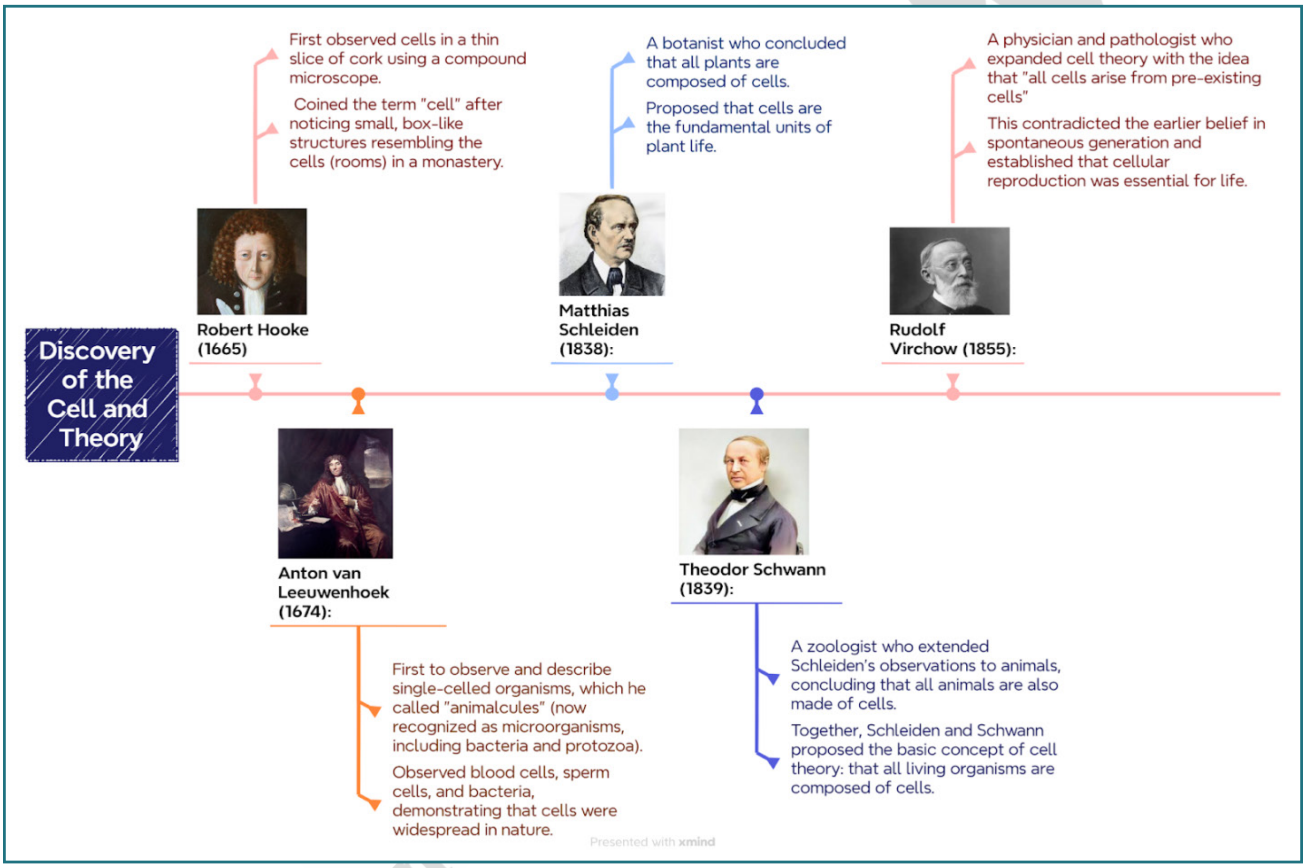
1 BIO TECHNOLOGY

1.1 CELL

- A **cell** is the basic structural, functional, and biological unit of all living organisms.
- Cells are often referred to as the "building blocks of life" because they carry out essential functions such as energy production, metabolism, and replication.

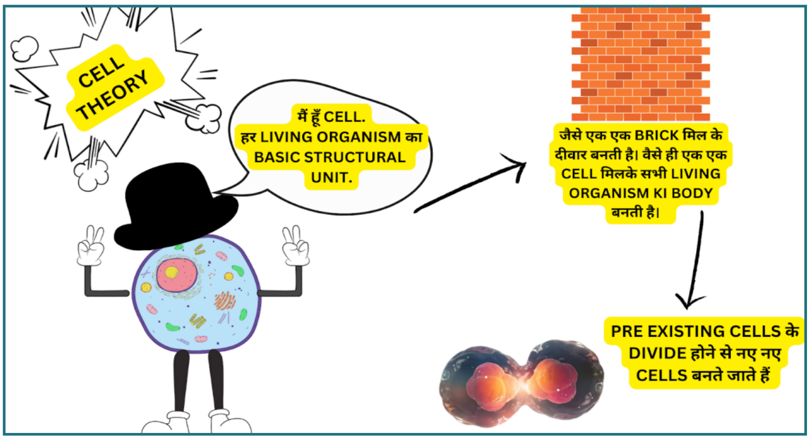
1.1.1 Discovery of Cells



1.1.2 Cell Theory

The cell theory states that:

1. All living organisms are made up of one or more cells.
2. The cell is the basic unit of structure and function in living organisms.
3. All cells arise from pre-existing cells through cell division.

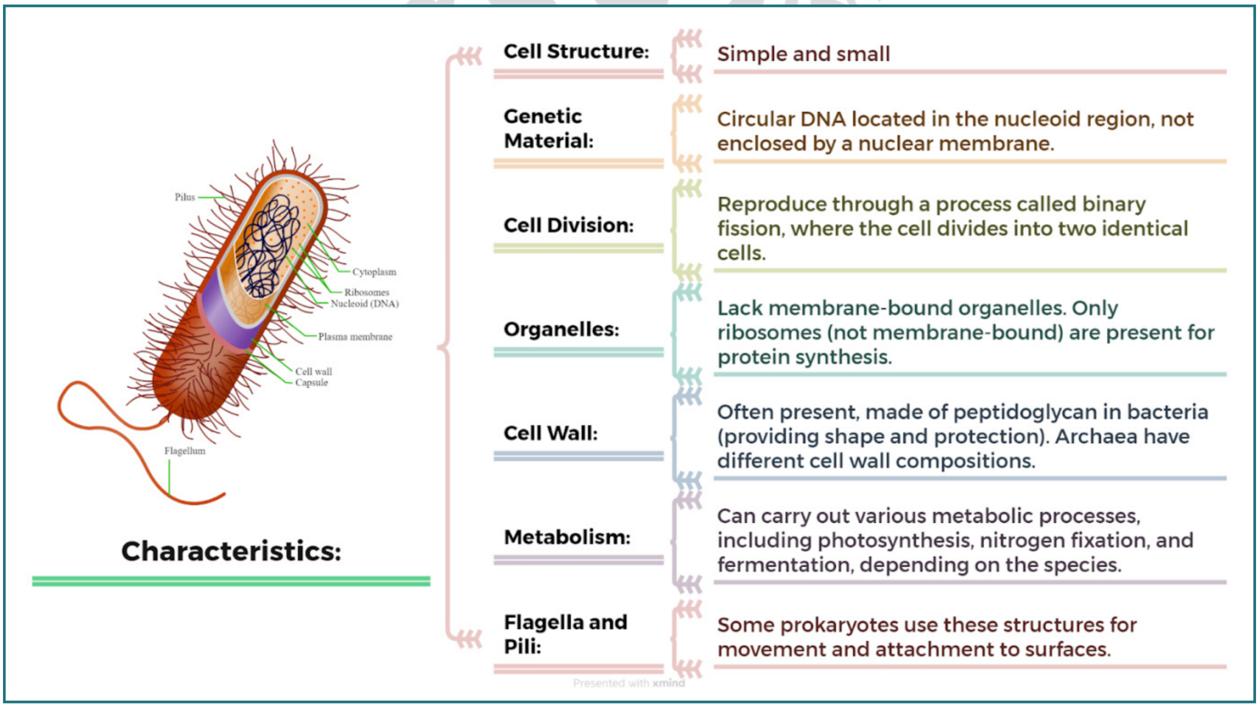


1.1.3 Types of cells

- Cells are basically of two types: Eukaryotic & Prokaryotic

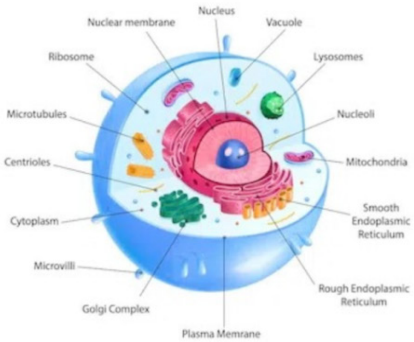
Prokaryotes

- Prokaryotes are single-celled organisms that lack a membrane-bound nucleus and other membrane-bound organelles.
- Their genetic material is not enclosed within a nucleus but is instead found in a region called the nucleoid.
- **Examples**: Bacteria and archaea are the two main groups of prokaryotes.



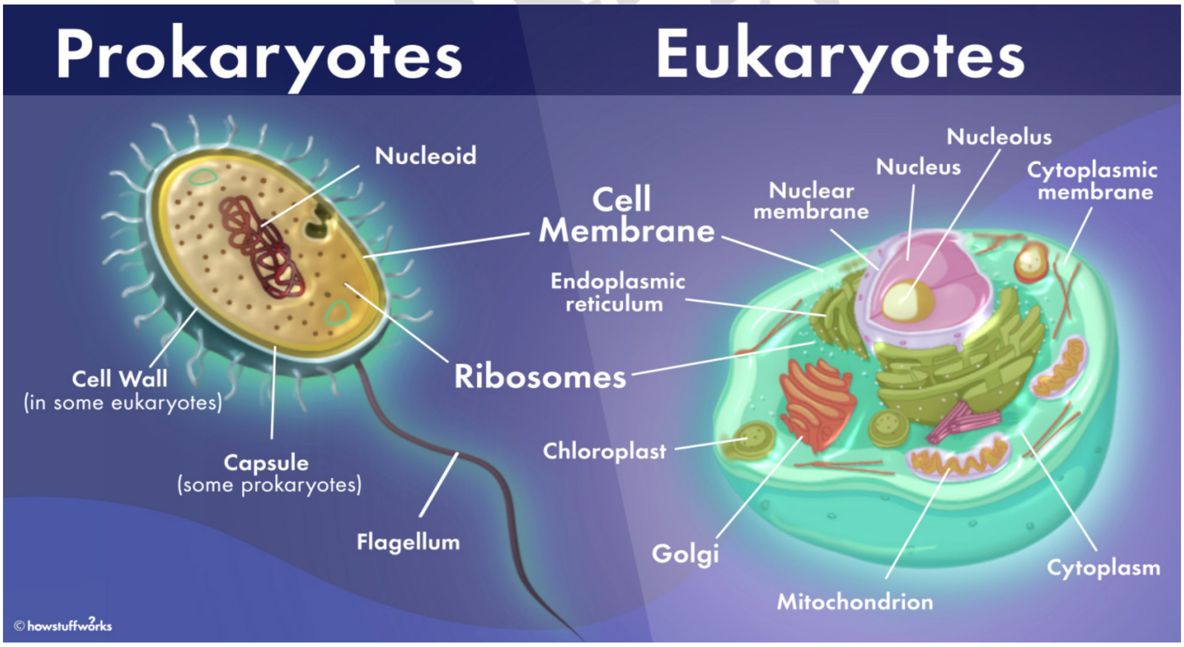
Eukaryotes

- Eukaryotes are organisms whose cells have a well-defined, membrane-bound nucleus and other membrane-bound organelles, making them structurally more complex than prokaryotes.
- **Examples**: Plants, animals, fungi, and protists are all eukaryotic organisms. They can be unicellular (like some protists) or multicellular (like plants and animals).



Characteristics:

Cell Structure:	Larger and more complex than prokaryotic cells
Genetic Material:	DNA is linear and organized into chromosomes within a membrane-bound nucleus.
Cell Division:	Reproduce through mitosis (for growth and repair) or meiosis (for producing gametes in sexual reproduction).
Organelles:	Contain various membrane-bound organelles such as mitochondria (for energy production), endoplasmic reticulum (for protein and lipid synthesis), Golgi apparatus (for packaging and transport), and sometimes chloroplasts (for photosynthesis in plants).
Cell Wall:	Present in some eukaryotes (e.g., plants and fungi), but absent in others (e.g., animals). In plants, the cell wall is made of cellulose; in fungi, it's made of chitin
Cytoskeleton	A well-developed network of protein filaments that maintains cell shape, aids in intracellular transport, and is involved in cell division.
Specialized Cells:	In multicellular eukaryotes, cells can differentiate into various types, such as muscle cells, nerve cells, and epithelial cells, each with specialized functions.

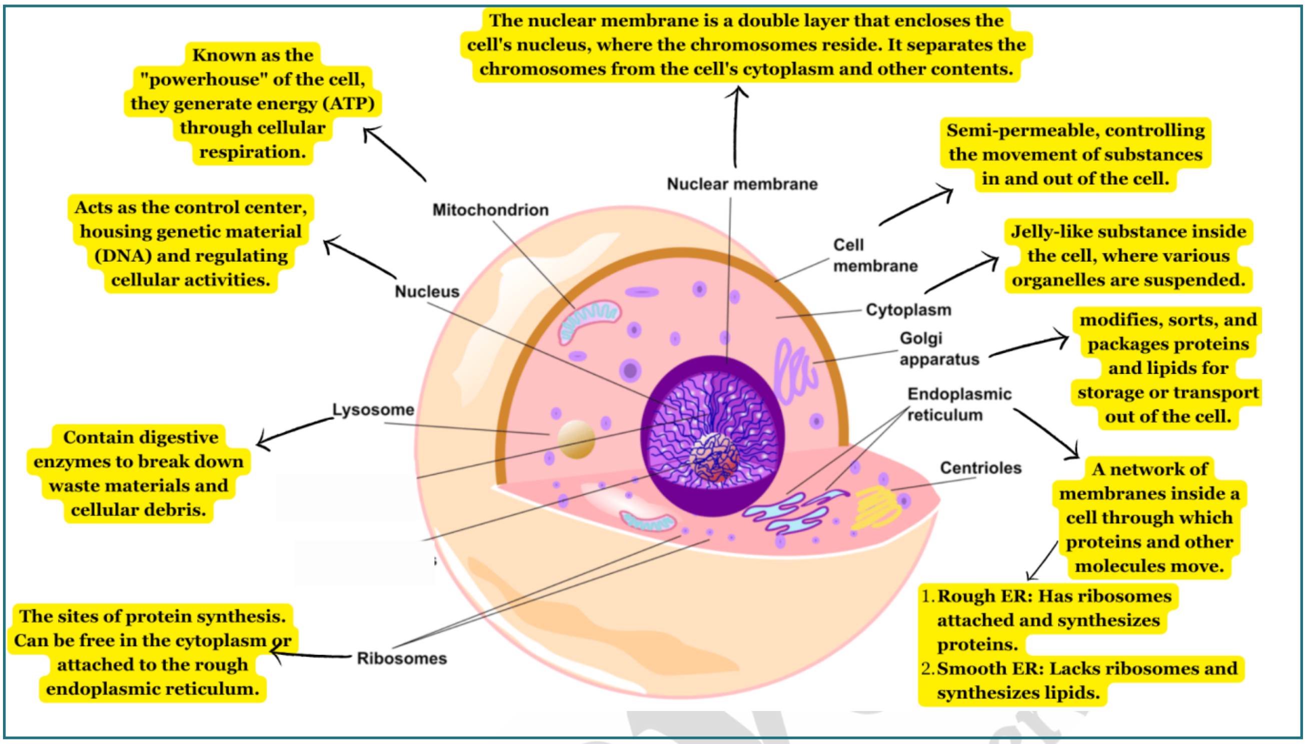


1.1.4 Cell Structure and Organelles

Cell Organelles

➲ A cell organelle is a subcellular structure that, like an organ in the body, has one or more specific tasks to carry out within the cell.

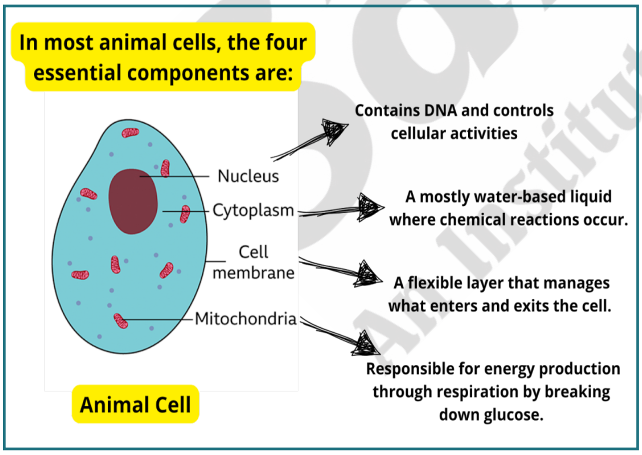
1.1.5 Key Components of a Cell



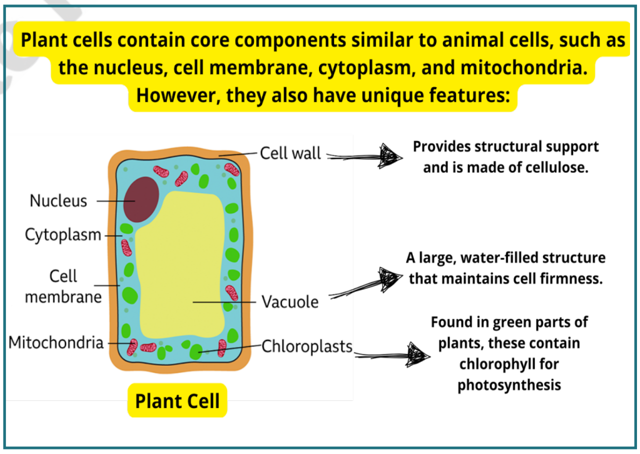
1.1.6 Subtypes of Eukaryotic Cells

- There are different types of eukaryotic cells. Plant and animal cells are both types of eukaryotic cells, meaning they have a defined nucleus and membrane-bound organelles.
- This nucleus houses DNA, differentiating them from prokaryotic cells, which lack such compartmentalization.

Animal Cells

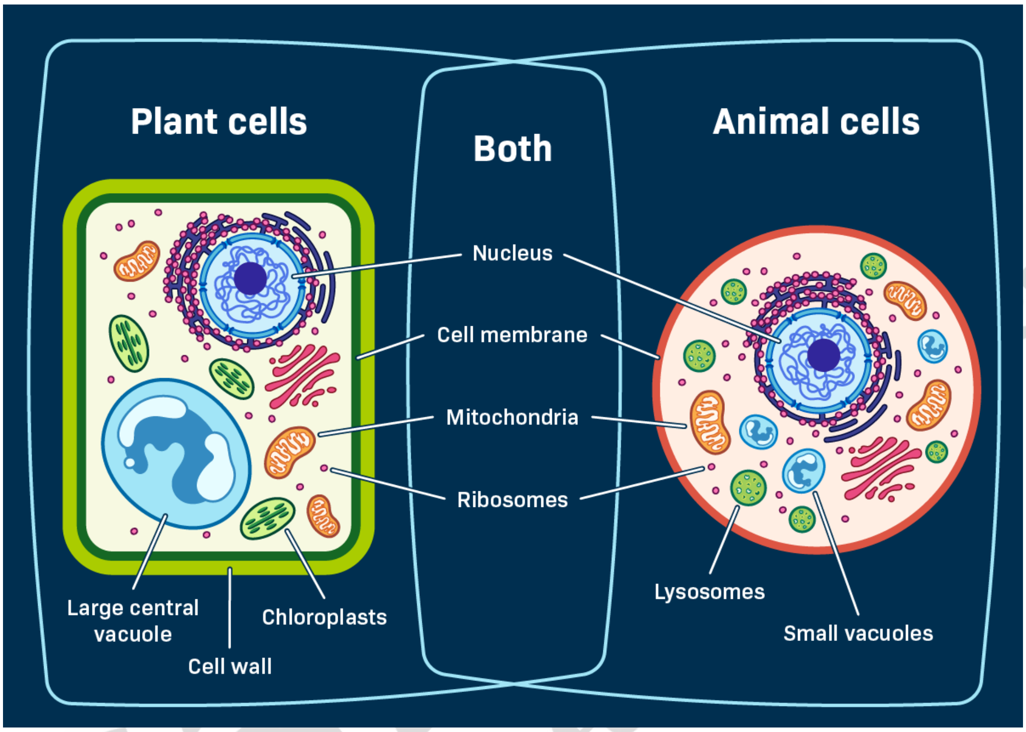


Plant Cells



PLANT CELL vs. ANIMAL CELL		
Feature	**Plant Cell**	**Animal Cell**
Cell Wall	Present, rigid	Absent
Nucleus Location	Peripheral cytoplasm	Centered
Reserve Food	Starch	Glycogen
Plastids	Present	Absent

Mitochondria	Fewer in number	Numerous
Centrosome	Generally absent (except in some lower forms)	Present
Cell Division	Cell plate method	Cleavage method
Cytokinesis Stage	Formation of a cell plate (due to rigid cell wall)	Formation of a cleavage furrow (pinching the cell membrane)
Glyoxysomes	Present in some cells	Absent



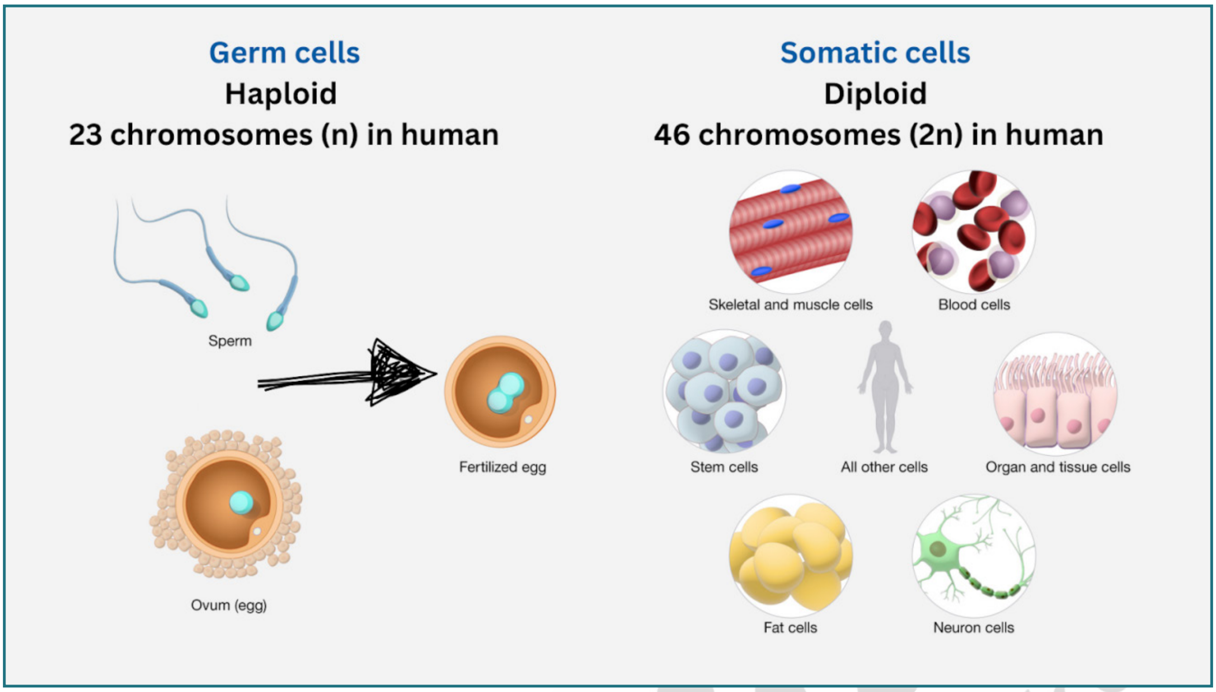
1.1.7 Cellular Organization in Multicellular Organisms

Somatic Cells

- A subtype of eukaryotic cells in multicellular organisms, somatic cells are all body cells except reproductive cells (sperm and egg), known as germ cells. E.g. skin cells, liver cells, muscle cells.
- In humans, somatic cells are diploid, containing two sets of chromosomes (one from each parent).
- They undergo mitosis to produce identical daughter cells, maintaining the same number of chromosomes.
- Mutations in somatic cells affect the individual but cannot be inherited by offspring.
- These cells play essential roles in growth, repair, and daily functions of tissues and organs.

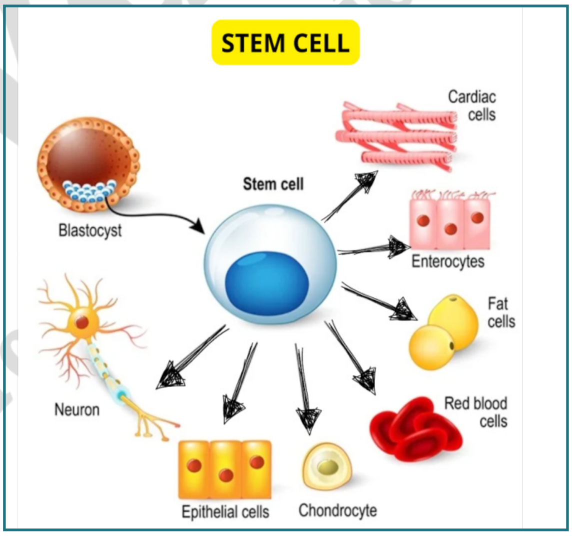
Germ Cells

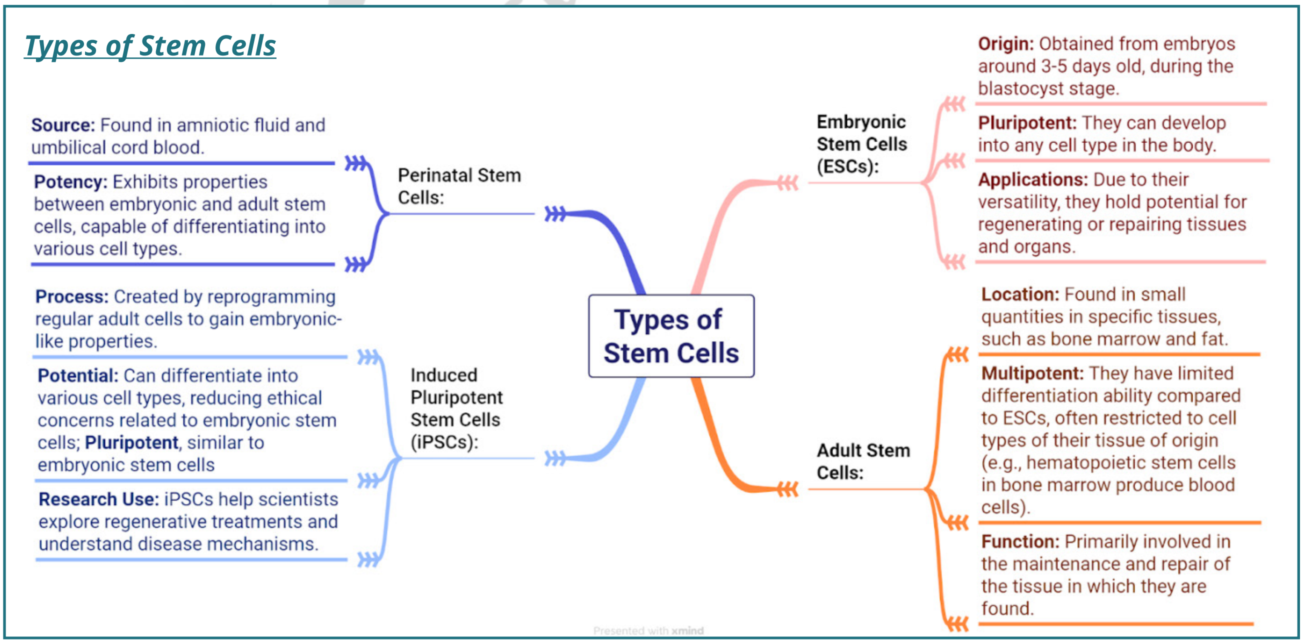
- Specialized cells involved in reproduction, located in the reproductive organs (ovaries in females, testes in males).
- They give rise to **gametes** (sperm in males and eggs in females), which carry half the genetic material to form a new organism upon fertilization.
- They undergo **meiosis** to produce gametes with half the chromosome number, ensuring genetic diversity in offspring.



Stem Cells

- These are undifferentiated cells with the unique ability to develop into different cell types and to self-renew.
- Stem cells can divide to create identical copies of themselves, helping in tissue maintenance and repair.
- Also, they can transform into specialized cell types with specific functions, like muscle, nerve, or blood cells.





Other types of Stem cells

- **Totipotent stem cells:** Produced from the fusion of an egg and sperm cell and can construct a complete, viable organism.
- **Pluripotent stem cells:** Pluripotent stem cells can divide into most, or all, cell types in an organism, but cannot develop into an entire organism on their own. Embryonic Stem Cells come under this category..
- **Multipotent stem cells:** Only those cells which are closely related family of cells Eg. The bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells.
- **Oligopotent stem cells:** Can differentiate into only a few cells
- **Unipotent cells:** One cell type, their own, include muscle stem cells.

1.1.8 Cell Division and Reproduction

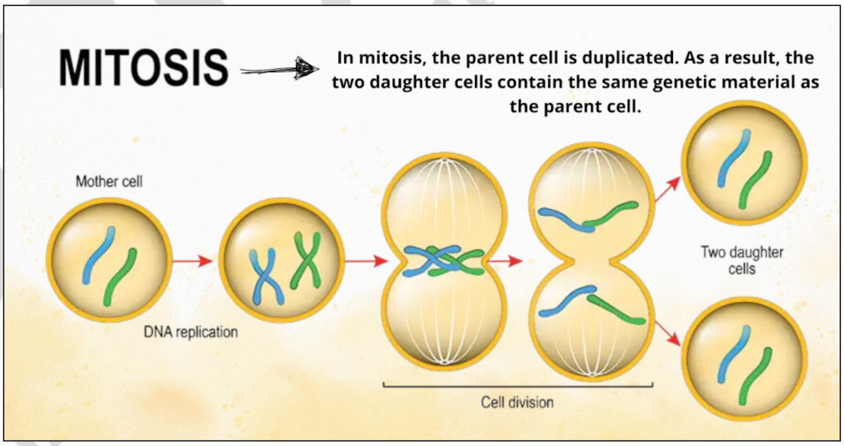
- Cells in organisms grow in two main ways:
    - **Cell replication** (division) to create more cells.
    - **Cell expansion** in volume.

Cell Division

- There are two types of cell division: mitosis and meiosis.
- Most often, cell division relates to mitosis, the process of making new body cells.

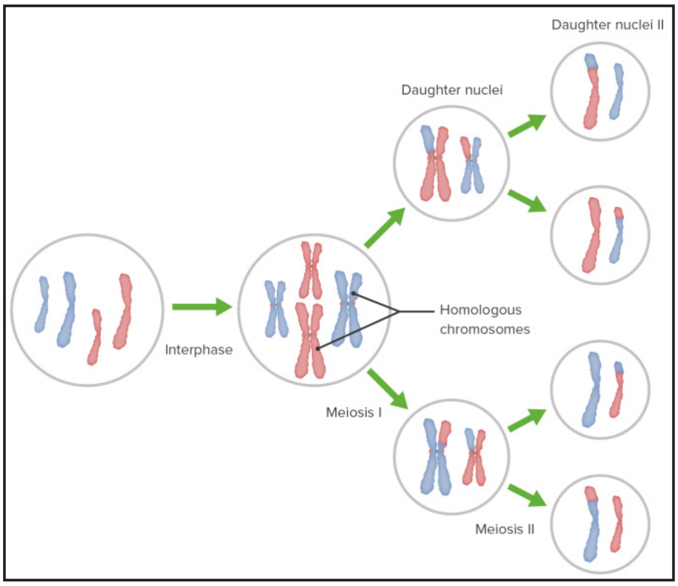
(1) Mitosis

- Process by which body cells (somatic cells) divide, producing identical daughter cells. It is used for growth, repair, and regeneration in multicellular organisms. It is a fundamental process for life.
- It is named for the thread-like appearance of chromosomes during division; discovered by Walther Flemming in 1882.
- It results in two genetically identical diploid cells.
- It occurs in both unicellular and multicellular eukaryotes.



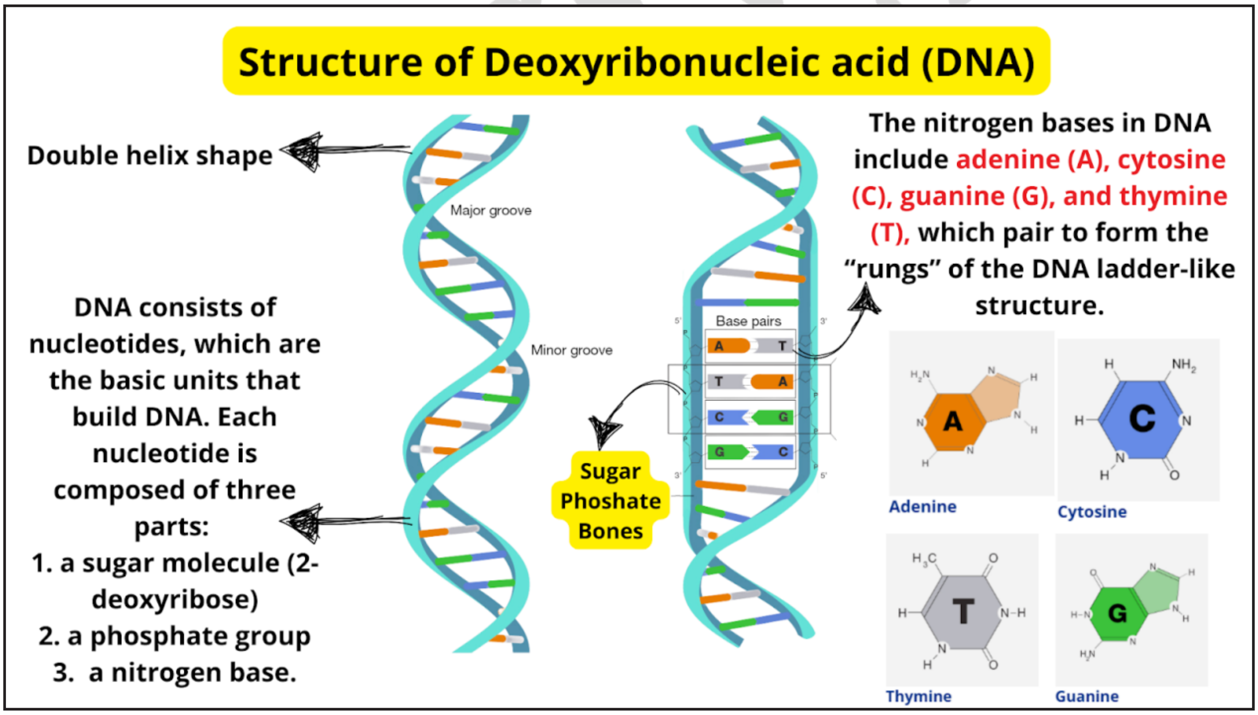
(2) Meiosis

- Meiosis is the type of specialized cell division that creates gametes (sperm and egg) with half the genetic material of the parent cell, enabling sexual reproduction and genetic diversity.
- It is named for the halving of genetic material; fusion of gametes observed by Oskar Hertwig in 1876.
- It results in four genetically unique haploid cells.
- It occurs only in organisms that reproduce sexually.



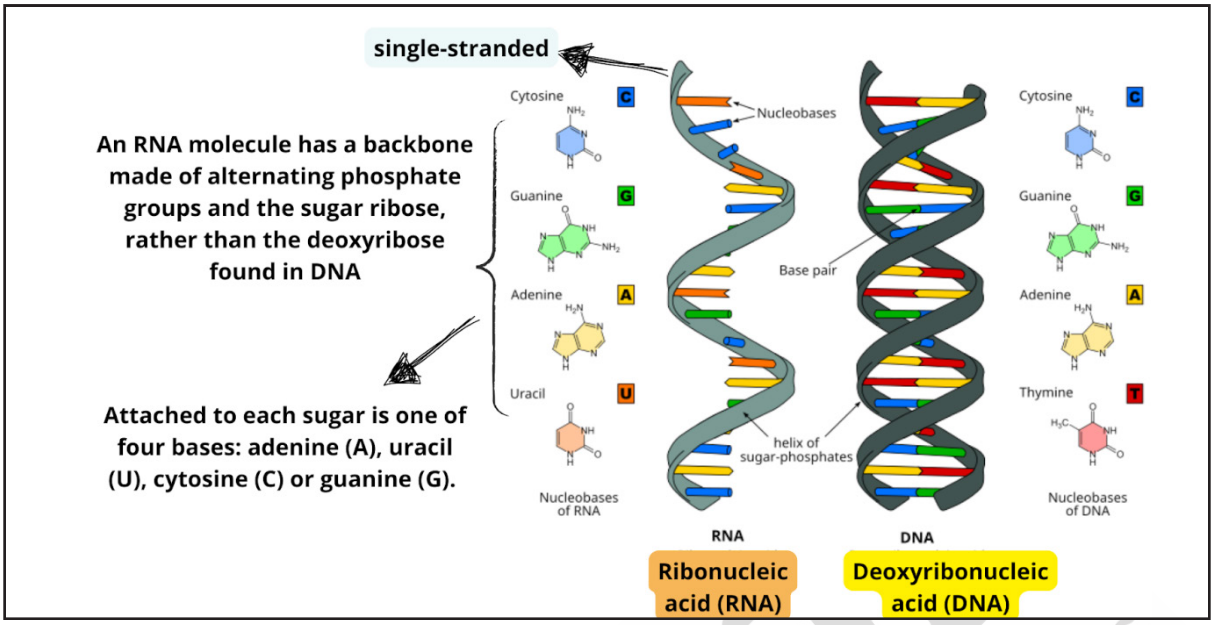
1.1.9 Deoxyribonucleic Acid (DNA)

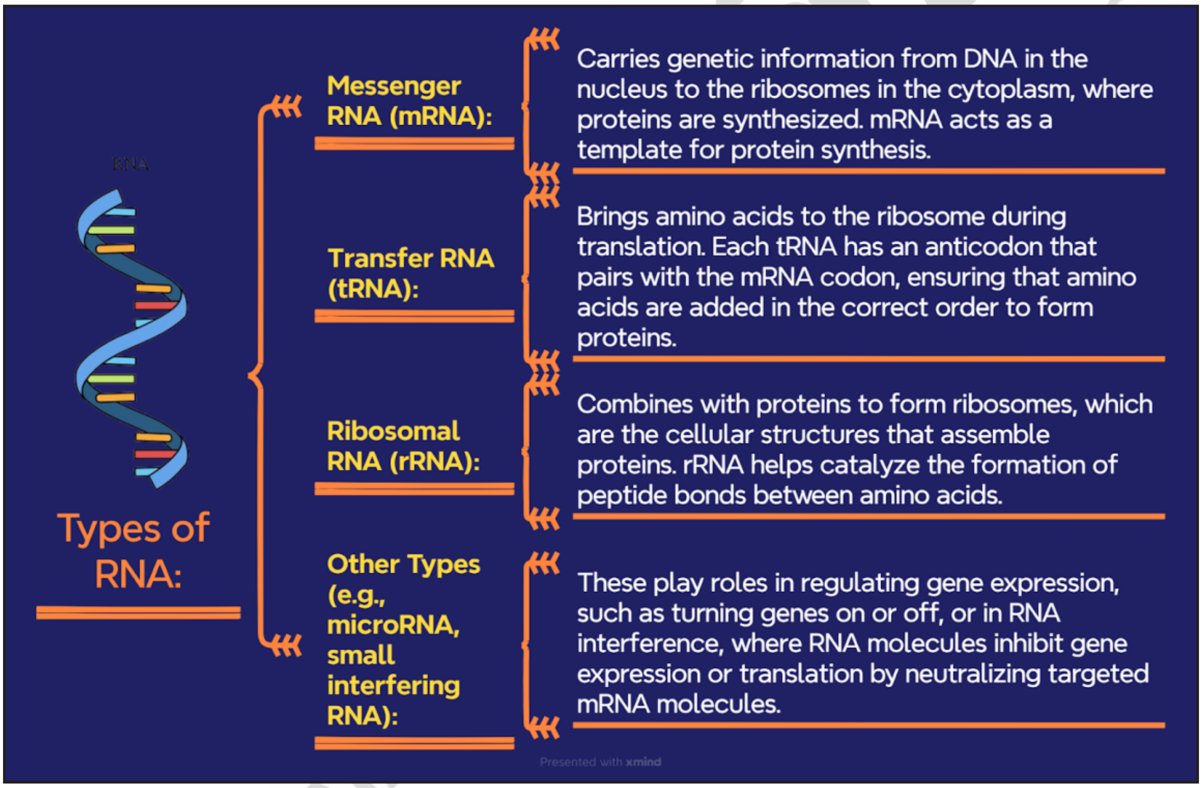
- Deoxyribonucleic acid (DNA) is a molecule that carries our hereditary material, meaning it contains the genetic information passed from one generation to the next.
- DNA holds the genes that make each organism unique, and it is fundamental to the structure and function of almost all living organisms.
- In eukaryotic cells (such as human cells), DNA is linear and contained within the nucleus. In prokaryotic cells (such as bacteria), DNA is circular and located in a region called the nucleoid.
- **DNA replication**
  - When cells divide for growth, repair, or reproduction, each new cell requires an **exact copy of DNA**.
  - Through **DNA replication**, the two strands of DNA separate, and cellular proteins create a new complementary strand for each original strand, resulting in two identical DNA molecules. Each new cell receives one copy of this DNA after cell division completes.
- Main Functions of DNA
  - DNA carries the instructions necessary for an organism's growth, development, and reproduction. This information is encoded in the sequence of nucleotide bases.
  - During protein synthesis, cells read groups of three DNA bases at a time (called codons) to form amino acids, which are the building blocks of proteins.
  - Proteins perform various essential roles within the body, including catalyzing metabolic reactions, supporting immune functions, and building cellular structures.



1.1.10 Ribonucleic Acid(RNA)

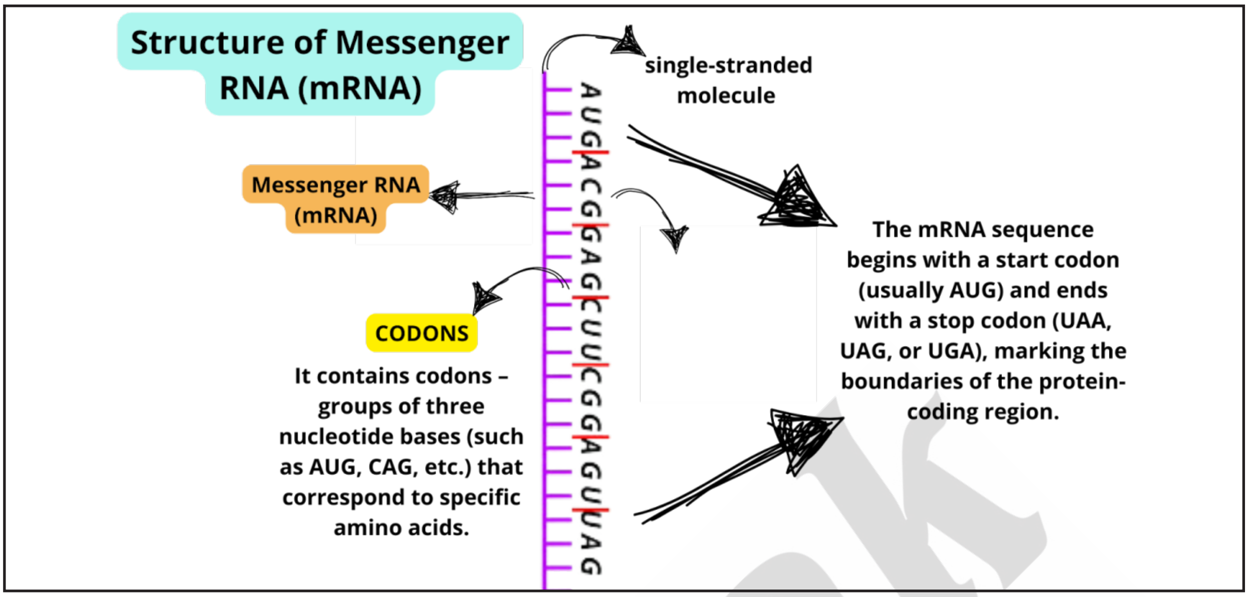
- Ribonucleic acid (abbreviated RNA) is a nucleic acid present in all living cells that has structural similarities to DNA. It is a molecule essential for various biological roles, primarily in coding, decoding, regulating, and expressing genes.
- RNA is similar to DNA, but it differs in structure, function, and types, playing a critical role in transferring genetic information from DNA to the cell's protein-making machinery



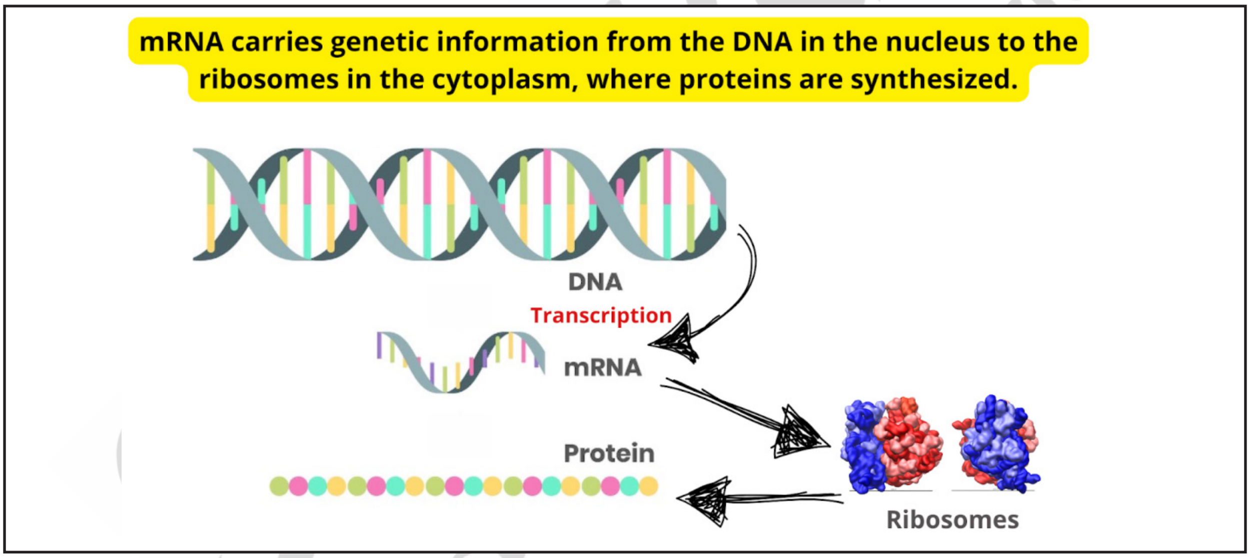


(1) MESSENGER RNA

- **Messenger RNA (mRNA)** is a type of RNA that plays a crucial role in the process of protein synthesis. It acts as a template for assembling amino acids into proteins according to the instructions encoded in a cell's DNA.
- mRNA is synthesized during **transcription**, a process in which an mRNA strand is created based on the DNA sequence of a gene.
- Enzymes called **RNA polymerases** bind to the DNA and "read" the gene sequence, creating a complementary mRNA strand.
- This mRNA strand is a temporary copy of the genetic code needed to make a specific protein.
- After transcription, mRNA leaves the nucleus and enters the cytoplasm.
- Ribosomes, the cell's protein-building machinery, bind to the mRNA strand and "read" the sequence in sets of three bases at a time.



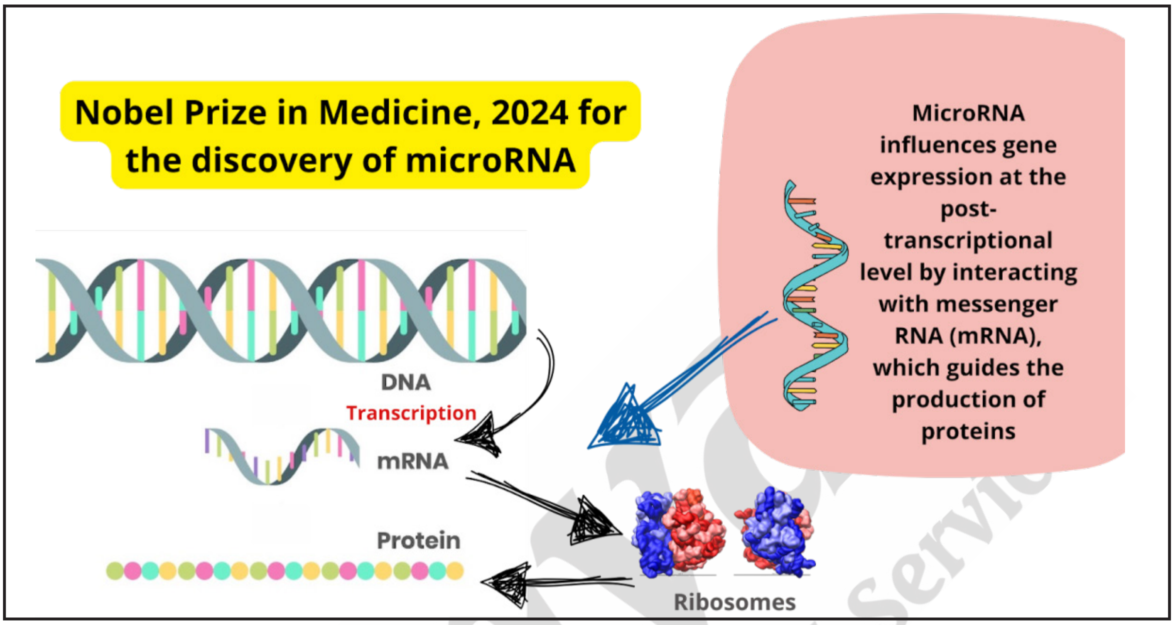
- Each codon corresponds to a specific amino acid, and **transfer RNA (tRNA)** molecules bring the correct amino acids to the ribosome in the sequence dictated by the mRNA.
- The ribosome links these amino acids together to form a protein.



(2) MICRO RNA

- **MicroRNA (miRNA)** is a small, non-coding RNA molecule that plays a key role in regulating gene expression.
- These tiny RNA molecules, typically about 21-23 nucleotides long, do not code for proteins but instead control the expression of other genes by interacting with messenger RNA (mRNA).
- This process of regulation helps control many cellular activities, from growth and development to immune responses and cell death
- **Nobel Prize in Medicine, 2024**
  - U.S. scientists Victor Ambros and Gary Ruvkun won the 2024 Nobel Prize in Medicine for the discovery of microRNA and and its essential role in regulating gene expression, which is crucial for the growth, specialization, and function of cells in multicellular organisms.

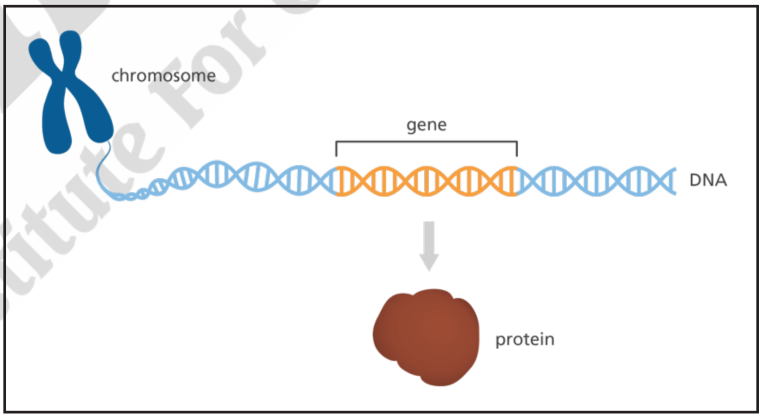
- Their findings explain how cells with identical genetic information can differentiate into various types like muscle and nerve cells, contributing to complex bodily structures and functions.
- As per the discovery, MicroRNA influences gene expression at the post-transcriptional level by interacting with messenger RNA (mRNA), which guides the production of proteins. Proteins are the primary building blocks of life, produced based on the DNA code.



1.1.11 GENES

(1) Gene

➲ A gene is a specific segment of DNA that contains the instructions for making a particular protein or set of proteins.



➲ Genes are fundamental units of heredity and are responsible for various traits and functions within an organism.

➲ Genes carry information that determines characteristics such as eye color, blood type, and other inherited traits.

➲ They also code for proteins that perform essential roles in cells, including those involved in growth, repair, and metabolism.

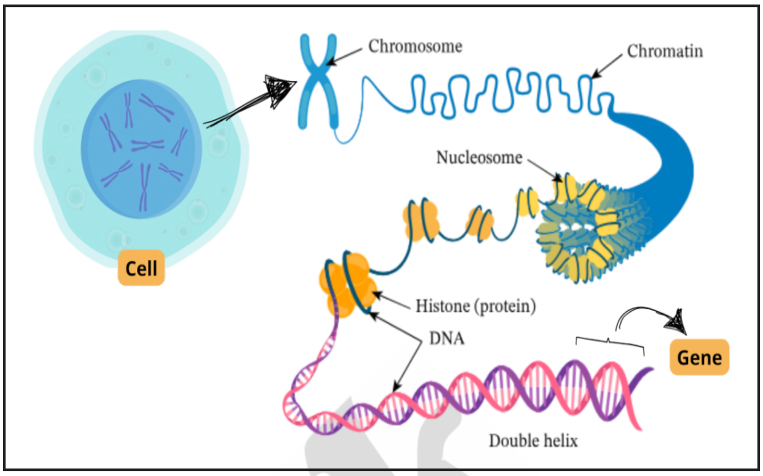
➲ Genes are located on chromosomes within the nucleus of a cell (in eukaryotic organisms) and are composed of sequences of nucleotides (A, T, C, and G).

(2) GENOME

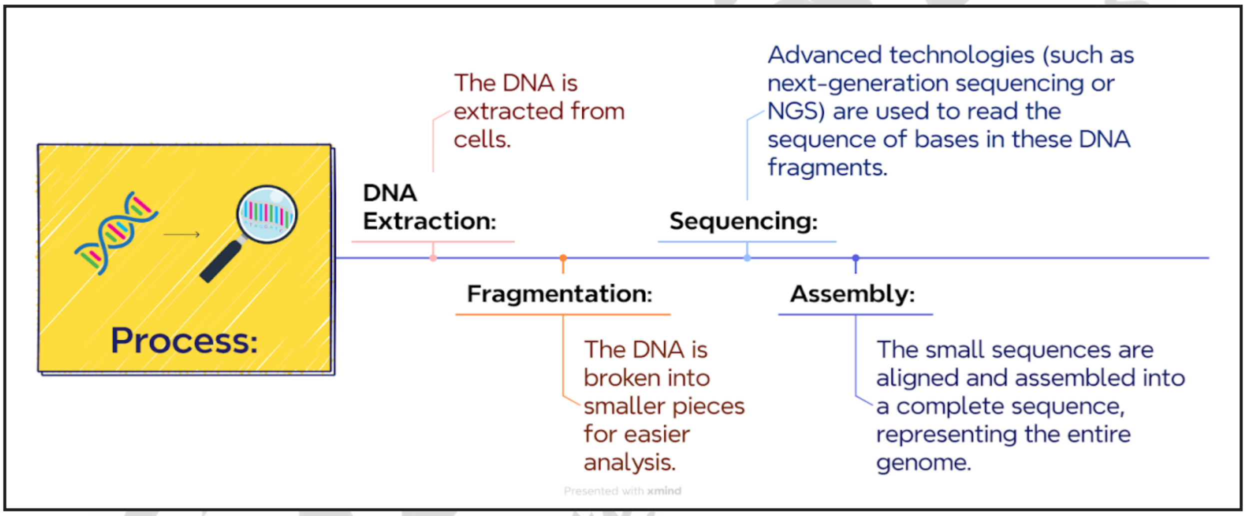
➲ A genome is the complete set of an organism's DNA, including all of its genes. It contains all the information necessary for building, maintaining, and running the organism.

➲ The genome includes both coding regions (genes) and non-coding regions (DNA sequences that don't directly code for proteins but play roles in regulating gene expression and other functions).

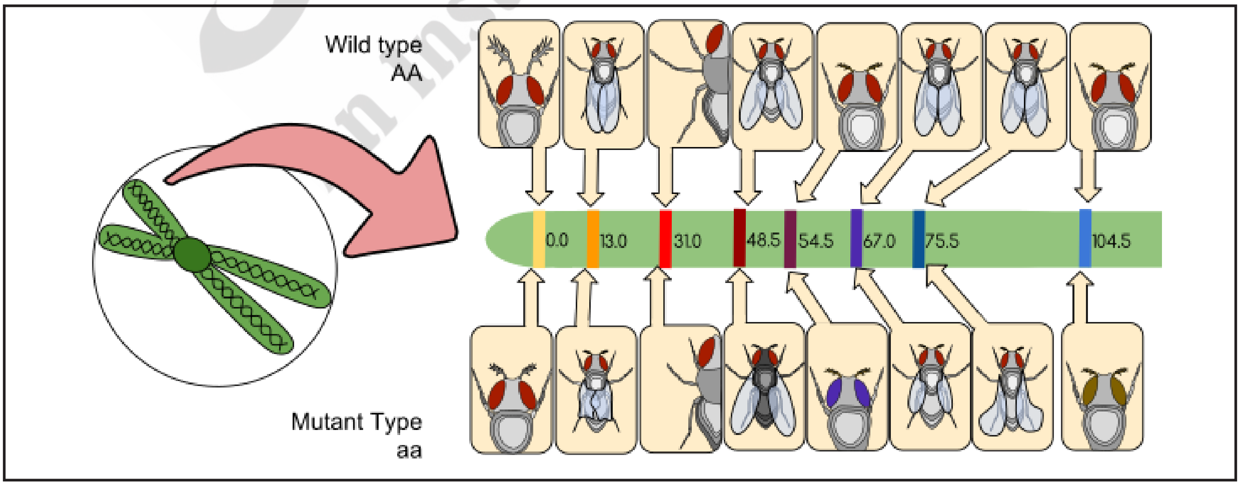
➲ The size of a genome varies significantly among different organisms. In humans, the genome consists of about 3 billion DNA base pairs organized into 23 pairs of chromosomes.



- The genome is considered important because it is a blueprint for all genetic information in an organism, influencing development, health, and inherited traits.
- **Related Terms**
  - **Genome sequencing** is the process of determining the complete DNA sequence of an organism's genome. It involves identifying the order of nucleotides (A, T, C, G) in the DNA.
  - The goal of genome sequencing is to understand the genetic makeup of an organism, which helps in identifying genes, understanding their functions, and studying the relationships between different species.



- **Genome mapping** is the process of creating a map that shows the locations of specific genes or other markers in the genome. It involves identifying the position of genes on chromosomes and determining their relative distances.



- The goal of genome mapping is to provide a "roadmap" of where genes are located on chromosomes, which helps in understanding the function of different regions of the genome and their roles in diseases and traits.

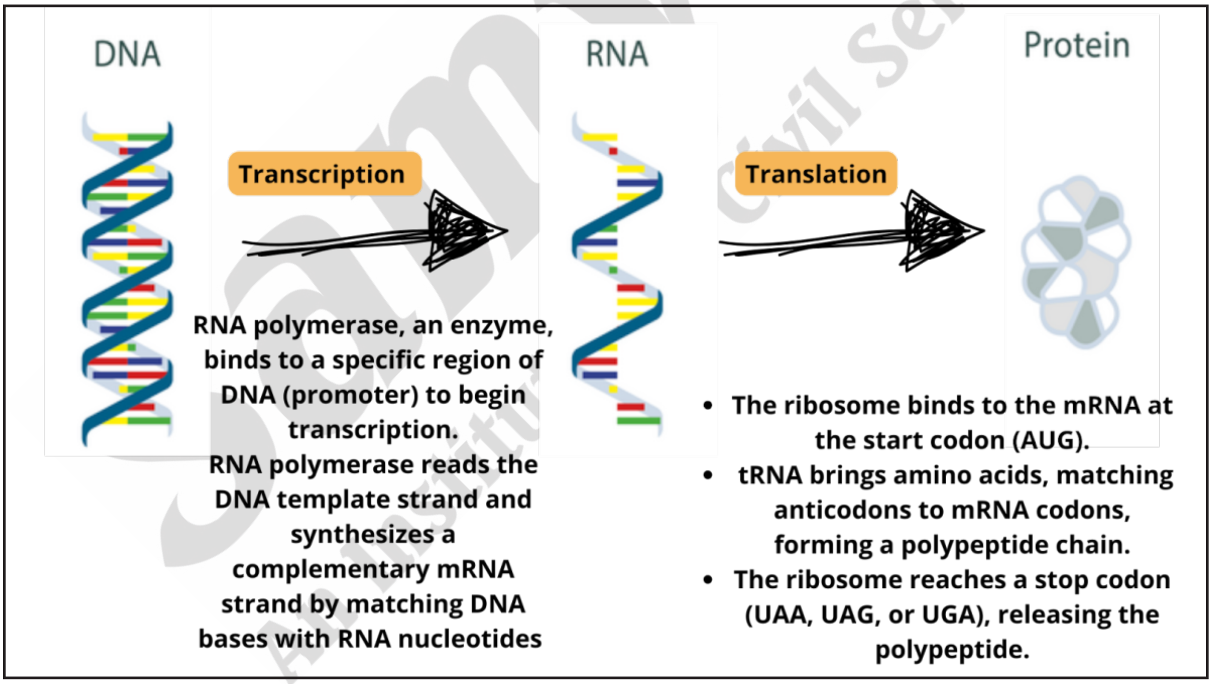
1.1.12 Transcription and Translation

Transcription

- Transcription is the process where the DNA sequence of a gene is copied into **messenger RNA (mRNA)**. It essentially transfers the genetic code from DNA to RNA.
- This process takes place in the **nucleus** of eukaryotic cells.
- The mRNA strand carries the genetic information from the DNA out of the nucleus to the ribosomes, where proteins will be assembled.
- Transcription is the first step in the pathway of gene expression, enabling genetic information in DNA to reach the protein-making machinery of the cell.

Translation

- Translation is the process by which the mRNA sequence is read by the **ribosome** to synthesize a **polypeptide chain (protein)**. It translates the genetic code into amino acid sequences, which form proteins.
- Translation occurs in the **cytoplasm** at the ribosomes, either in the cytosol or attached to the endoplasmic reticulum.
- Translation decodes the information in mRNA to form proteins, which are essential for cell structure, function, and regulation.
- Translation is the final step in gene expression, where the information in the gene ultimately becomes a functional product that carries out activities within the cell.



1.2 RELATED TECHNOLOGY AND PROJECTS

1.2.1 GENE EDITING

- Gene Editing refers to a powerful and innovative technology that enables precise modifications to an organism's DNA (genetic material). This technology has the potential to revolutionize biology, medicine, and agriculture by allowing scientists to alter specific genes to achieve desired outcomes.

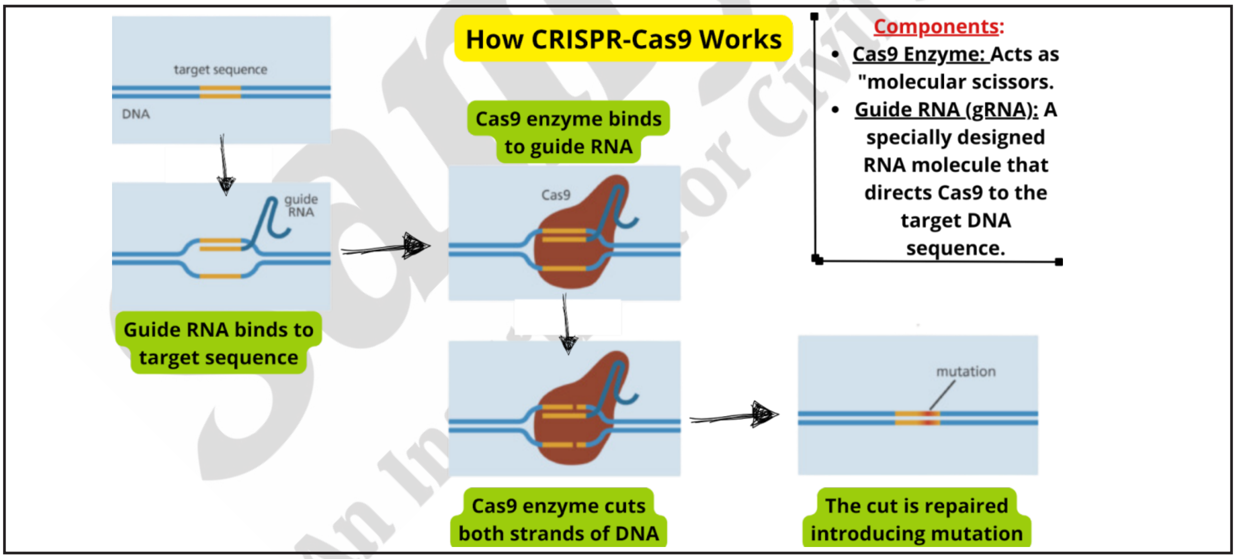
How Gene Editing Works ?

- Gene editing involves the **insertion, deletion, or replacement** of DNA in an organism’s genome using **engineered nucleases** or molecular scissors.

1. **Key Tools in Gene Editing**
   Four families of engineered nucleases are commonly used:
   - **Meganucleases**
   - **Zinc Finger Nucleases (ZFNs)**
   - **Transcription Activator-Like Effector Nucleases (TALENs)**
   - **CRISPR-Cas System (Clustered Regularly Interspaced Short Palindromic Repeats)**
2. **Mechanism**
   - These nucleases create site-specific **double-strand breaks (DSBs)** in DNA.
   - The breaks are repaired via **end-joining** or **homologous recombination**, leading to targeted mutations.
   - **CRISPR-Cas9**, the most well-known gene-editing tool, provides unparalleled precision and efficiency in targeting and modifying DNA sequences. It has democratized gene editing due to its simplicity and wide applicability.

(1) CRISPR-Cas9

- **CRISPR-Cas9** is a groundbreaking gene-editing technology that allows scientists to precisely alter DNA within living organisms
- **CRISPR** stands for **Clustered Regularly Interspaced Short Palindromic Repeats**. These are sequences found in the DNA of bacteria that act as a defense mechanism against viruses.
- **Cas9** is an enzyme that acts like "molecular scissors." It can be guided to a specific location in the DNA by a piece of RNA and cut the DNA at that precise spot.



(2) Transcription Activator-Like Effector Nucleases (TALENs): A Pioneer in Gene Editing

- TALENs are engineered nucleases composed of a TALE DNA-binding domain and a DNA-cleaving domain. They allow scientists to target and cut DNA at specific sequences, making them a critical step in the evolution of gene editing technologies.
- TALENs consist of two primary components:
  - **TALE DNA-Binding Domain**: This domain binds to specific sequences of DNA. It is derived from naturally occurring **Transcription Activator-Like Effectors (TALEs)** that bacteria use to interact with plant cells.
  - **DNA Cleavage Domain**: This is a **nuclease** that cuts the DNA strands at the targeted location.
- **Mechanism of Action**: By fusing these two domains, TALENs can be programmed to bind to nearly any desired DNA sequence, enabling scientists to create **double-strand breaks**

(DSBs) at precise genomic locations. The cell's natural repair mechanisms then fix these breaks, allowing scientists to make targeted mutations or insertions.

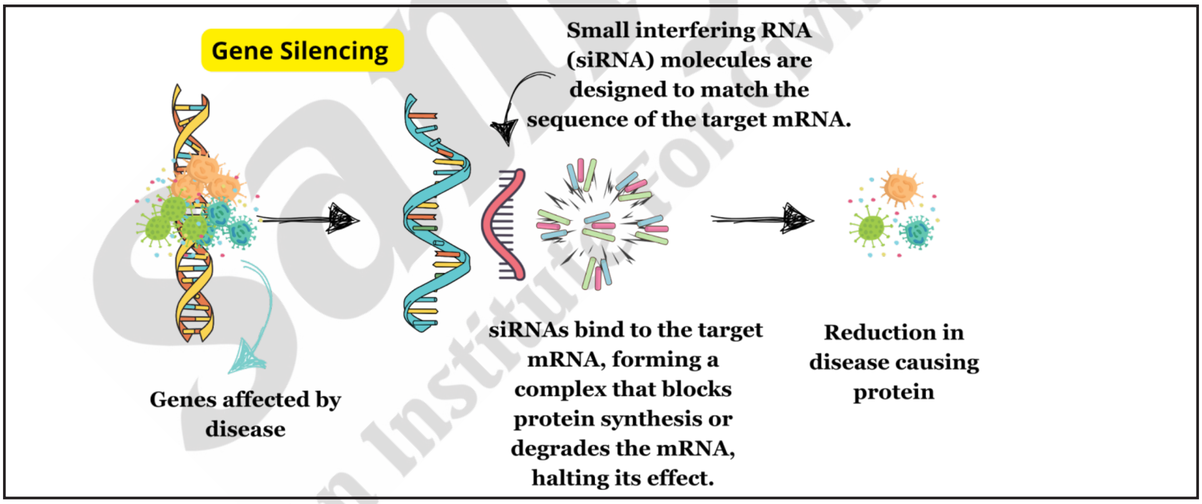
- **Applications**: TALENs are used for gene editing or **genome editing in situ**, a process where DNA is edited directly within the organism. This technology is used for **gene therapy**, the creation of genetically modified organisms (GMOs), and in **biotechnological research**..

(3) Zinc Finger Nucleases (ZFNs)

- **Zinc Finger Nucleases (ZFNs)** are artificial enzymes created by fusing a **zinc finger DNA-binding domain** to a **DNA-cleavage domain**. Zinc fingers are small protein motifs that can be engineered to bind to specific DNA sequences, allowing ZFNs to target and modify genes at precise locations within the genome.
- **Design and Mechanism**: The **zinc finger DNA-binding domain** is customizable, which enables ZFNs to bind to almost any specific DNA sequence. The **DNA-cleavage domain** is typically derived from the FokI enzyme, which cuts the DNA at the targeted location. The combination of the DNA-binding and DNA-cleaving domains results in a tool that can induce **double-strand breaks (DSBs)** at chosen genomic sites.
- **Applications**: Like other gene editing tools, ZFNs leverage the cell's endogenous repair mechanisms, such as **non-homologous end joining (NHEJ)** or **homologous recombination**, to introduce targeted mutations, corrections, or insertions in the genome.

(4) Gene silencing

- Gene silencing is a technique used to "turn off" specific genes, particularly those involved in diseases.
- Unlike genetic modification, it doesn't alter the DNA but suppresses gene expression, reducing the production of harmful proteins.



(5) GENE-DRIVE TECHNOLOGY (GDT)

- Gene-drive technology is a genetic engineering method designed to modify the genetic makeup of organisms, ensuring specific genes are inherited preferentially, bypassing Mendelian inheritance rules.
- It was conceptualized by Austin Burt, a professor at Imperial College London.
- **Process**:
  - A protein cuts DNA at a non-coding region of the mosquito genome.
  - The natural DNA repair mechanism incorporates a *drive sequence* into the damaged region, ensuring selective gene inheritance.
- **Applications:**
  - **Eradication of Malaria**: Used to target malaria-carrying mosquitoes, modifying them to block *Plasmodium* parasite development or reduce their lifespan.

- **Controlled Testing**: Conducted in India, Brazil, and Panama under outdoor, controlled conditions.

Mendelian inheritance

It refers to the principles of genetic inheritance established by Gregor Mendel, based on his experiments with pea plants. These rules describe how traits are passed from parents to offspring through genes:

- **Law of Segregation:** Each organism carries two alleles for a trait, and these alleles separate during gamete formation, ensuring offspring receive one allele from each parent.
- **Law of Independent Assortment:** Genes for different traits are inherited independently of each other, provided they are on different chromosomes.
- **Law of Dominance:** Some alleles (dominant) mask the expression of others (recessive).

1. 2.2 GENE THEREPY

- Gene therapy is a medical technique aimed at treating or preventing diseases by altering an individual's genetic material.
- Gene therapy differs from traditional drugs by addressing the root genetic causes of diseases directly within cells rather than just managing symptoms.
- <u>**Methods:**</u>
  - **Viral Vectors**: Viruses are engineered to carry therapeutic genes into cells.
  - **Non-viral Vectors**: Direct delivery using nanoparticles.

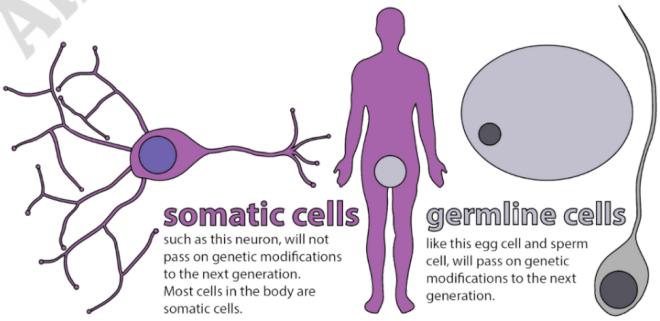
<u>How It Works:</u>

Replacement Therapy: Introducing healthy copies of faulty or missing genes.

- **Gene Editing: Using tools like CRISPR to correct defective genes.**
- **Gene Silencing: Inactivating harmful genes that cause diseases.**
- **Introducing New Genes: Adding genes to help fight diseases.**

(1) Types of Gene Therapy

Somatic cell gene therapy | **Germ line therapy**



Somatic cell gene therapy involves modifying or replacing faulty genes in somatic (non-reproductive) cells to treat or prevent diseases. Unlike germ line therapy, these changes are not inherited by future generations

Germ line therapy involves modifying the DNA in germ cells (sperm or eggs) or early-stage embryos. This ensures that genetic changes are passed on to future generations.

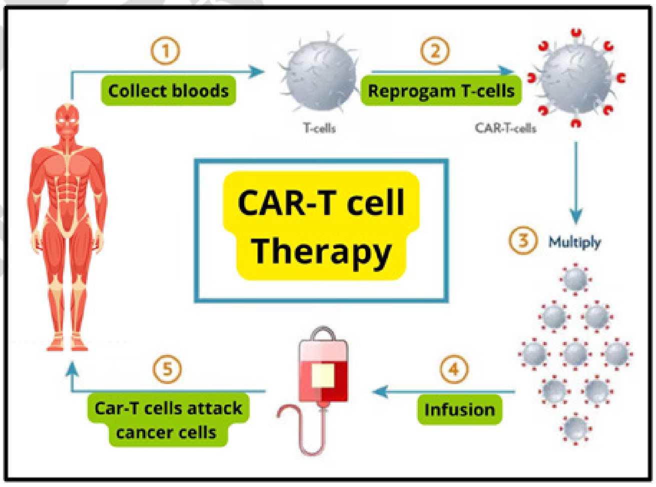
- **ex vivo approach**
  - In gene therapy, the ex vivo approach involves removing specific cells from a patient's body, modifying their genetic material in a laboratory (e.g., using a viral vector), and then reintroducing the corrected cells back into the body.
  - This method allows for precise control over gene editing and is often used for conditions like blood disorders.
- **in vivo approach**
  - The in vivo approach involves directly delivering therapeutic genetic material to the target cells inside the patient's body using vectors like viruses or nanoparticles.
  - This is useful for treating localized or systemic conditions but poses challenges in targeting accuracy.

(2) Applications of Gene Therapy

- Gene therapy has diverse applications in treating various genetic and acquired conditions:
- Used to address inherited disorders such as cystic fibrosis, hemophilia, and muscular dystrophy by replacing defective genes with functional ones.
- Enhances immune response (e.g., CAR-T therapy), introduces tumor suppressor genes, or delivers oncolytic viruses to kill cancer cells.
- Aims to repair damaged heart tissues or improve blood vessel function.
- Treats conditions like Parkinson's disease by targeting specific genetic mutations.
- Investigated for improving immunity against diseases like HIV.

1.2.3 CAR-T CELL THERAPY

- Chimeric Antigen Receptor (CAR)-T cell therapy is a revolutionary immunotherapy that genetically engineers a patient's T cells to target and destroy cancer cells.



- **Process**:
  - **Collection**: T cells are extracted from the patient's blood.
  - **Engineering**: T cells are genetically modified to express CARs, specialized proteins enabling them to recognize cancer-specific antigens.
  - **Multiplication**: Modified T cells are cultured to increase their numbers.
  - **Infusion**: The engineered T cells are reintroduced into the patient's body to attack cancer cells.
- **CART-T Cell Therapy : Applications**
  - Treatment of Hematological cancers.
  - Expanding applications to solid tumors
  - Treatment of Rare Genetic Disorders
  - Treatment of autoimmune conditions
  - Addressing Relapsed or Refrractory Cancers
  - Advancement in precision medicine.