a second organism where it can express it. During this technique the DNA to be cloned is integrated with the plasmid.

Hence each vector molecule should be cleaved with same restriction site at a single position to open the circular form so that the new DNA fragment can be inserted at these complementary sites.



# Cloning Vector Characteristics

**Cloning vector:** is a small DNA molecule capable of self-replication inside the host cell. Cloning vector is used for replicating donor DNA fragment within host cell.

## Characteristics of a cloning vectors

1. It must be small in size.
2. It must be self-replicating inside host cell.
3. It must possess restriction site for restriction endonuclease enzymes.
4. Introduction of donor DNA fragment must not interfere with replication property of the vector.
5. It must possess some marker gene such that it can be used for later identification of recombinant cell.
6. It must possess multiple cloning site.

# Types of Cloning Vectors

## 1. Plasmid (PBR322)

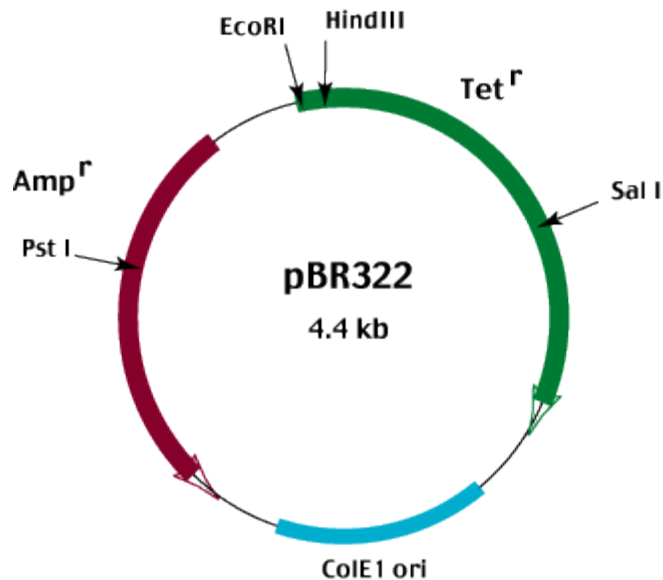
It is isolated from *E. coli*

Size: 4361 bp (**base pair**)

Cloning limit: 0.1-10 kb (**kilo base**)

Marker gene: Ampicillin and Tetracycline resistant gene

Restriction site for various restriction endonucleases



# Types of Cloning Vectors

## 2. Bacterial artificial chromosome (BAC):

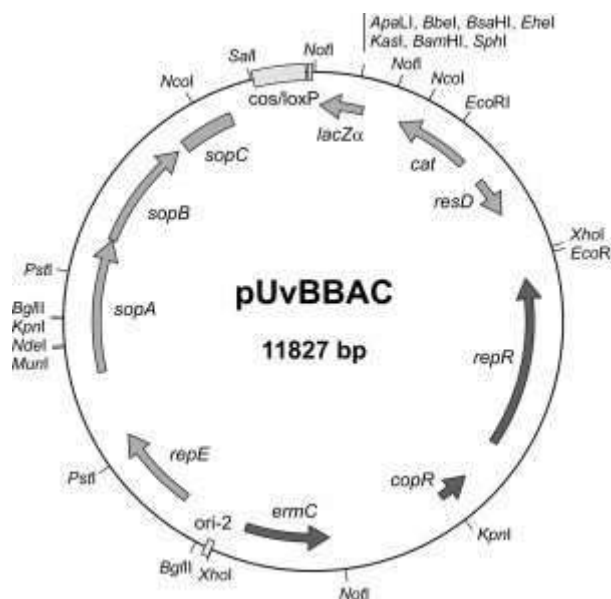
It is artificially synthesized plasmid

size: 11827 bp

It is modification of bacterial F-plasmid

Cloning limit: 35-300 kb

Marker gene: chloramphenicol resistant gene and lactose metabolizing gene (LacZ)



## Types of Cloning Vectors

### 3. Yeast artificial chromosome (YAC):

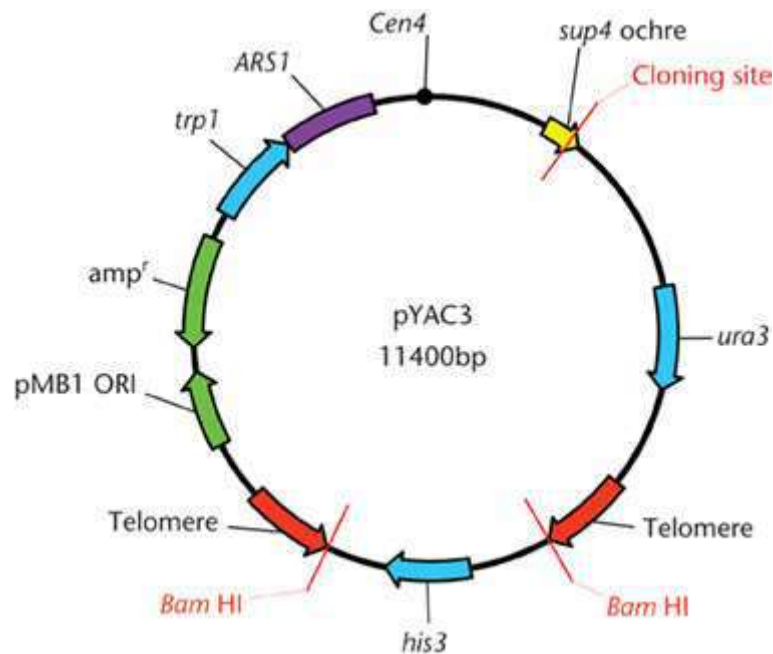
It is an artificial chromosome having yeast centromere isolated from *Saccharomyces cerevisiae* and ligated to bacterial plasmid

size: 11400 bp

It has telomere sequence

Marker: similar as for identification of yeast cell

Cloning limit: 100-1000 kb



# Types of Cloning Vectors

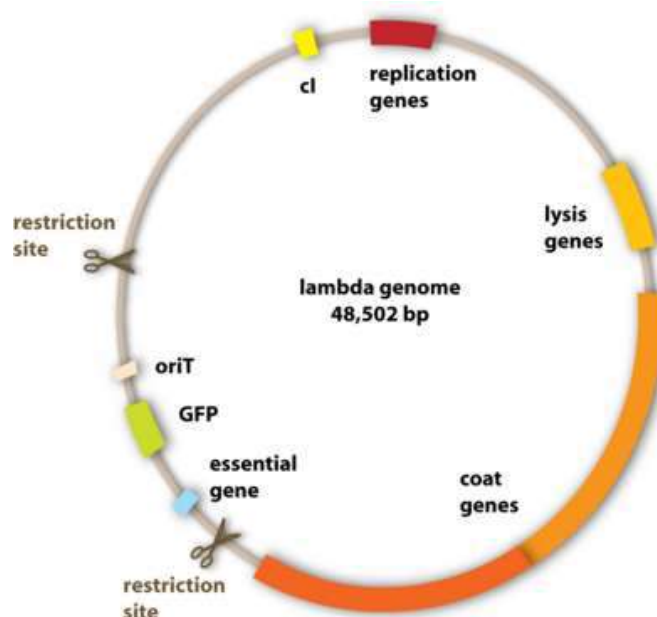
## 4. $\Lambda$ -Bacteriophage:

It is a phase (Virus) genome

size: 48502 bp

One third of the bacteriophage genome is non-essential, so that it can be cut, removed and replaced by donor DNA fragment during cloning.

It can recombinant only 4-5 Kbp of donor DNA fragment



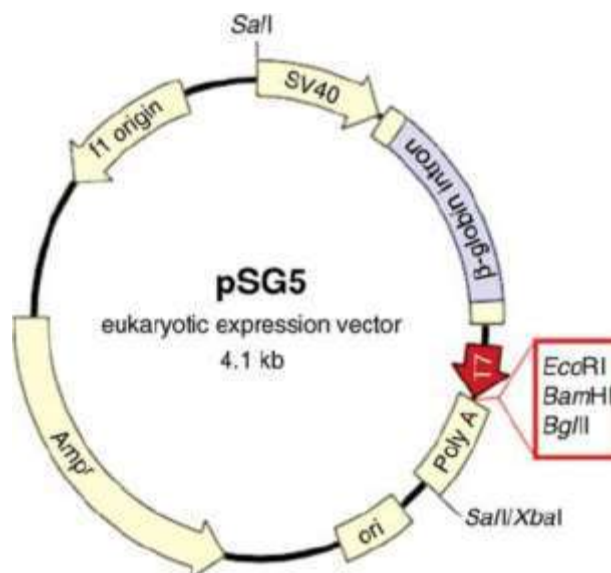
# Types of Cloning Vectors

## 5. Expression vector:

E.g. Eukaryotic expression vector pSG5

size: 4100 bp

An eukaryotic vector modified in such a way that it can be expressed in prokaryotic cell known as expression vector.



# Types of Cloning Vectors

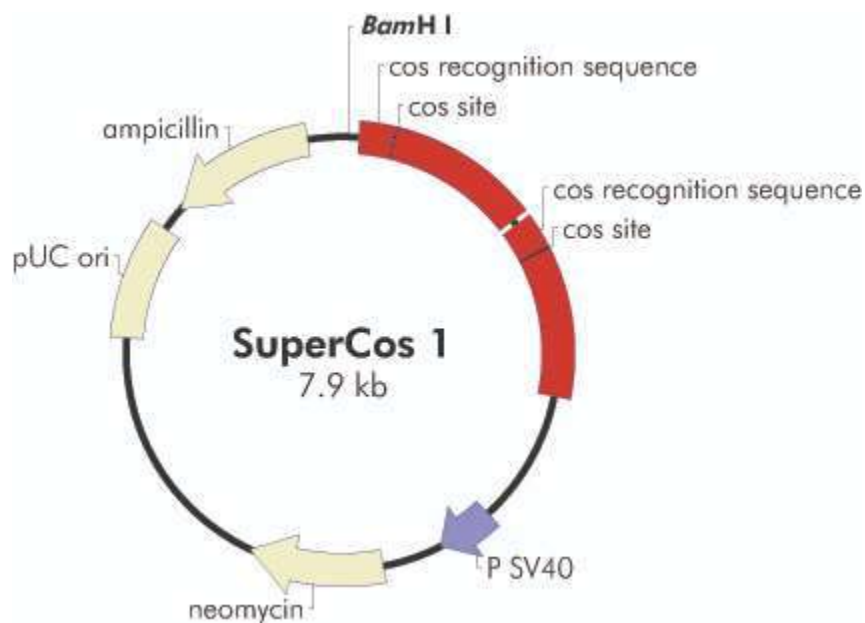
## 6. Cosmid:

Example: super COS1

size: 7900 bp

It has combined feature of both phage and plasmid

Cloning limit: 30-50 kb

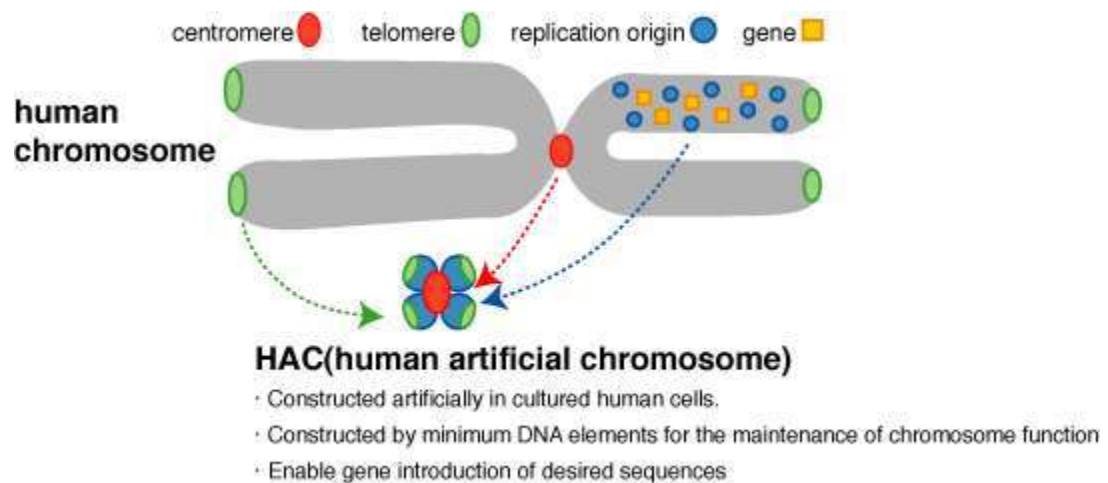


# Types of Cloning Vectors

## 7. Human artificial chromosome (HAC):

It is artificially synthesized chromosome used to transfer human gene

Cloning limit; No limit, it can carry large segment of DNA





# KEY POINTS

**Restriction endonucleases:** are enzymes that recognizes a specific DNA base sequence (recognition sequence, recognition site, restriction sequence, restriction site) and cleaves both strands of DNA at or near that site.

**Types of Restriction Endonucleases.**

**Type I, Type II & Type III.**

**Target Sites for Endonucleases.**

RE recognizes a specific recognition site (base sequence) on the DNA and makes a **cut** at this site **only**. These **target sites are four to six (4-6) nucleotides long**.

They exhibit **palindromic symmetry** Madam I'm Adam or 2002.

**Nature of Cut Ends.**

1. **Blunt cut ends.**
2. **Sticky cut ends.**



# KEY POINTS

## Uses of Restriction Endonucleases.

1. Cutting the gene.
2. Cutting the vectors.

## Cloning Vector Characteristics.

Small in size.

Self-replicating.

Possess restriction site.

Introduction of donor DNA fragment.

Possess some marker gene.

Possess multiple cloning site.

## Types of Cloning Vectors.

Plasmid

BAC

YAC

$\lambda$ -Bacteriophage

Expression vector

Cosmid

HAC



## ***Lecture 15 Questions Practice***

### **Q1:- Define the followings:**

- a. Restriction endonuclease.

### **Q2:- Enumerate the followings:-**

- a. Enumerate briefly, the types of restriction endonucleases.
- b. Enumerate only, the types of cloning vectors.

### **Q3:- MCQ**

1. Many restriction enzymes make staggered, single-stranded cuts, producing short single-stranded projections at each end of the cleaved DNA that called?
  - a. Blunt ends.
  - b. Dead ends.
  - c. Sticky ends.
  - d. Non-sticky ends.

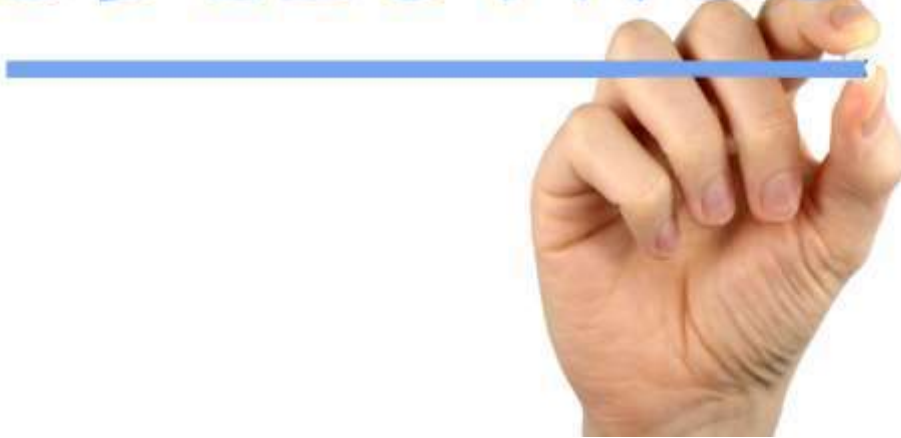


# Molecular Biology

## 16<sup>th</sup> lecture

### Brief Outline of Molecular Cloning

# OBJECTIVES



- Introduction.
- Molecular Cloning Techniques.
- Steps of Molecular Cloning.



# Introduction

**Molecular cloning:** is the collection of experimental procedures required to isolate and expand a specific fragment of DNA into a host organism in order to create a large number of identical copies.

**Molecular cloning:** is the set of experimental techniques used to generate a population of organisms carrying the same molecule of recombinant DNA.

Molecular cloning allowing the study of a single DNA sequence of interest. It is a powerful technique that permits the generation of complex combinations of DNA fragments for the most disparate applications.



# Introduction

Molecular cloning process is key to most modern **biomedical basic research** studies and **powerful tool** in **translational applications**.

Cloning genes is now **so simple** and **efficient** that it has become a **standard laboratory technique**.

This is first **assembled *in vitro*** and then **transferred** to a **host organism** that can direct its **replication** in **coordination** with its **growth**. This is usually achieved in an **easy-to-grow, non-pathogenic** laboratory bacterial strain of ***Escherichia coli***.



# Introduction

A single modified *E. coli* cell carrying the desired **recombinant DNA** can easily be grown in an exponential fashion to generate virtually **unlimited identical copies** of this **DNA**.

Molecular cloning can be seen as an *in vivo* PCR reaction, in which a desired piece of DNA can be isolated and expanded. However, molecular cloning allows more **flexibility, better fidelity, higher yields, and lower costs** than a PCR.

Development of molecular cloning techniques started with the discovery of bacterial enzymes known as **restriction endonucleases**.

These restriction endonucleases allow researchers to break up large DNA fragments into smaller pieces that are then joined with other DNA molecules (vectors) using an enzyme called **DNA ligase**.



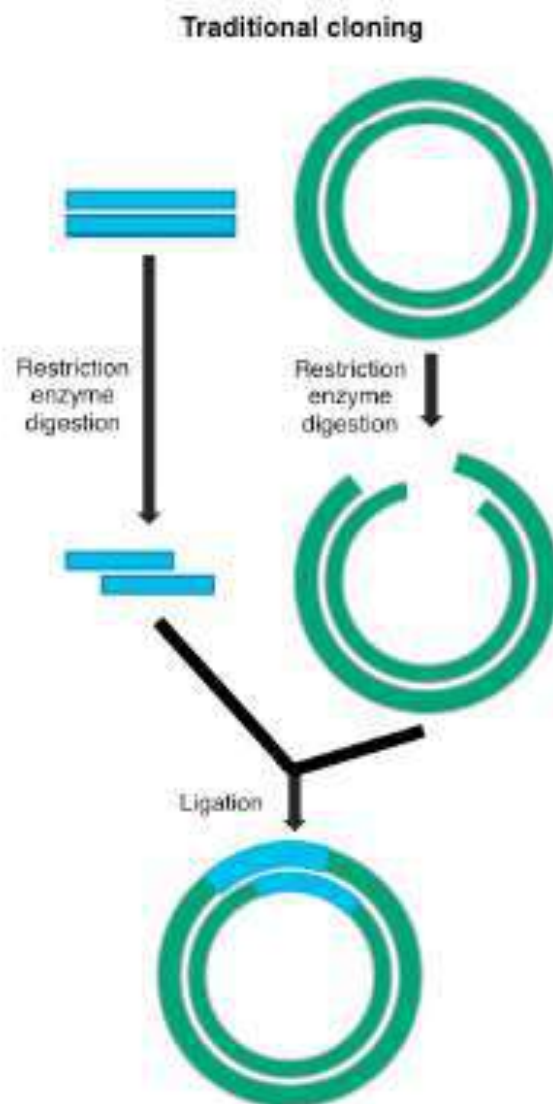
# Molecular Cloning Techniques

1. **Cut-and-paste cloning technique:** is commonly referred to as **traditional** or **conventional cloning** and it still widely used today.

Restriction endonucleases generate either **sticky ends**, in which the DNA fragment has a single-stranded overhang (either on the 3' or 5' ends), or **blunt ends**, in which no overhang is present.

For a sticky end fragment ligation to be successful, the two overhangs to be joined must have complementary base pairing. However, this is not a requirement of a blunt end ligation, making it much more flexible. On the other hand, blunt end ligation is much less efficient than sticky end ligation due to a lack of binding stability of the two fragments.

# Molecular Cloning Techniques

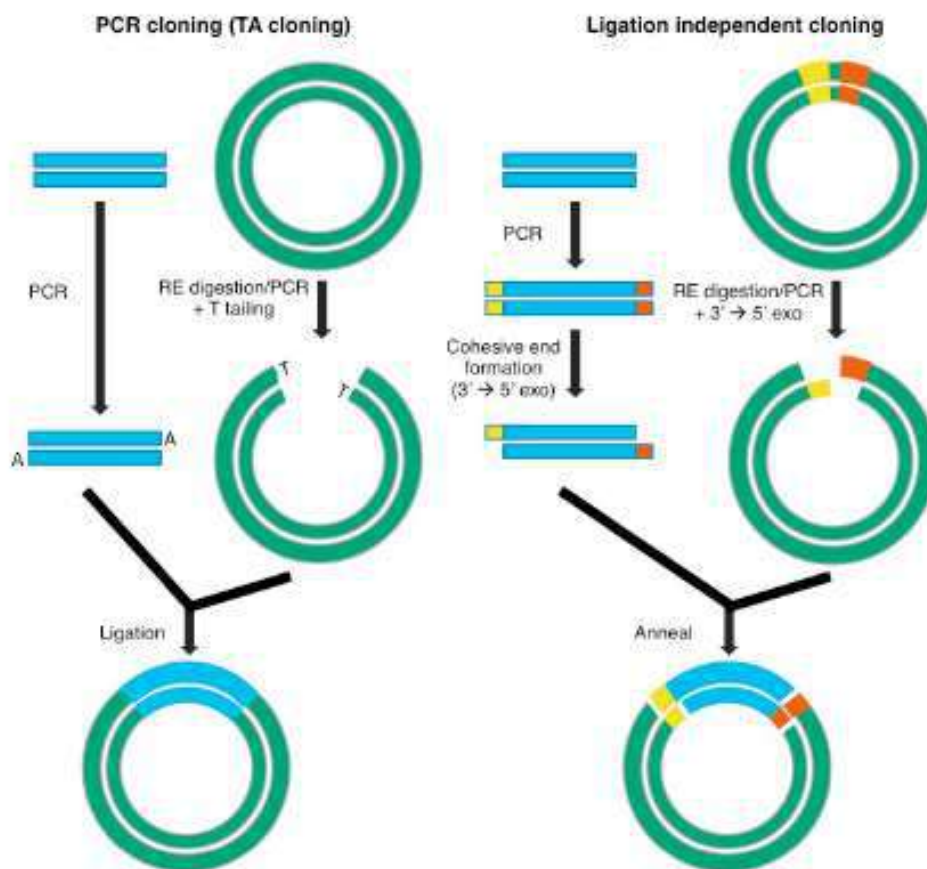




# Molecular Cloning Techniques

2. **PCR cloning technique:** it involves the direct ligation of a PCR-generated DNA fragment without using restriction enzymes to cut the insert.
3. **Ligation independent cloning (LIC) technique:** is usually carried out by adding short sequences of DNA to the fragment to be cloned that are homologous to the destination vector (this is easily accomplished using modified primers during the PCR amplification). Complementary cohesive ends between the vector and insert are then formed using enzymes with 3' to 5' exonuclease activity (which digest back 3' end to create 5' end overhangs), and the resulting two molecules are then mixed together and annealed. The resulting plasmid has four single-stranded DNA incisions that are efficiently repaired by the host organism. Importantly, the resulting product does not contain any new restriction enzyme sites, nor other unwanted sequences, and is therefore *scar-free*.

# Molecular Cloning Techniques



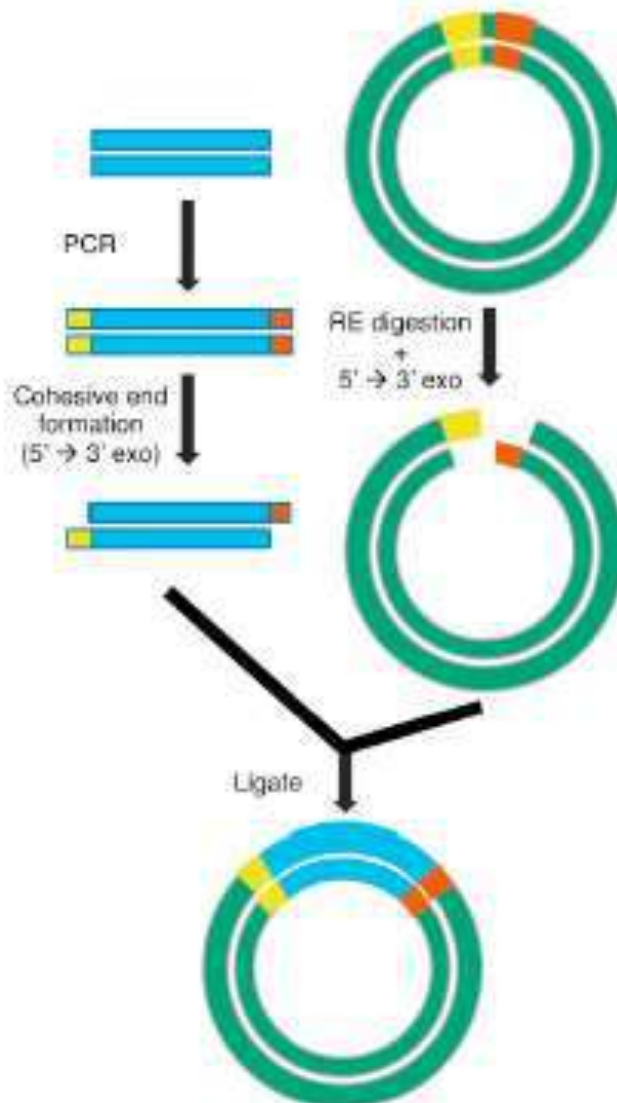


# Molecular Cloning Techniques

4. **Seamless cloning technique:** is a group of techniques that allow sequence-independent and scar-free insertion of one or more DNA fragments into a vector. The most well known of these methods is the **Gibson Assembly Method**, in which up to **ten fragments** can be easily combined. Similarly to LIC, this relies on the addition of regions of homology at each end of the fragments to be cloned. Then, the combined action of an exonuclease (which digest back 5' end to create 3' ends compatible overhangs), a DNA polymerase (which fills in gaps in the annealed fragments), and a DNA ligase (which seals the incisions in the assembled DNA) allows the generation of the recombinant DNA.

# Molecular Cloning Techniques

## Seamless cloning (Gibson cloning)





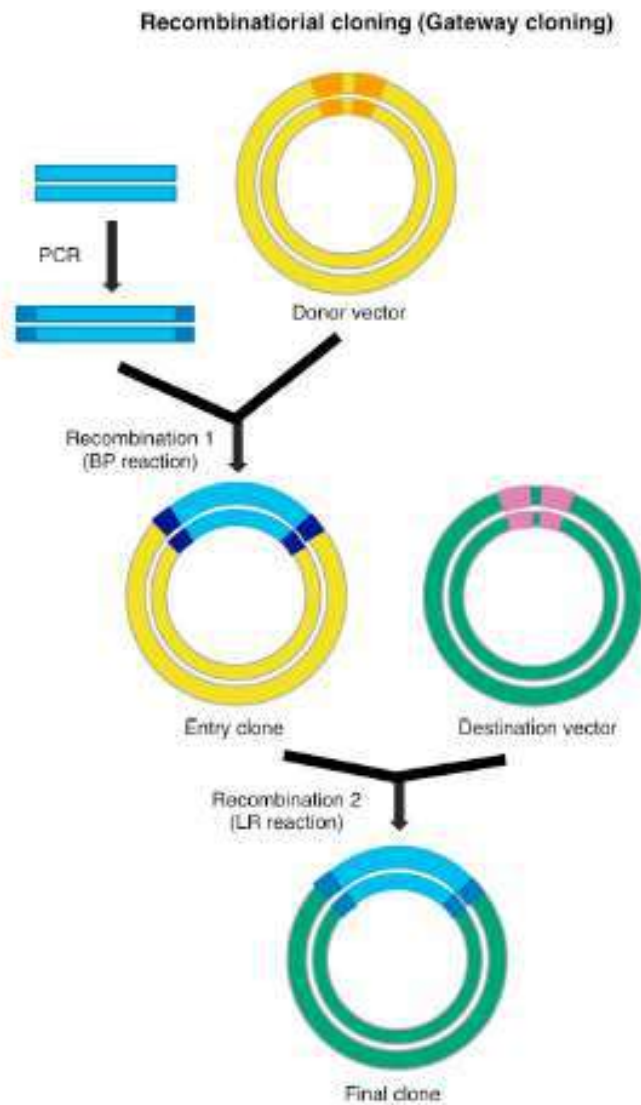
# Molecular Cloning Techniques

5. **Recombinatorial cloning technique:** it uses site-specific DNA recombinases enzymes capable of **swapping pieces** of DNA between two molecules containing the appropriate sequences (known as recombination sites).

The most widely used system in this category is the **Gateway Cloning System**, which relies on two proprietary enzyme mixes (**BP Clonase** and **LR Clonase**) to swap a DNA fragment across various recombination sites.

First the appropriate recombination sites are inserted by PCR on either side of the insert to be cloned, and then this is recombined with a **donor vector** to create an **entry clone**. This entry clone is recombined again with a **destination vector** (the required final vector) to make the final construct.

# Molecular Cloning Techniques





# Steps of Molecular Cloning

**A traditional molecular cloning experiment can be divided into nine steps:**

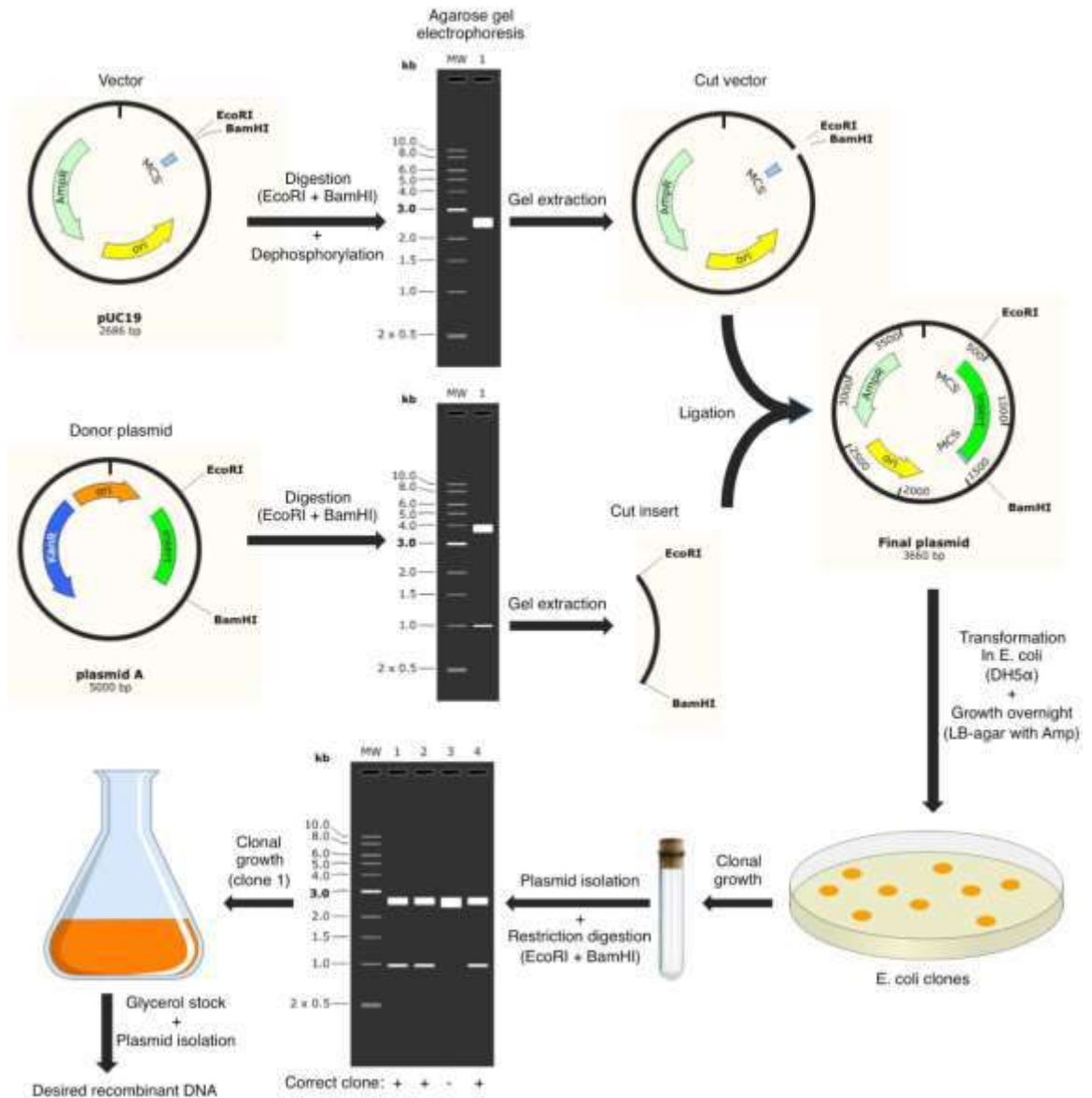
1. Selection of the host organism.
2. Selection of cloning vector.
3. Preparation of the vector.
4. Preparation of the insert.
5. Generation of the recombinant DNA.
6. Introduction of the recombinant DNA into the host organism.
7. Selection of the clones of organisms containing the vectors.
8. Screening for clones with the desired recombinant DNA molecules.
9. Expansion and isolation of the recombinant DNA.



## Steps of Molecular Cloning

Importantly, before performing a cloning experiment it is always recommended to perform an ***in silico* simulation** of the procedure using dedicated software for DNA sequence manipulation (several free and commercial options are available). This same software is also useful to align DNA sequences and create high-quality plasmid maps.

# Steps of Molecular Cloning





# KEY POINTS

**Molecular cloning:** is the collection of experimental procedures required to isolate and expand a specific fragment of DNA into a host organism in order to create a large number of identical copies.

**Molecular cloning:** is the set of experimental techniques used to generate a population of organisms carrying the same molecule of recombinant DNA.

1. **Cut-and-paste cloning technique.**
2. **PCR cloning technique.**
3. **Ligation independent cloning (LIC) technique.**
4. **Seamless cloning technique.**
5. **Recombinatorial cloning technique**



# KEY POINTS

1. Selection of the host organism.
2. Selection of cloning vector.
3. Preparation of the vector.
4. Preparation of the insert.
5. Generation of the recombinant DNA.
6. Introduction of the recombinant DNA into the host organism.
7. Selection of the clones of organisms containing the vectors.
8. Screening for clones with the desired recombinant DNA molecules.
9. Expansion and isolation of the recombinant DNA.



## ***Lecture 16 Questions Practice***

### **Q1:- Define the followings:**

- a. Molecular cloning.

### **Q2:- Enumerate the followings:-**

- a. Enumerate molecular cloning techniques.
- b. Enumerate the steps of molecular cloning.

### **Q3:- MCQ**

1. Gibson Assembly Method is type of?
  - a. Cut-and-paste cloning technique.
  - b. PCR cloning technique.
  - c. Ligation independent cloning (LIC) technique.
  - d. Seamless cloning technique.
  - e. Recombinatorial cloning technique.



# Molecular Biology

17<sup>th</sup> lecture

## Applications of Recombinant DNA Technology

# OBJECTIVES



- Introduction.
- Applications of rDNA technology in Medicine.
- 1. Medical diagnosis of diseases.
- 2. Gene therapy.
- 3. Production of vaccines.



# Introduction

The **rDNA technology** is an important technique of **biotechnology** because it can be applied in medicine and genetics. In **medicine**, any **diseases** can be treated with this technology and new **hormones** can also be produced.

Recombinant DNA technology is the technique of **genetic engineering** in which recombinant DNA is prepared by **cutting** the **DNA** into **small fragments** and **joining different fragments** together taken from **different organisms**.

This technique makes it possible to take any gene from any specie and place this gene in any other organism or specie. It is similar to **cloning** because when the foreign gene is incorporated in an organism like bacteria then multiple copies are made through cloning to use the gene in different applications.



# Applications of rDNA Technology in Medicine

Recombinant DNA technology had **made it possible** to treat **different diseases** by **inserting new genes** in place of **damaged and diseased genes** in the **human body**.

It has brought many revolutionary changes in the field of medicine and introduced such methods of treating diseases and delivering the drug.

**The top nine applications of recombinant DNA technology are:-**

1. Medical diagnosis of diseases.
2. Gene therapy.
3. Production of vaccines.
4. DNA typing (DNA fingerprint).



## Applications of rDNA Technology in Medicine

5. Production of hormones (Insulin and Growth hormone).
6. Production of monoclonal antibodies.
7. Production of antibiotics.
8. Production of interferon.
9. Production of Enzymes.



# 1. Medical Diagnosis of Diseases

The **rDNA technology** acts as a **tool** to **diagnose diseases**. This involves the **construction** of **probes** (short, single strands of radioactive or fluorescent DNA, used to identify the complementary DNA).

These **probes** are used to **identify** the **infectious agents**, such as *Salmonella spp* (food poisoning), *Staphylococcus spp* (pus), HIV, hepatitis virus, etc.

With the help of this technique the infected child can also be identified. This can be done by testing the DNA of prospective parents for any genetic disorder for example, the parents are not carrier of a certain disorder.



# 1. Medical Diagnosis of Diseases

Examples of medical diagnosis of diseases are:-

## a. Phenylketonuria:

In this case, **phenylalanine** fails to get converted into **tyrosine**. This causes **disturbances** in **metabolism** resulting in **mental retardation**. It is possible to cure this disease using rDNA technique in early periods of pregnancy.

## b. Thalassemia Genes:

In this case, syntheses of **alpha** and **beta globin chains** are **reduced** and the excess chains precipitate and cause **hemolytic anemia** and spleen enlargement.

Human globin genes have been **identified** and **sequenced**. Alpha and beta globin genes are closely linked. Human globin gene has also been **developed** and **cloned**. Still lot of work needs to be done to cure this disease.



# 1. Medical Diagnosis of Diseases

## c. Haemophilia Gene:

Haemophilia is a **sex linked disease** in human where blood clotting does not take place normally due to the absence of **clotting factor VIII**.

Using gene cloning technique (rDNA technology), the **clotting factor VIII gene** was cloned to express in mammalian cell lines and produce the protein VIII responsible for blood clotting.



## 2. Gene Therapy

Gene therapy means to change a **faulty gene** with a **normal, healthy gene**. Gene therapy can be used to correct a rare disease, like **sickle cell anemia**, which is caused by single mutation and killer diseases such as **Severe Combined Immuno Deficiency (SCID)**.

Gene therapy is used to produce **recombinant therapeutic bio chemicals** such as insulin, somatotropin, somatostatin, interferon, human blood clotting factor VIII, etc.

Several protocols have been developed for expression and introduction of genes in humans, but the clinical efficiency has to be demonstrated conclusively. Success of gene therapy is dependent on the development of better **gene transfer vector** for **sustained, long-term expression of foreign gene** as well as better understanding of **gene physiology of human disease**.



## 2. Gene Therapy

There are two gene transfer strategies:

- a. **In vivo** approach which involves introduction of **genes directly** into the **target organ** of an individual. This is done in patients, therefore called as **patient therapy**.
  
- b. **Ex vivo (in vitro)** approach where the cells are isolated for gene transfer in vitro followed by transplantation of genetically modified cells back into the patients.



### 3. Production of Vaccines

#### Hepatitis B Vaccine

Hepatitis B (HepB) is a major public health problem worldwide. Approximately 30% of the world's population, or about 2 billion persons, have serologic evidence of hepatitis B virus (HBV) infection. Of these, an estimated 350 million have chronic HBV infection and at least one million chronically infected persons die each year from liver cancer (HBV is known human carcinogen) and cirrhosis.

the HepB vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure. At least 85-90% of HBV-associated deaths are vaccine-preventable.



### 3. Production of Vaccines

Hepatitis B vaccine (rDNA) is a preparation of hepatitis B surface antigen (HBsAg), a component protein of hepatitis B virus, this antigen may be adsorbed on a mineral carrier such as aluminum hydroxide or hydrated aluminum phosphate. The antigen is obtained by recombinant DNA technology.

Hepatitis B vaccine (rDNA) is produced by the expression of the viral gene coding for HBsAg in yeast (*Saccharomyces cerevisiae*) or mammalian cells such as CHO cell line (Chinese hamster ovary), purification of the resulting HBsAg and the rendering of this antigen into an immunogenic preparation (vaccine).

The vaccine may contain the product of the S gene (spike protein), a combination of the S gene and pre-S2 gene products (middle protein) or a combination of the S gene, the pre-S2 gene and pre-S1 gene products (large protein).



### 3. Production of Vaccines

Recently, the transfer of hepatitis-B surface antigen gene in **tobacco plant** and expression of this recombinant gene in tobacco followed by partial purification of protein antigen from the plant showed possibility of producing this antigen through higher plants (Molecular Farming).

When this protein was injected into mice, it provoked antibody response similar to that obtained with yeast derived commercially available vaccine.

This is clear that gene product obtained from **two different organisms** has same property and **transgenic plants** can be used as source of **antibodies**. Attempts are being made to produce many more molecules by cultivation of transgenic higher plants.



# KEY POINTS

## **Introduction.**

### **Applications of rDNA Technology in Medicine**

1. Medical diagnosis of diseases.
2. Gene therapy.
3. Production of vaccines.
4. DNA typing (DNA fingerprint).
5. Production of hormones (Insulin and Growth hormone).
6. Production of monoclonal antibodies.
7. Production of antibiotics.
8. Production of interferon.
9. Production of Enzymes.



# KEY POINTS

## **1. Medical Diagnosis of Diseases.**

- a. Phenylketonuria.
- b. Thalassemia Genes.
- c. Haemophilia.

## **2. Gene therapy.**

- a. In vivo approach.
- b. Ex vivo (in vitro) approach.

## **3. Production of vaccines.**

Hepatitis B Vaccine and producing it in transgenic plants.



## ***Lecture 17 Questions Practice***

### **Q1:- Define the followings:**

- a. Probe.

### **Q2:- Enumerate the followings:-**

- a. Enumerate three example of medical diagnosis of diseases using rDNA technology?
- b. Enumerate the two gene transfer strategies.

### **Q3:- MCQ**

1. Thalassemia happens due to?
  - a. Reduction of alpha globulin chains synthesis.
  - b. Reduction in beta globulin chains synthesis.
  - c. Synthesis of faulty hemoglobin.
  - d. All the answers.
2. Success of gene therapy is dependent on?
  - a. Development of better gene transfer vector.
  - b. Development of sustainable vector.
  - c. Development of long term expression vector.
  - d. All the answers.

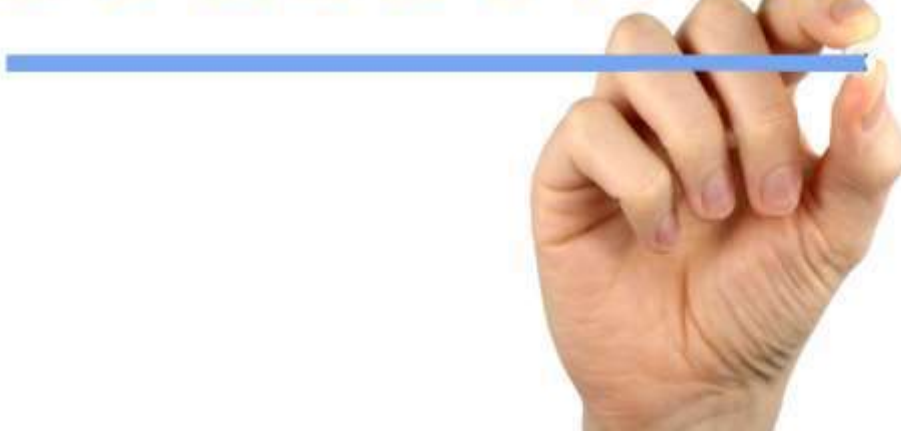


# Molecular Biology

18<sup>th</sup> lecture

Applications of Recombinant DNA  
Technology Continues,,

# OBJECTIVES



- 4. DNA typing (DNA fingerprint).
- 5. Production of Hormones (Insulin and Human Growth hormone).
- 6. Production of monoclonal antibodies.
- 7. Production of antibiotics.
- 8. Production of interferon.
- 9. Production of enzymes.



## 4. DNA Typing (DNA Fingerprint)

DNA typing is a technique in which biological samples help in solving forensic problems.

For DNA typing, biological samples like blood, skin, semen or hair are collected from the crime scene. A portion of a sample is analyzed to confirm that there is sufficient amount of intact DNA (undegraded) for the further analysis.

Intact DNA, it is digested with a restriction enzyme, and the fragments are separated on an Agarose gel and transferred by blotting onto a nylon membrane.

This nylon membrane is then hybridized sequentially with four or five separate radio-labeled probes that each recognizes a distinct DNA sequence. After each hybridization reaction, the bands where the probe has bound to the digested DNA sample are visualized by autoradiography and the banding pattern for each sample is noted.



## 4. DNA Typing (DNA Fingerprint)

A commonly used set of probes for this type of analysis consists of **human mini-satellite DNAs**. These sequences occur throughout the human genome and consist of tandemly repeated sequences. The length of the repeats range from 9 to 40bp, and the number of repeats in the mini-satellites ranges from about 10 to 30. Unrelated individuals generally have different length of mini-satellites. However, a child will inherit one mini-satellite DNA sequence from mother and one from father.

The human mini-satellites DNA sequences are highly variable, and the chance of finding two individuals in the population with the same DNA fingerprint is about one in  $10^5$  to  $10^8$ . An individual's DNA banding pattern based on mini-satellites DNA sequences is almost as unique as his/her fingerprints.

In addition to forensic applications of DNA typing, this technique may be used to determine the paternity of a child.



## 5. Production of Hormones

### a. Production of Insulin

Human gene for insulin production has been incorporated into bacterial DNA and such genetically engineered bacteria are used for large scale production of insulin.

Amongst the earliest uses of biotechnology in pharmaceutical manufacturing is the use of rDNA technology to modify *E. coli* to produce human insulin (1978).

Researchers produced artificial genes for each of the two protein chains that comprise the insulin molecule.

The recombinant plasmids were inserted into *E. coli*, which were induced to produce 100,000 molecules of either chain A or chain B human insulin. Then the two protein chains were then combined to produce insulin molecules.



## 5. Production of Hormones

### b. Production of Human Growth Hormone

In 1979, researchers produced human growth hormone by inserting DNA coding for human growth hormone into a plasmid that was implanted in *E. coli*.

The gene that was inserted into the plasmid, was isolated by a type of restriction enzyme which acts at restriction sites for human growth hormone. It was used to produce a DNA fragment of 551 base pairs.

Then a chemically synthesized DNA adaptor fragment containing an **ATG initiation codon** was produced with the codons for the first through 23<sup>rd</sup> amino acids in human growth hormone. The two DNA fragments were combined to form a synthetic-natural hybrid gene.



## 6. Production of Monoclonal Antibodies

The development of monoclonal antibodies in 1975 led to a medical revolution. The body normally produces a wide range of antibodies, immune system proteins that eliminate microorganisms and other foreign invaders. By fusing antibody-producing cells with myeloma cells, researchers were able to generate antibodies that would, like "magic bullets" enhance in on specific targets including unique markers, called antigens, on the surfaces of inflammatory cells.

After their invention in 1970's the monoclonal antibodies (mAbs) earned the reputation of "magic bullet" in particular against tumor specific antigens and infectious diseases.

Present antibody based therapeutics include unconjugated mAbs, antibody drug conjugates (ADC1), antibody based radio-conjugates (ARC), bispecific antibodies (BsAb) recognizing two different antigens, Ab fragments and fc-fusion proteins. Antibodies and antibody fragments can be relatively easily modified by molecular biological techniques.



## 7. Production of Antibiotics

Antibiotics are the chemical substances which are used against bacterial infections. They can be produced by microorganisms as well as in the laboratory. They have the ability to destroy bacteria or other harmful microbes which cause infections in the body.

Recombinant DNA technology is used to produce antibiotics. For instances, *Penicillium* and *Streptomyces* fungi are used for mass production of famous antibiotics **Penicillin** and **Streptomycin**. Genetically modified efficient strains of these fungi have been developed using rDNA technology to greatly increase the yield of these antibiotics.



## 8. Production of Interferon

Interferon is virus-induced glycoprotein produced by virus-infected cells. Interferon is **antiviral in action** and act as first line of defense against viruses causing serious infections, including breast cancer and lymph nodes malignancy.

Natural interferon is produced in very small quantity from human blood cells, which is not cost effective. It is now possible to produce interferon by recombinant DNA technology at much cheaper rate. **Interferon alpha** is used to treat lymphoma and myelogenous leukemia.



## 9. Production of Enzymes

Some useful enzymes can also be produced by recombinant DNA technology. For example, the enzyme **Urokinase**, which is used to **dissolve blood clots**, has been produced by genetically engineered microorganisms using rDNA technology.



#### **4. DNA Typing (DAN Fingerprint)**

Forensic work → enough DNA → restriction enzymes → hybridization with the right probe → discover the identity of the paternity.

#### **5. Production of hormones**

- a. Insulin hormone      b. Human growth hormone

#### **6. Production of monoclonal antibodies**

mAbs → antibody drug conjugates (ADC1), antibody based radio-conjugates (ARC), bispecific antibodies (BsAb) recognizing two different antigens, Ab fragments and fc-fusion proteins. Antibodies and antibody fragments can be relatively easily modified by molecular biological techniques.

#### **7. Production of Antibiotics**

Such as Penicillin and Streptomycin



# POINTS

## 8. Production of Interferon

Interferon is virus-induced glycoprotein produced by virus-infected cells. Interferon is **antiviral in action**.

## 9. Production of enzymes

Urokinase to dissolve blood clots.



## ***Lecture 18 Questions Practice***

### **Q1:- Define the followings:**

- a. Interferon.

### **Q2:- MCQ**

1. In addition to forensic applications of DNA typing, this technique may be used to?
  - a. Determine the genetic diseases of a child.
  - b. Determine the paternity of a child.
  - c. Determine the mutations of a child.
  - d. Determine the sex of a child.
2. Are types of monoclonal antibodies?
  - a. Unconjugated mAbs.
  - b. Antibody drug conjugates (ADC1).
  - c. Antibody based radio-conjugates (ARC).
  - d. Bispecific antibodies (BsAb).
  - e. Antibodies fragments and fc-fusion proteins.
  - f. All the answers.

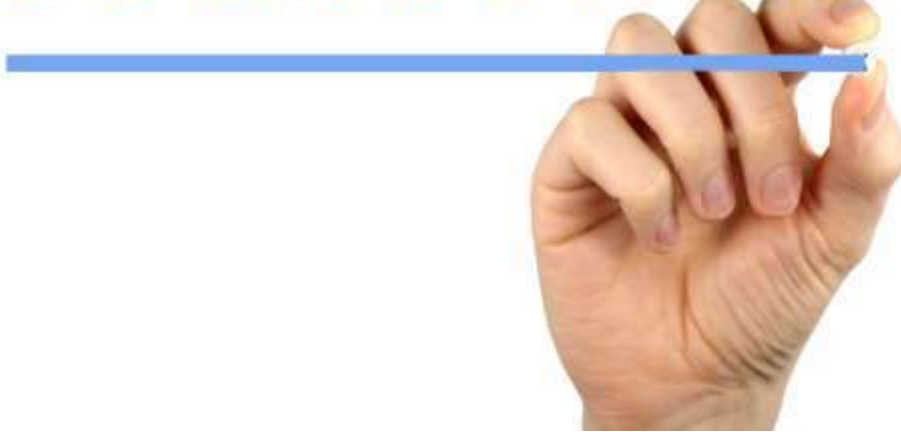


# Molecular Biology

19<sup>th</sup> lecture

Disorder of Cell Growth

# OBJECTIVES



- Cellular adaptation.
- Hypertrophy.
- Hyperplasia.
- Atrophy.
- Metaplasia.
- Disorders of cell growth.
- Causes of Developmental disturbances.
- Organ specific Growth Disturbances.



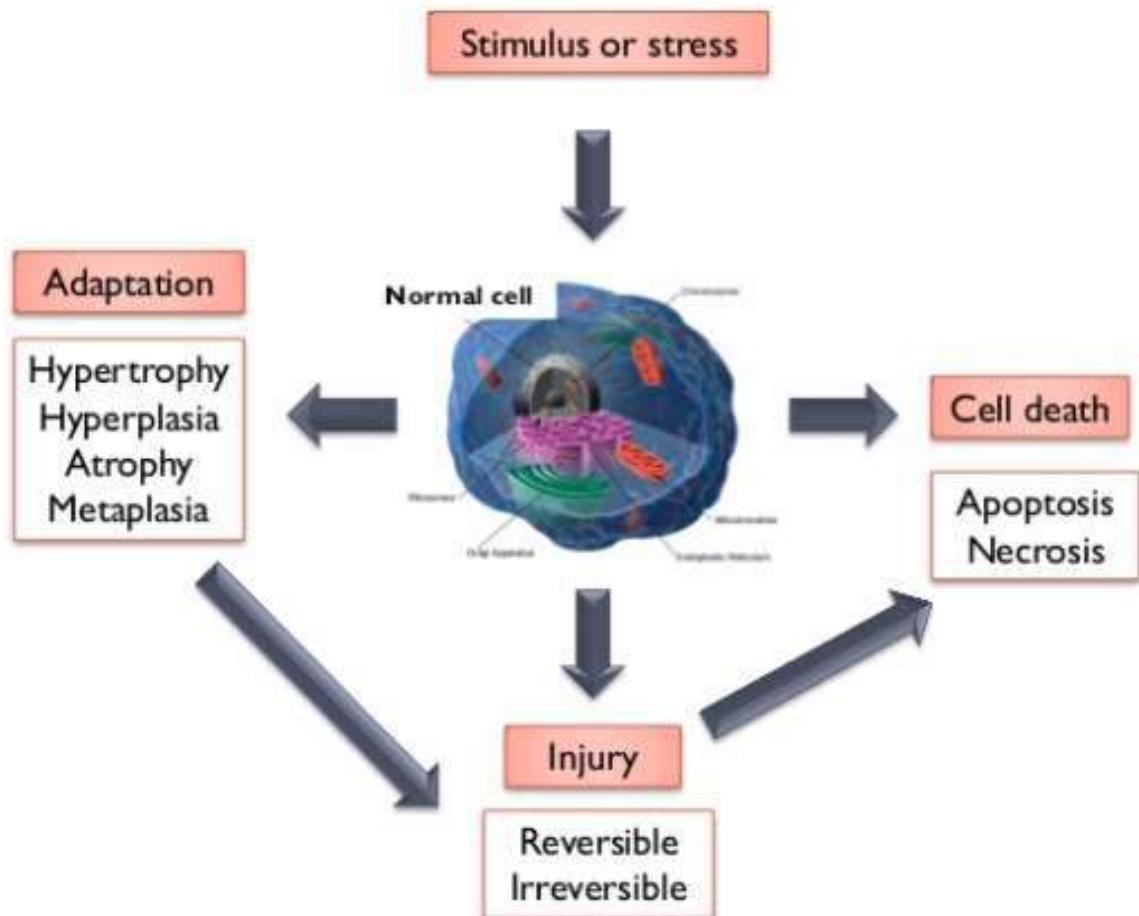
# Cellular Adaptation

**Cellular adaptation** is reversible changes in size, number, phenotype, metabolic activity or function of the cells in response to change in their environment.

## Types of cellular adaptation:-

1. **Physiologic adaptation:** in response to normal stimulation by hormones.
2. **Pathogenic adaptation:** in response to stress to escape injury. It has many forms such as **hypertrophy, hyperplasia, atrophy and metaplasia**.

## Cellular Adaptation Continues,,





# Hypertrophy

**Hypertrophy** is increase in the size of the cells which leads to increase in the size of organ to increase the functional capacity.

- No new cells, just larger cells.
- Increase in metabolic activity → increase protein synthesis → decreased protein degradation.

Types of hypertrophy includes:-

1. **Physiologic hypertrophy**, such as skeletal muscle after exercise and pregnant uterus.
2. **Pathologic hypertrophy**, which includes **a) adaptive hypertrophy** such as left ventricle in case of hypertension, **b) compensatory hypertrophy** such as removal of a kidney and, **c) hormonal hypertrophy** such as acromegaly due to excessive growth hormone.



# Hyperplasia

**Hyperplasia** is increase in the number of cells which leads to increase in the size of organ . It may occur with **hypertrophy** and often in response to the same stimuli.

Types of hyperplasia includes:-

1. **Physiologic hyperplasia**, which includes **a) hormonal hyperplasia** such as female breast during puberty and, **b) compensatory hyperplasia** such as partial removal of liver.
2. **Pathologic hyperplasia**, which includes **a) excessive hormonal growth stimulation** such as endometrial hyperplasia due to imbalance of estrogen and progesterone hormones leading to abnormal menstrual bleeding or thyroid gland enlargement due to increase in TSH (thyroid stimulating hormone).



## Hyperplasia Continues,,

**b) Wound healing hyperplasia**, which is due to response of connective tissue cells growth factors and, **c) hyperplasia due to viral infection** such as skin warts.

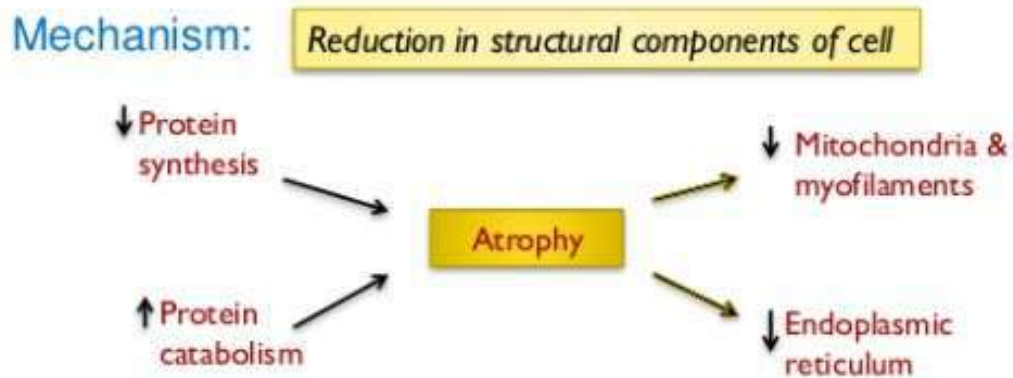
### Hyperplasia and cancer

Pathologic hyperplasia considered as fertile soil in which cancerous proliferation may eventually occur. For example, endometrial hyperplasia increase the risk of endometrial cancer.

# Atrophy

**Atrophy** is shrinkage in the size of cell due to loss of cell substance (structural components of cell are reduced). When a large number of cells involved, the entire tissue or organ diminishes in size.

## Mechanism of atrophy.



*The cells retreats to a smaller size at which survival is still possible.  
New equilibrium is achieved between cell size & diminished blood supply  
or trophic stimulation.*



# Atrophy Continues,,

## Types of atrophy:-

1. **Physiological atrophy**, such as the atrophy of thymus gland after puberty, decreased workload (disuse) after limb fracture and atrophy because of loss of hormonal stimulation such as atrophy of ovaries and uterus after menopause.
2. **Pathological atrophy**, such as **a) loss of innervation**, **b) starvation and malnutrition**, **c) senile atrophy** (atrophy of the heart) and **d) pressure atrophy**.



# Metaplasia

**Metaplasia** is transformation of one type of tissue to another type of the same differentiation.

Cells sensitive to particular pressure are replaced by other cell types better able to withstand the adverse environment.

## **Mechanism of metaplasia:-**

It induced by altered differentiation pathway of tissue stem cells (genetic reprogramming of stem cells).



# Metaplasia Continues,,

## Metaplasia types:-

1. **Epithelial metaplasia**, such as **a) squamous cell metaplasia** of respiratory tract of smokers and urinary bladder “stratified” squamous epithelium due to Bilharziasis, **b) odontogenic epithelial metaplasia** and **c) glandular metplasia** of gastric acid reflux.
2. **Connective tissue metaplasia**, such as **a) cartilage to bone** in old age and **b) muscle tissue to bone**.



# Disorders of Cell Growth

**Disorders of cell growth** are developmental disturbances which caused by congenital conditions or hereditary conditions.

1. **Congenital defects or anomalies**, are structural defects that are present **at, before or after** birth, but not necessarily inherited and they either have cosmetic or functional significance.
2. **Hereditary defects or anomalies**, are diseases or conditions that transmitted by genes, some become apparent after birth (Down Syndrome) and some others years after birth (Alzheimer's disease).



# Causes of Developmental Disturbances

1. **Genetic factors** such as Down Syndrome.
2. **Environmental influences**, such as most viral infections, drugs, maternal disease status, pregnant smokers and irradiations.
3. **Multifactorial inheritance**, which includes the genetic factors with environmental influences.
4. **Unknown causes**.



## Organ specific Growth Disturbances

**Agenesis**, complete absence of an organ.

**Aplasia**, incomplete development of an organ.

**Hypoplasia**, underdevelopment of an organ.

**Atresia**, absence of an opening of a hollow visceral organ such as rectum (Atresia ani) or of duct of salivary gland or bile duct.



# KEY POINTS

**Cellular adaptation** is reversible changes in size, number, phenotype, metabolic activity or function of the cells in response to change in their environment. Physiologic adaptation OR Pathogenic adaptation.

**Hypertrophy** is increase in the size of the cells which leads to increase in the size of organ to increase the functional capacity. Physiologic hypertrophy OR Pathogenic hypertrophy.

**Hyperplasia** is increase in the number of cells which leads to increase in the size of organ . It may occur with **hypertrophy** and often in response to the same stimuli. Physiologic hyperplasia OR Pathogenic hyperplasia.

**Atrophy** is shrinkage in the size of cell due to loss of cell substance (structural components of cell are reduced). When a large number of cells involved, the entire tissue or organ diminishes in size. Physiologic atrophy OR Pathogenic atrophy.



# KEY POINTS

**Metaplasia** is transformation of one type of tissue to another type of the same differentiation.

Epithelial metaplasia OR connective tissue metaplasia

**Disorders of cell growth** are developmental disturbances which caused by congenital conditions or hereditary conditions.

Congenital defects or anomalies OR Hereditary defects or anomalies,

## **Causes of Developmental disturbances**

Genetic factors, Environmental influences, Multifactorial inheritance, Unknown causes.

## **Organ specific Growth Disturbances**

Agensis, Aplasia, Hypoplasia, Atresia