

Biology For Engineers

Module 1

1. Introduction: Why do engineers need to study biology?
2. Chemical foundations and basic chemistry of the cell, and cells as a unit of life.
3. Physical and chemical principles involved in the maintenance of life processes.
4. Cell structures and functions- Ultrastructure and functions of cellular components (Prokaryotic & Eukaryotic cells)
5. Cell wall, Plasma membrane, Endoplasmic reticulum, other organelles
6. Transport Across the cell membrane, cell signaling, nerve impulse conduction

Module 2

1. Exothermic and endothermic versus endergonic and exergonic reactions; Concept of K_{eq} and its relation to standard free energy
2. Spontaneity; ATP as an energy currency
3. Aerobic Respiration (Glycolysis and Krebs cycle)
4. Synthesis of glucose (Photosynthesis), Energy yielding and energy-consuming reactions
5. Concept of energy charge, Morphology of chromosomes; The cell cycle, and phases
6. Mitosis and meiosis

Module 3

1. Laws of heredity: Mendelian and Non-Mendelian
2. Molecular genetics: Structure of DNA & RNA
3. Mutations- Cause, types, and effects on Species
4. Origin of life- Haldane and Oparin's concepts
5. Evolution: Modern concept of natural selection and speciation- Lamarckism, Darwinism/ Neo-Darwinism
6. Bioinformatics- brief idea

Module 4

1. Concept of single-celled organisms, the concept of species and strains
2. Identification and classifications of microorganisms; Microscopy
3. Ecological aspects of single-celled organisms
4. Sterilization and media composition; Growth kinetics
5. Microbial diseases, epidemiology, and public health
6. Human immune mechanism- Types of immunities
7. Antigen-antibody reactions- Applications in human health
8. Immunological disorders: Auto-immune diseases

Module 5

1. Amino acids & proteins- Classification based on function and structure
2. Protein synthesis- components and regulatory mechanism
3. Enzymes- An overview
4. Carbohydrates and Lipids
5. Nucleic Acids
6. Basic concepts on Totipotency and cell manipulation
7. Biotechnology- Basic concepts of recombinant DNA technology
8. Plant & Animal tissue culture- Methods and uses in agriculture, medicine and health
9. Biological indicators, Biofuel
10. Bio-sensors, Biochips, Nano biomolecules

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7. Basic Introduction

A vision of the future:

Imagine a world:

- where there is abundant and healthy food for everyone
- where the environment is resilient and flourishing
- where there is sustainable and clean energy
- where good health is the norm

Each of these goals is a daunting challenge. Furthermore, none can be attained independently of the others—we want to grow more food without using more energy or harming natural environments, and we want new sources of energy that do not contribute to global warming or have adverse health effects. The problems raised by these fundamental biological and environmental questions are interdependent and “solutions” that work at cross purposes will not in fact be solutions.

Fortunately, advances in the life sciences have the potential to contribute innovative and mutually reinforcing solutions to reach all of these goals and, at the same time, serve as the basis for new industries that will anchor the economies of the future. Here are some of the many different ways in which the life sciences could contribute to meeting these challenges:

- A wide variety of plants with faster maturation, drought tolerance, and disease resistance could contribute to a sustainable increase in local food production.
- Food crops could be engineered for higher nutritional value, including higher concentrations of vitamins and healthier oils.
- Critical habitats could be monitored by arrays of remote sensors, enabling early detection of habitat damage and providing feedback on the progress of restoration efforts.
- Water supplies and other natural resources could be monitored and managed using biosensors and other biologically based processes.
- Biological systems could remove more carbon dioxide from the atmosphere, thus helping to maintain a stable climate; the carbon they capture could be used to create biologically based materials for construction and manufacturing.
- Biological sources could contribute at least 20 percent of the fuel for transportation through a 10-fold increase in biofuel production.
- Bio-inspired approaches to producing hydrogen could provide another affordable and sustainable source of fuel.
- Biologically inspired approaches to capturing solar energy could increase the efficiency and lower the cost of photovoltaic technology.
- Manufactured products could increasingly be made from renewable resources and be either recyclable or biodegradable.
- Industrial manufacturing processes could be designed to produce zero waste through a combination of biological treatment of byproducts and efficient recycling of water and other manufacturing inputs.
- Greater understanding of what it means to be healthy could lead to health care focused on maintaining health rather than reacting to illness.
- Individualized risk profiles and early detection could make it possible to provide each person with the right care at the right time.

Integration of the biological sciences with physical and computational sciences, mathematics, and engineering promise to build a wider biological enterprise with the scope and expertise to address a broad range of scientific and societal problems.

However, science and technology alone, cannot solve all of our food, energy, environmental, and health problems. Political, social, economic, and many other factors have major roles to play in both setting and meeting goals in these areas. Indeed, increased collaboration between life scientists and social scientists is another exciting interface that has much to contribute to developing and implementing practical solutions. But the life sciences have the potential to provide a set of tools and solutions that can significantly increase the options available to society for dealing with problems.

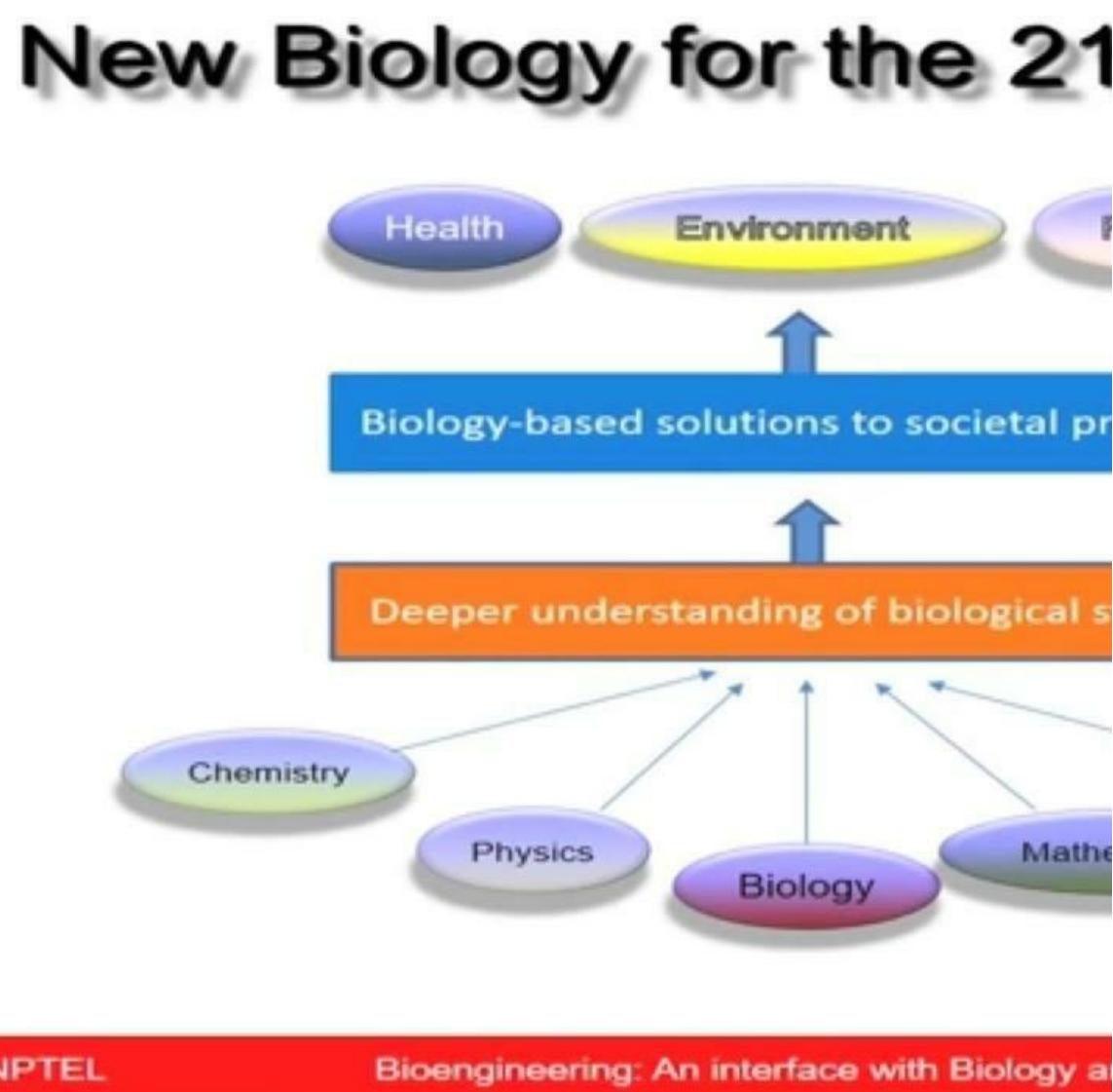


Figure. 1.1: New Biology for the 21st Century

Why do engineers need to study biology?

Major global problems require attention from both biologists and engineers:

1. To feed the growing world's population: The world population is projected to grow to 9.3 billion by 2050. We need fertile soil for agriculture. As it is getting depleted as well as its availability is getting reduced day by day, we need some technical solutions and alternatives to meet the need.
2. To diagnose and treat deadly diseases like HIV, TB, Cancer, etc.
3. Pollution problems
4. Energy sources- to find alternative energy sources. It is also a major challenge.

These problems require inter-disciplinary skills for effective solutions. Genetic Engineering and Biotechnology aim to provide promising solutions. Healthcare devices, applications, biosensors, etc are used simultaneously for healthcare management. Smart medical devices are being developed by engineers having an understanding of biology that act as a boon to society.

We can have a better understanding by citing a few examples in both ways:

1. Bio-inspired engineering: How biology has inspired engineers to solve big problems
2. How Engineering has brought a great revolution in solving various medical, environmental, and industrial problems.
3. Bio-inspired engineering:
 - Green Shield Construction Chemicals private limited, used "lotus effect" in waterproofing. They are producing many products which have the property of water and stain repellence.
 - Creation of sustainable buildings: lessons from Termites. These buildings are based on the principle of how termite mounds maintain stable temperature by passive cooling. Example: Eastgate building, Zimbabwe-Air conditioning system modeled on self-cooling mounds termites.
 - Fog harvesting technology: Technology developed by IIT, Mumbai alumnus, Material Science and Engineering, MIT. He took lessons from the beetle.
 - Bullet train: Bullet-shaped nose has been remodeled based on the structure of the beak of a kingfisher and the pantograph was redesigned using the serrated feather of an owl to reduce the noise pollution created by it. This new design has solved the problem of the "Shinkansen sonic boom", increased the speed of the train by 10%, and made it 15% more efficient as well.
 - Aeroplanes have been designed based on the bird's flying principle.



Figure 1.2: Bullet Train remodeled by observing the structure of bird.



URL: <http://news.mit.edu/2011/fog-harvesting-0421>



MOOC-NPTEL

Bioengineering: An interface with Biology a

Figure 1.3: Fog Harvesting Technology

“Natural world is full of excellent designs that we can learn from. Now, there is a need for interaction between good biologists and engineers.

We need good engineers to convert the biological observations into useful devices.”

- Anonymous

How has engineering brought a great revolution in solving problem in various fields such as medicine, the environment, and industries?

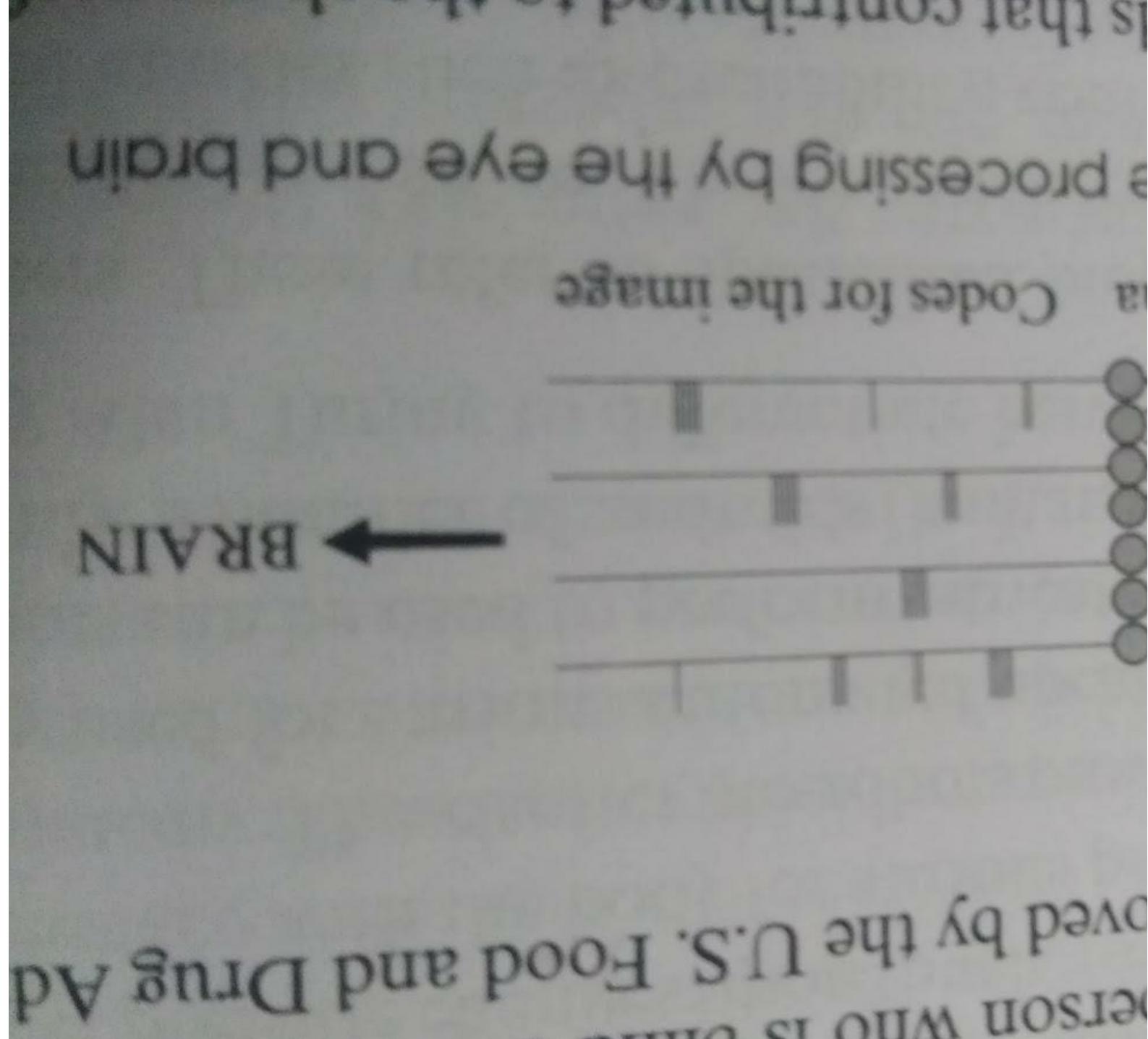


Figure 1.4: Image processing by the eye and brain

How do we see and interpret?

- When the eyes see something, the lens projects an image onto the retina.
- Photoreceptor cells of the retina convert it into appropriate codes.
- The codes get converted to corresponding electrical signals by the cells located just behind the retina.
- The electrical signals are conveyed to the brain where the image is interpreted.
- All this happens in milliseconds or less so that we are able to visually perceive the happenings around us in real-time.

When the retina gets diseased, the relevant cells die and the image can't be converted into appropriate codes.

Is there any solution? Yes, the Prosthetic Retina!

The prosthetic retina is a device that is able to convert optical images into codes and then to electrical signals which can be transmitted to the brain. The prosthetic is so small to be placed inside the eye at the retina and it is connected to the cells that transmit signals to the brain.

Different engineering fields were involved in developing the prosthetic retina:

Computer Science principles were used to develop the relevant codes. Principles of electrical engineering, material engineering, biological engineering, etc. were used to construct the device to ensure its biocompatibility and to facilitate its implantation for long-term use in the eye.

Few more examples: Biosensors, Bio-chips, Bio-pesticides, Bio-fertilizers, Concrete Self-heal, Bio-filters, Nanoparticles, etc. are things that have been manufactured based on the integrated principles of biology and engineering. Medical Diagnostics Apparatus: SPHYGMOMANOMETER, STETHOSCOPES, OPHTHALMOSCOPES, PULSE OXIMETERS, FETAL DOPPLER, ECG MACHINE, etc. Genetic engineering: It needs sophisticated apparatus and

equipment for gene manipulation.

All the above technologies need engineering principles in integration with biological ones to serve mankind. And, currently, almost all the tech giants are focusing on some or other biological-driven projects to deal with many problems related to human health, energy sources, or the environment and this forms the basis for integrating biology with engineering.

1. Chemical foundations and basic chemistry of the cell

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Elements that may possibly be essential for some species: Li, Cd, Sn, Br

Elements believed to be essential for some other species: Sr, Ba, W, As

Percentage of elements in the human body: 99% of the human body is comprised of 11 elements. These elements are Hydrogen, carbon, nitrogen, oxygen, phosphorous, sulphur, chlorine as phosphates, sulphates and sodium, magnesium, potassium, and calcium as bulk metal ion nutrients.

Elements Percentage in human body

Hydrogen	62.8
Oxygen	25.4
Carbon	9.4
Nitrogen	1.4
Others	1.0

Comparison of the composition of elements present in the human body, seawater, and earth's crust:

Composition of the human body (%) Composition of seawater (%) Composition of earth's crust (%)

H (63)	H (66)	O (46.6)
O (25.4)	O (33)	Si (27.7)
C (9.5)	Cl (0.33)	Al (8.1)
N (1.4)	Na (1.1)	Fe (6.0)
Ca (0.31)	Mg (0.13)	Ca (5)
P (0.22)	S (0.017)	Na (2.3)
Cl (0.03)	Ca (0.04)	K (1.5)
K (0.06)	K (0.04)	Mg (3)
S (0.05)	C (0.0014)	Ti (0.44)
Na (0.03)	Br (0.0005)	H (0.14)
Mg (0.01)		C (0.20)

From the above table, we can infer that the composition of the human body is more similar to seawater than the earth's crust.

All living organisms are made up of the same chemicals though their relative amounts may be different. Most of the elements exist in the form of compounds i.e. in a combined state. These compounds are divided into two main categories:

1. Inorganic Compounds
2. Organic Compounds

Inorganic Compounds:

They usually lack Carbon and are structurally simple. Their molecules have only a few atoms and cannot be used by cells to perform complicated biological functions.

They include Na, K, Ca, Mg, water, many salts of Na or Ca in chloride or carbonate forms, acids and bases, compounds of sulfate and phosphate, etc. They have either ionic or covalent bonds. Water makes up 55-60% of a lean adult's total body mass.

Organic Compounds:

They always contain carbon and usually hydrogen. They are formed of covalent bonds. Most of them are large molecules and many are made up of long chains of carbon atoms. Organic compounds make up 38-43% of the human body. Many organic molecules are large and have unique characteristics that allow them to carry out complex functions. Important organic compounds are carbohydrates, lipids, proteins, nucleic acids, etc.

The study of the chemistry of living organisms is known as biochemistry.

The molecules or compounds obtained from the living tissue are called Biomolecules.

The different types of biomolecules present in the cell are collectively called Cellular Pool.

The compounds of Carbon are central to life on this planet/ Carbon forms the basis of life on this earth. The properties of carbon make it the backbone of the organic molecules which form living matter. These properties are:

- Carbon is a versatile element because it can form four covalent bonds.
- A carbon atom can form a covalent bond with other carbon atoms and can form a long chain. This property is called catenation.
- Carbon skeletons can vary in length, branching, and ring structure.
- The functional groups of organic molecules are the parts involved in chemical reactions.
- Organic molecules important for life include relatively small monomers as well as

- large polymers.

In fact, there are nearly 10 million carbon-based compounds in living things! However, the millions of organic compounds can be grouped into just four major types: carbohydrates, lipids, proteins, and nucleic acids. DNA is the genetic material. It carries all the genetic information. Proteins catalyze all the reactions in our body and also form essential compounds in blood, muscle, bone, skin, etc. Carbohydrates are energy molecules. Lipids are structural compounds as well as provide energy to the body.

*Silicon also has similar properties as carbon but, carbon forms the molecules or compounds that are comparatively more stable due to its smaller size.

1. A cell is called “a unit of life”!

A cell is the smallest unit of a living thing. Living things, whether made of one cell called a unicellular organism (like bacteria) or many cells called a multicellular organism (like a human). A cell can perform all the functions of life processes. The macromolecules carbohydrates, proteins, lipids, and nucleic acids make up all of the structural and functional units of cells. In unicellular organisms, cells are independent, single-celled organisms that take in nutrients, excrete wastes, detect and respond to their environment, move, breathe, grow, and reproduce. In a multicellular organism, cells are the basic building blocks of all organisms. We can find levels of organizations. Several cells of common origin form a group and perform a particular function form tissue; several tissues combine to form an organ (your stomach, heart, or brain); Several organs make up an organ system (such as the digestive system, circulatory system, or nervous system). Several systems that function together form an organism (like a human being). There are many types of cells all grouped into one of two broad categories: prokaryotic and eukaryotic. For example, both animal and plant cells are classified as eukaryotic cells, whereas bacterial cells are classified as prokaryotic.



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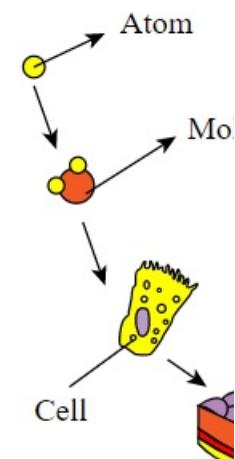
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- Organ system A group of organs that work together to perform a specific function. The body's different organ systems are the circulatory system, lymphatic system, reproductive system, muscular system, and endocrine system.
- Organism An organism is the most complex level of organization. It contains all the body functions. All the atoms, molecules, and cells work together to help in the sustenance of life. Human is the most complex organism on earth.



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Figure 1.6: Organisation level

Life processes

There are certain basic vital processes that are essential for an organism to stay healthy and to maintain the proper functioning of the body's organ systems and are necessary for survival. These basic essential activities performed by an organism are called as life processes.

These are:

- Nutrition, digestion, and absorption
- Organization
- Responsiveness
- Homeostasis
- Transportation
- Metabolism
- Respiration

- Reproduction
- Excretion

Organization

At all levels of the organizational scheme, there is a division of labor. Each component has its own job to perform in cooperation with others. Even a single cell, if it loses its integrity or organization, will die.

Responsiveness

Responsiveness or irritability is concerned with detecting changes in the internal or external environments and reacting to that change. It is the act of sensing a stimulus and responding to it. Homeostasis, any self-regulating process by which biological systems tend to maintain stability while adjusting to conditions that is optimal for survival. If homeostasis is successful, life continues; if unsuccessful, disaster or death ensues. The stability attained is actually a dynamic equilibrium, in which continuous change occurs yet relatively uniform conditions prevail.

Nutrition

Nutrition is the process where an entity takes food and utilizes it for energy. It is a pivotal biological process that helps living beings to obtain their energy from various sources. Nutrients are the substances which provide nutrition based on body requirements. Mode of nutrition varies from one species to another. All green plants exhibit autotrophic nutrition as they synthesize their food by the process of photosynthesis, using light, carbon dioxide, and water. Animals are grouped into a heterotrophic mode of nutrition, as they depend on plants for food. All vertebrates, including humans and some unicellular organisms such as amoeba exhibit the holozoic mode of nutrition. Digestion is the chemical breakdown of the ingested food (Carbohydrates, Proteins, Lipids) into absorbable molecules (glucose, galactose and fructose, amino acids, dipeptides and tripeptides, fatty acids, monoglycerides, cholesterol and lysolecithin).

Absorption: The absorbable molecules such as glucose, galactose and fructose, amino acids, dipeptides and tripeptides, fatty acids, monoglycerides, cholesterol and lysolecithin can get absorbed by the cells. Inside the cells these are metabolized to form different important elements or compounds which are necessary for our body. These are further transferred into the blood from the cells to get transported via blood to different sites wherever it is required to perform various functions of life. It refers to the movement of nutrients, water, and electrolytes from the lumen of the small intestine into the cell, then into the blood.

Transportation

Transportation or transportation systems in plants and animals are entirely different. In animals, transportation is carried out through the circulatory system. This system includes the heart, blood, and blood-carrying blood vessels.

Plants have particular tissues called vascular tissues for the conduction and transportation of materials throughout the plant parts. Vascular tissues include xylem and phloem. Xylem conducts water and minerals from roots to the shoot system while phloem transports prepared food from leaves to other plant parts.

Metabolism

Metabolism is the chemical process in which different types of chemical reactions are involved in controlling the living state of the cells in an organism. It is broadly classified into catabolism and anabolism. Catabolism: The metabolic process in which energy is released. Anabolism: The metabolic process in which energy is stored for further requirements.

Respiration

Respiration includes the exchange of gases as well as the burning of food. Animals have a well-defined respiratory system for respiration. In the process of respiration, glucose is broken down to extract energy. It is a redox reaction that can take place with or without oxygen and takes place in the mitochondria of the cell and releases energy in the form of ATP. Respiration is mainly of two types- aerobic respiration and anaerobic respiration.

Reproduction

The biological process of reproducing their own offspring determines the continuity of species, generation after generation. The basic types of reproduction are sexual and asexual reproduction. Sexual Reproduction: The process of reproducing their own offspring by the involvement of the two parents. Asexual Reproduction: The process of reproducing their own offspring by the involvement of a single parent.

Excretion

Elimination of toxic waste substances from the body is called excretion. There are various modes of excretion and it generally differs with the different types of living species. Plants have different modes of excretion. The oxygen during photosynthesis and carbon dioxide during respiration are given out through a structure called stomata. Excess water is removed by transpiration. They shed dead cells and even plant parts like leaves. Waste products are also stored in vacuoles and leaves that fall off. Other waste products include gums and resins, etc. Humans have a well developed excretory system consisting of a couple of kidneys, ureter, urinary bladder, and urethra. The kidney has a structural unit called the nephron where the blood is filtered. After filtration, the pure blood circulates back to other parts and extracted waste products are passed into the ureter. The urinary bladder collects urine, which is excreted through the urethra.

1. Physical and chemical principles involved in the maintenance of life processes.
2. The transport of molecules and ions through the cell membrane forms the basis of all cellular mechanisms. It includes:
 3. Passive Transport: Energy is not required to transport the molecules or ions across the membrane. Here, molecules or ions move along the concentration gradient. Example: Osmosis, diffusion and facilitated diffusion
 4. Active Transport: It needs energy for transporting the molecules and ions across the cell membrane. Molecules and ions move against the concentration gradient.
 5. Membrane potential and action potential are also important for the life processes like responsiveness, adaptation, homeostasis, etc. This will be discussed a little later in this module.
 6. Cell

1. A brief description of the discovery of a cell and formulation of cell theory

A cell can be defined as the structural and functional unit of life. It was first observed by Robert Hook in 1665. He observed a thin slice of cork (plant part) under the microscope. It was like a honey-comb structure with empty spaces inside or compartments surrounded by the firm cell wall. Whatever he observed was the dead plant cells. Anton van Leeuwenhoek first discovered free-living algae Spirogyra cells in water in the pond in 1674 with the improved microscope. The living cells were first discovered by Antony Van Leeuwenhoek. He observed living cells and called them 'animalcules'. Some small 'animalcules' are now called bacteria. In 1831, Robert Brown observed the nucleus in the cell. In 1855, Rudolph Virchow observed that new cells arise from preexisting cells. In 1838, M.J. Schleiden, a German Botanist studied many plants section under a microscope and came to the conclusion that, "all plants are made up of cell". In 1838, T.S. Schwann, a German Zoologist came to the similar conclusion that "all animals are made up of cell". In 1839, Schleiden and Schwann put forth a theory called "cell theory".

The cell theory can be summarised as:

1. The cell is the fundamental unit of structure and function in living things.
2. All organisms are made up of one or more cells.
3. Cells arise from other cells through cellular division.

The expanded version of the cell theory can also include:

- Cells carry genetic material passed to daughter cells during cellular division
- All cells are essentially the same in chemical composition
- Energy flow (metabolism and biochemistry) occurs within cells
 - 1. Cell size and shape

The size, structure and shape of the cells are not fixed. These depend upon their specialized functions and location as well. Neurons are long and branched as it has to pass the information in the form of action potential from sensory organs to the brain and from the brain to the sensory organs. Parenchyma and RBCs are equidimensional. The cell size ranges from 0.2 μm to 2mm. Some cells are encased in a rigid wall while some have a thin flexible cell membrane as their outer covering.

Few examples are:

Cell Shape	Example
Variable cells	
Fixed shape	
Spherical/Oval cells	Amoeba, Leucocytes
Flattened cells	Squamous epithelium, endothelium, and upper layer of epidermis
Cuboidal cells	Thyroid gland follicles
Columnar cells	The cells lining the intestine
Discoidal cells	Erythrocytes
Spindle shaped cells	Smooth muscle fibres
Elongated cells	Nerve cells
Branched cells	Pigment cells of skin

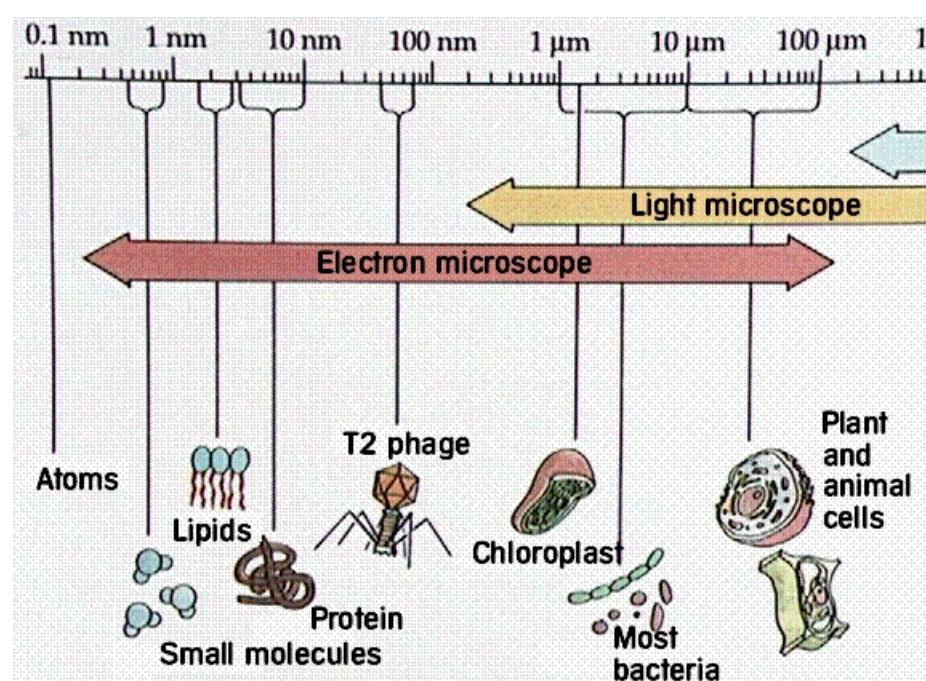


Figure 1.7: Cell size

• 1. Classification of cells

Depending upon the organisation, cells are categorized into two types

- 1. Prokaryotic cells
- 2. Eukaryotic cells

1. Prokaryotic cells: The word prokaryotes are derived from Greek word 'pro' means primitive or old and karyon means nucleus. Prokaryotic cells are primitive type of cells. They do not possess true nucleus or membrane bound organelles.

Structure and function of a Prokaryotic cell:

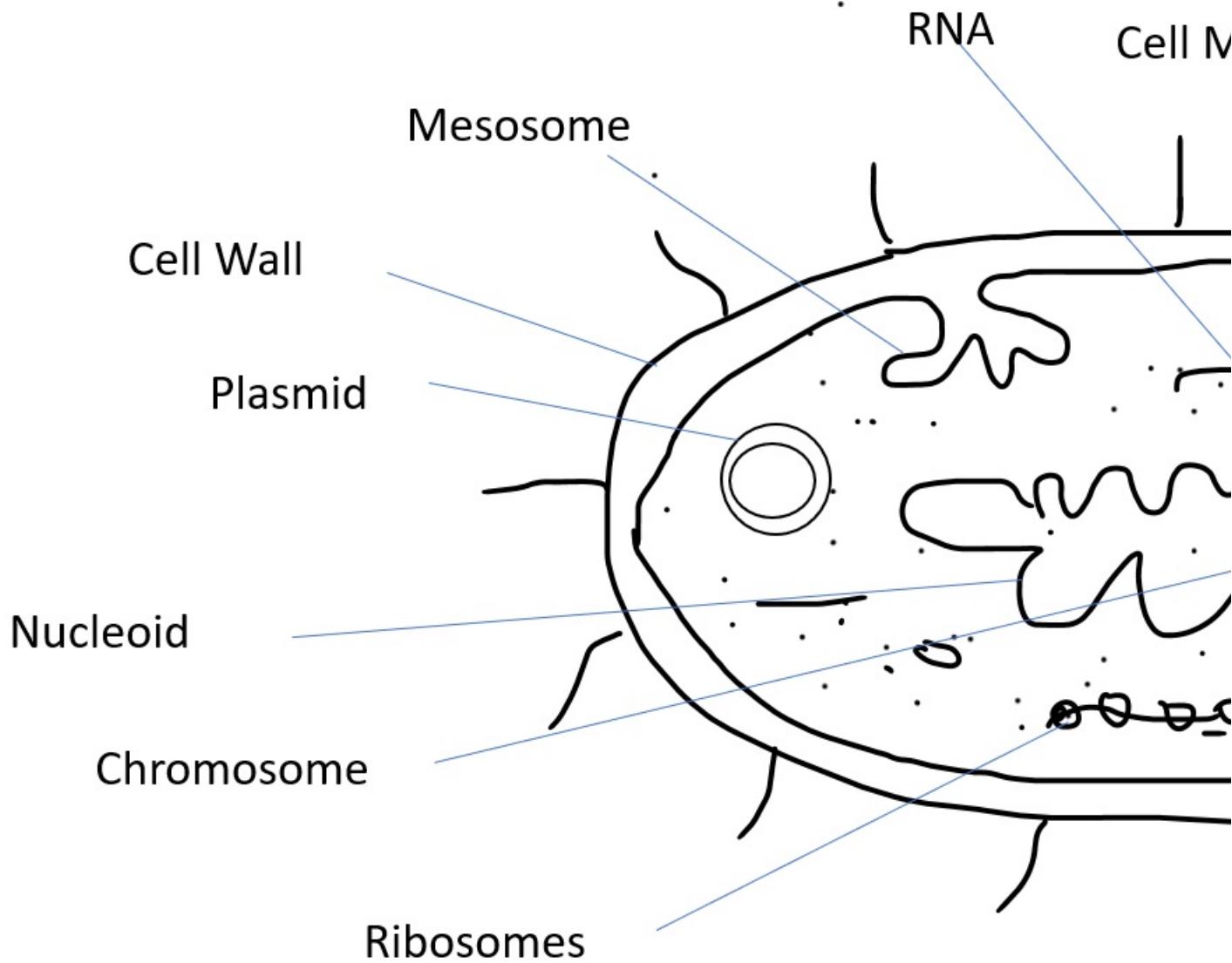


Diagram: Structure

Figure 1.8: Structure of a typical prokaryotic cell

a. Nucleoid: Most bacteria have a genome that consists of a single DNA that is several million base pairs in size and is "circular". The region where chromosome is present is known as nucleoid.

b. Plasmid: In addition, bacteria may have one or more self-replicating smaller circular DNA molecules, called plasmids. Plasmids provide additional characteristics to the bacteria. Example: R plasmid provides a bacteria resistivity from antibiotics, CF plasmid is responsible for the formation of a structure called pili which helps

in conjugation, etc.

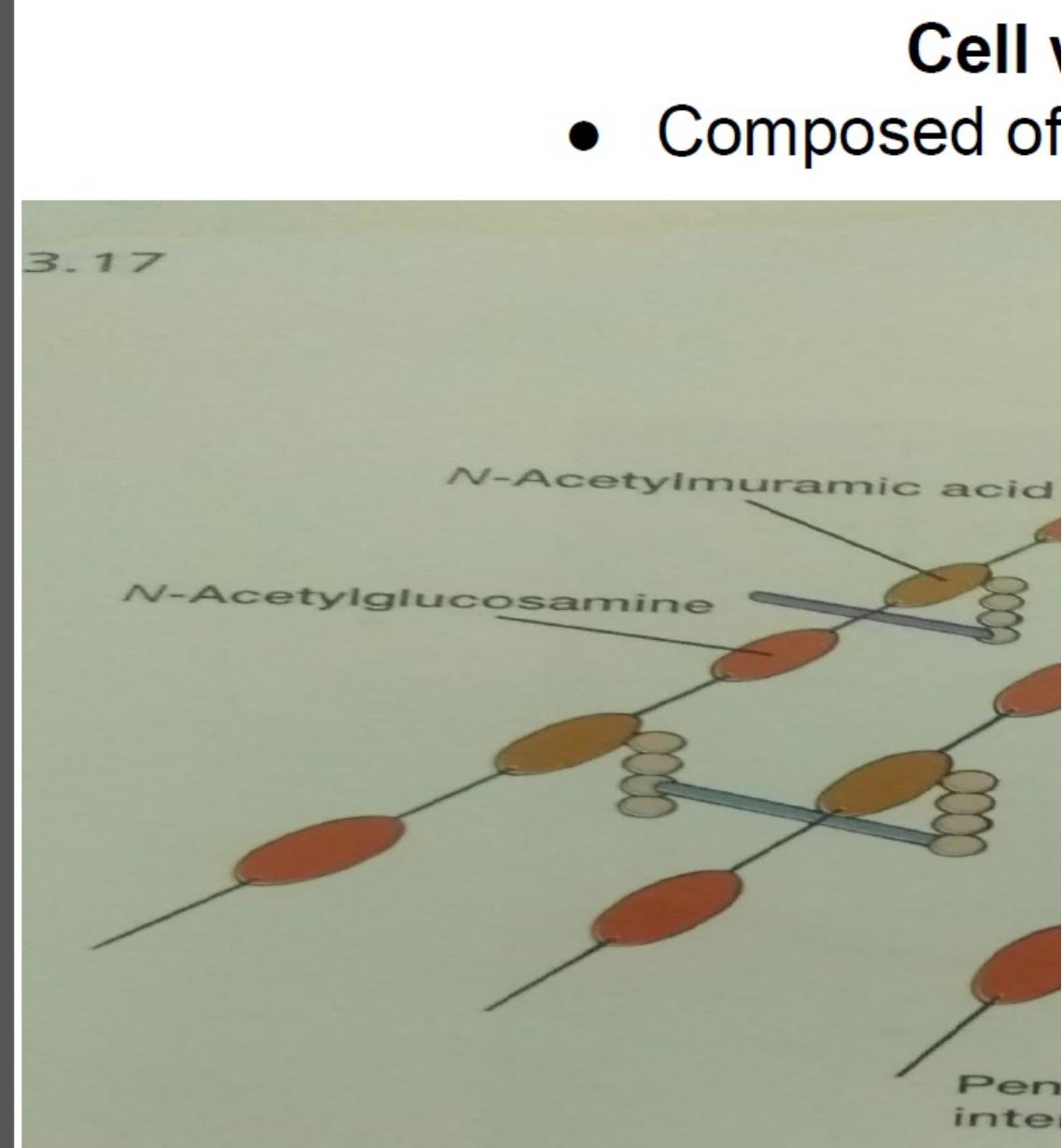
c. Cell envelope: All the structures present outside the plasma membrane form the cell envelope. This includes the cell wall and capsule or slime layer (if present). Capsule/Slime layer is made up of polysaccharides called glycocalyx. Both capsule and slime layer are made up of glycocalyx but the difference is; capsule is firmly bound to the cell wall and slime layer is loosely attached to the cell wall. Capsule or Slime layer surrounds the cell wall. It does not present in all prokaryotic cells. Some may have capsule or some other may possess slime layer and some do not have either capsule or slime layer. This layer makes the cell more infectious.

d. Cell wall: It is the outermost layer of a prokaryotic cell. It is composed of peptidoglycan or murein. It protects the cell from environmental stress and also provides mechanical strength.

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Cell wall



Figure 1.9: Bacterial cell wall components

On the basis of the difference in the cell wall composition it is of two types: Gram positive and Gram negative cell wall.

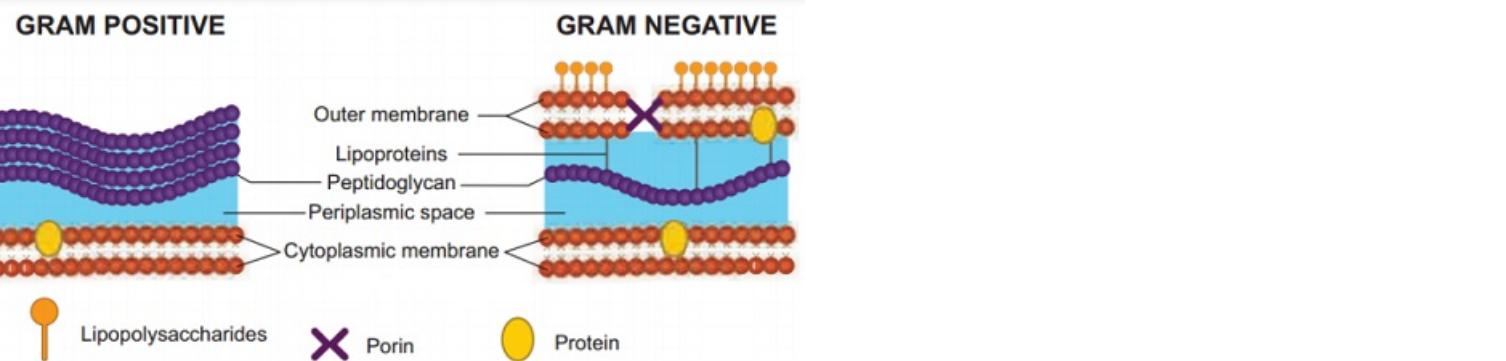


Figure 1.10: Differences in the cell wall of gram-positive and gram-negative bacteria

Gram-positive bacteria stain blue when it is stained with the Gram-stain procedure while gram-negative bacteria do not take gram stain and appear pink in colour.

Periplasmic space: The space between the plasma membrane and the cell wall is called periplasmic space. It is filled with a loose network of peptidoglycan. It can be said to be a gel-like structure and present in both gram-positive and negative bacteria. But, in gram-negative bacteria it is prominent.

Outer membrane: It is present in gram-negative bacteria. It is an additional membrane outside the peptidoglycan periplasmic space. It is composed of lipopolysaccharide and lipoprotein. It helps in attachment to the animal cells and helps in causing disease.

e. **Cell membrane/Plasma Membrane:** Cell membrane surrounds the fluid-filled structure cytoplasm. It is semi-permeable as well as selectively permeable in nature. It is composed of proteins, lipids, and carbohydrates.

- Bacterial plasma membrane usually has a higher proportion of proteins than do Eukaryotic membrane.
- The reason is bacterial cell membrane has to perform various functions that are carried by other organelle membranes in eukaryotes.
- So, phospholipids constitute about 40% of the cell membrane and proteins form about 60% of the cell membrane.
- Asymmetric in nature
- Selectively permeable-allows passage of water and uncharged molecules up to the molecular weight of 100dalton.

Bacterial cell membrane shows diversity in membrane lipids.

A few common names are Phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, cardiolipin, glycolipids, sphingolipids, etc.

Bacteria have their own special membrane adaptations in the form of hopanoids, the bacterial equivalent of membrane sterols. Hopanoids have 5 rings, and do not require oxygen for their biosynthesis.

f. **Cytoplasm:** Jelly-like fluid substances surrounded by the plasma membrane is the cytoplasm. Here, nucleoid, RNA, polyribosomes, cell inclusions, etc. are present.

g. **Mesosome:** Mesosomes are infolding of the cell membrane where more proteins are present. It has many important functions:

- Respiration
- Helps in DNA segregation
- In photosynthetic bacteria it performs the function of photosynthesis

h. **Flagella:** Flagella helps in the locomotion of the organism.

i. **Pili:** It helps in conjugation.

j. **Fimbria:** It helps the organisms to get attached to the substratum.

k. 70S ribosomes are present which help in protein synthesis with the help of RNAs.

1. Eukaryotic Cell

Eukaryotic cells have a nucleus enclosed within the nuclear membrane and form large and complex organisms. Protozoa, fungi, plants, and animals all have eukaryotic cells.

The features of eukaryotic cells are as follows:

1. Eukaryotic cells have the nucleus enclosed within the nuclear membrane.
2. Apart from nucleus cell has also other membrane bound organelles which perform specific functions.

3. Flagella and cilia are the locomotory organs in a eukaryotic cell.
4. A cell wall is the outermost layer of the eukaryotic cells such as plant and fungi.
5. The cells divide by a process called mitosis.
6. The eukaryotic cells contain a cytoskeletal structure.
7. The nucleus contains a single, linear DNA, which carries all the genetic information.

Nucleus

Nuclear envelope: membrane enclosing the nucleus. Protein-lined pores allow material to move in and out.

Chromatin: DNA plus associated proteins.

Nucleolus: condensed region where ribosomes are formed.

Peroxisome: metabolizes waste

Endoplasmic reticulum
Rough: associated with ribosomes; makes secretory and membrane proteins.
Smooth: makes lipids.

Cytoskeleton

Microtubules: form the mitotic spindle and maintain cell shape.

Centrosome: microtubule-organizing center.

Intermediate filaments: fibrous proteins that hold organelles in place.

Microfilaments: fibrous proteins; form the cellular cortex.

Plasma membrane

Lysosome: digests food.

Golgi apparatus: modifies proteins.

Cytoplasm

Mitochondria: produce energy.

Vacuole

Plasmodesmata: channels connect two plant cells

Cell wall: maintains cell shape

Plasma membrane

Cytoplasm

Central vacuole: filled with cell sap that maintains pressure against cell wall

Cytoskeleton: microtubules intermediate filaments microfilaments

Endoplasmic reticulum
smooth
rough

Chloroplast: site of photosynthesis

Figure 1.11: An Animal Cell and A Plant Cell

Structure Of Eukaryotic Cell

The eukaryotic cell structure comprises the following:

Plasma Membrane

- The plasma membrane separates the cell from the outside environment.
- It comprises specific embedded proteins, which help in the exchange of substances in and out of the cell.

Various models were proposed regarding the structure of plasma membrane:

- 1. Davson-Danielli Model/Sandwich Model
 2. Robertson Model
 3. Fluid Mosaic Model

Sandwich Model: It was proposed by James Danielli and Hugh Davson in 1935. The model describes a phospholipid bilayer that lies between two layers of globular proteins and it is trilaminar and lipoproteins.

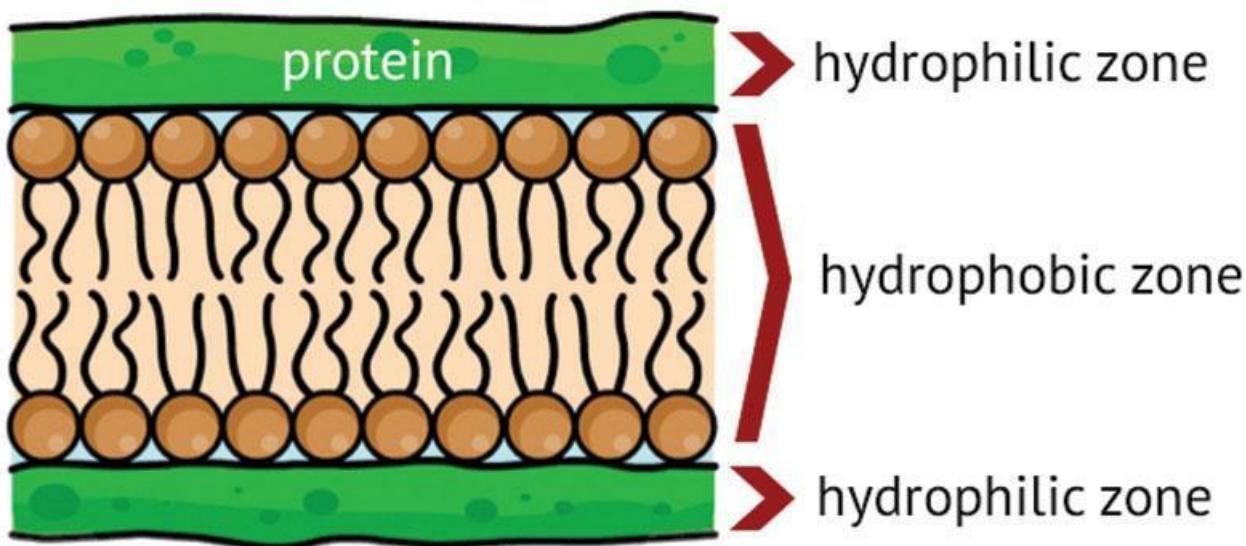
In this arrangement, the association between the surface proteins and bimolecular lipid leaflet would be maintained primarily by electrostatic interactions between the polar ends of each lipid molecule and charged amino acid side chains of the polypeptide layers. Either electrostatic or van der Waals bonds could bind other groups to the outer protein surface.

Danielli and Davson proposed that such a membrane would exhibit selective permeability, being capable of distinguishing between molecules of different sizes and solubility properties and also between ions of different charges.

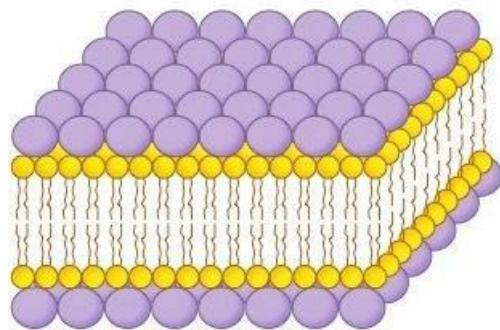
From the speed at which various molecules penetrate the membrane, they predicted the lipid bilayer to be about 6.0 nm in thickness, and each of the protein layers of about 1.0 nm thickness, giving a total thickness of about 8.0 nm.

Lipid bilayer composed of phospholipids (hydrophobic tails inside, hydrophilic heads outside). Proteins coat the outer surface. Proteins do not permeate the lipid bilayer.

Sandwich (Davson-Danielli) model of cell membrane



Davson-Danielli Model (1935)



Proteins form distinct layers (*sandwich*)

Figure 1.12: Davson-Danielli Model of plasma membrane

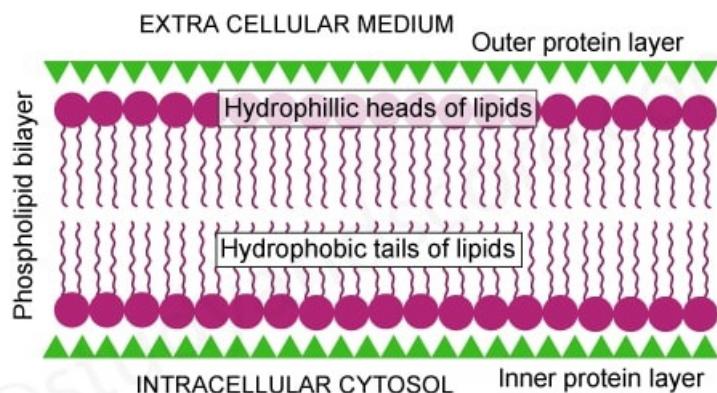
Support of Davson-Danielli model

- The Danielli-Davson model got support from electron microscopy.
- In high magnification electron micrographs, membranes appeared as two dark parallel lines with a light-colored region in between.
- Proteins appear dark in electron micrographs and phospholipids appear light – possibly indicating proteins layers either side of a phospholipid core.
- The total thickness of the membranes too turned out to be about 7.5 nm.

Problems in the Davson-Danielli Model

- It assumed all membranes were of a uniform thickness and would have a constant lipid-protein ratio.
- It assumed all membranes would have symmetrical internal and external surfaces (i.e. not bifacial)
- It did not account for the permeability of certain substances (did not recognize the need for hydrophilic pores)
- The temperatures at which membranes solidified did not correlate with those expected under the proposed model.

Robertson Model: J. David Robertson modified the sandwich model in 1959. He said that lipid bilayer is covered by β -protein molecules. He also gave the concept of the unit membrane. The unit membrane concept says that all membranes (plasma membrane as well as membranes of the cell organelles) have an underlying bilayer composed of phospholipids embedded between the protein layers.



UNIT MEMBRANE MODEL OF PLASMA MEMBRANE -Robertson

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Figure 1.13: Unit Membrane Model of Plasma Membrane

Fluid Mosaic Model: The lipids and integral membrane proteins diffuse laterally within the plane of the membrane; hence the “fluid mosaic model” of cell membranes. The fluid Mosaic Model of Plasma membrane was given by Singer and Nicolson in 1972. However, because of the hydrophobic inner core, phospholipids and integral membrane proteins do not spontaneously cross the lipid bilayer or flip across it from one side to the other. Cell membrane thickness- 5 to 10nm. Lipids that can pack more tightly (like saturated fatty acids and sterols) make membranes more rigid and stronger, but less fluid. Anything that disrupts close packing of lipids, such as higher temperatures or unsaturated fatty acids with kinks or bends, make membranes more fluid.

Membrane proteins

1. Peripheral proteins or extrinsic proteins:
 - a. loosely connected to the membrane and can be easily removed.
 - b. Soluble in aqueous solution.
 - c. makeup 20-30% of total membrane proteins.
2. Integral Proteins or intrinsic proteins:
 - a. Forms about 70 to 80% of the total membrane proteins.
 - b. Not easily extracted from membranes.
 - c. Insoluble in aqueous solutions when freed of lipids.
 - d. Amphiphatic i.e it possesses both hydrophilic and hydrophobic regions. Hydrophobic regions are buried in the lipid bilayer. Hydrophilic region projects from the lipid layer.

Binding proteins, transport proteins, sensing proteins, and enzymes are a few most important membrane proteins.

Cell membranes also have carbohydrates in minute quantities, in the form of oligosaccharides, covalently attached to membrane proteins (glycoproteins) or to lipids (glycolipids).

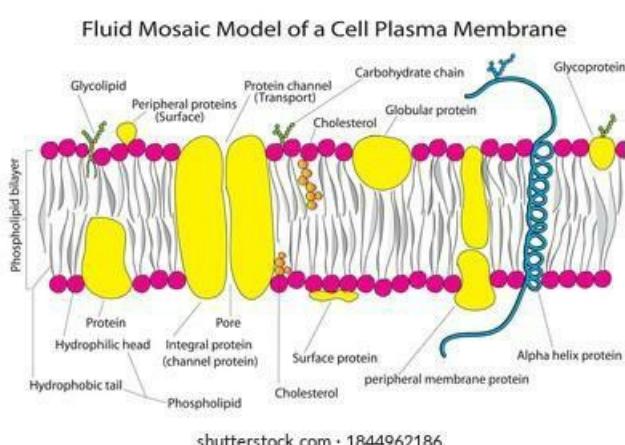


Figure 1.14: Fluid Mosaic Model of Plasma Membrane

The Function of Plasma Membrane:

- It protects the integrity of the interior cell.
- Provides support and maintains the shape of the cell.
- Helps in regulating cell growth by regulating endocytosis and endocytosis.

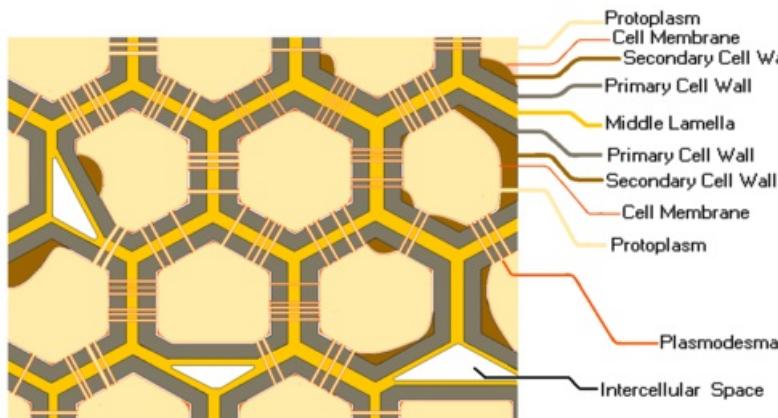
- It also plays an important role in cell signaling and communication.
- Acts as a selectively permeable membrane by allowing the entry of only selected substances into the cell.

Cell Wall

It is the outer, rigid layer that lies outside the plasma membrane. It is mostly found in the algae, fungi, plant cells, and some protists. In algae cell wall is made of cellulose, galactans, mannans, and minerals like calcium carbonate. In fungi, the cell wall is composed of chitin.

Plant Cell Wall

1. Middle lamella- Composed of mainly calcium pectate.
2. Primary cell wall- Composed of cellulose microfibrils aligned in any directions, hemicellulose and proteins.
3. Secondary Cell Wall- It is also made up of cellulose microfibrils but these are aligned in parallel layers. Apart from cellulose microfibrils hemicellulose and proteins are also present. It has the deposition of suberin, lignin, cutin, and fatty acids.



Placement of plant's cell wall (extracellular matrix) and its major parts (highly diagrammatic)

Figure 1.15: Plant cell wall structure

Functions of Plant Cell Wall:

- Cell wall prevents over-expansion of the cell when water enters.
- It provides mechanical strength.
- It maintains cell shape by regulating the direction of cell growth.
- Cell wall regulates intercellular transport.
- It protects the plant from infectious organisms and the external environment.
- It also functions as a storage unit by storing carbohydrates for use in plant growth, especially in seeds.

Cytoskeleton

The cytoskeleton is present inside the cytoplasm, which consists of microfilaments, microtubules, and fibres to provide perfect shape to the cell, anchor the organelles, and stimulate cell movement.

Endoplasmic Reticulum

It is a network of small, tubular structures that divides the cell surface into two parts: luminal and extraluminal.

Endoplasmic Reticulum is of two types:

- Rough Endoplasmic Reticulum contains ribosomes on its surface and thus helps in protein synthesis.
- Smooth Endoplasmic Reticulum that lacks ribosomes and is therefore smooth. It is the site of lipid synthesis.

The endoplasmic reticulum helps in transporting proteins, lipids, and other molecules throughout the cytoplasm.

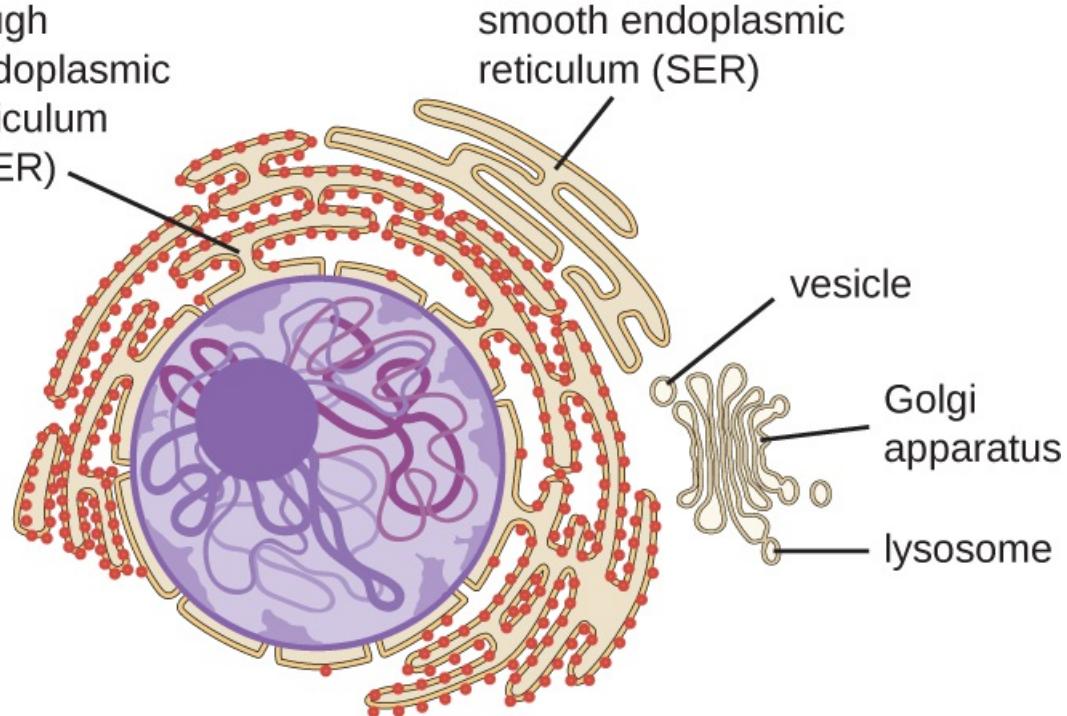
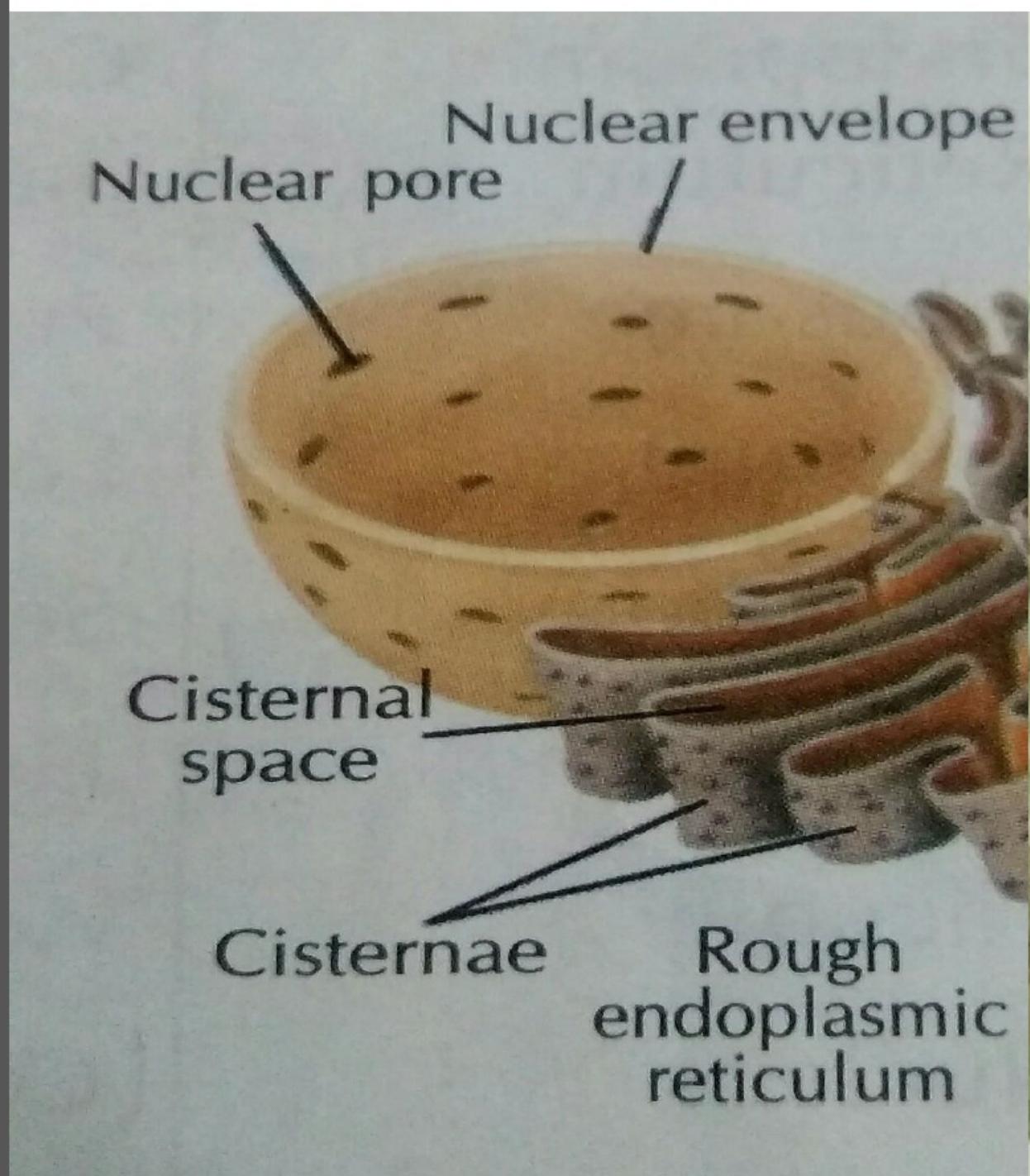


Figure: 1.16: Endoplasmic Reticulum

Nucleus



Nucleus



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Figure 1.17: Nucleus

- The nucleoplasm enclosed within the nuclear envelope contains DNA and proteins.
- The nuclear envelope consists of two layers- the outer membrane and the inner membrane. Both the membranes are permeable to ions, molecules, and RNA material.
- Ribosome production also takes place inside the nucleus.

Golgi Apparatus

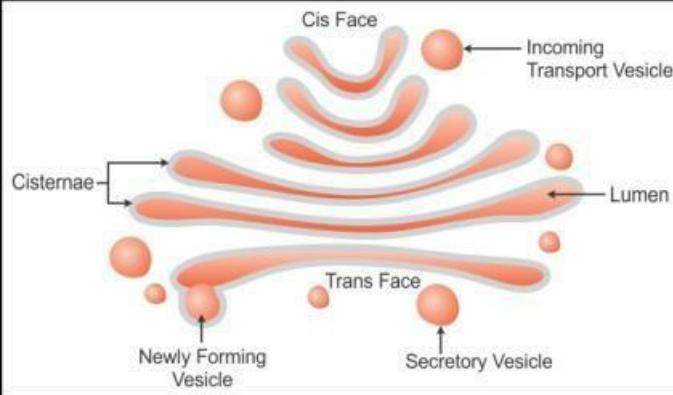
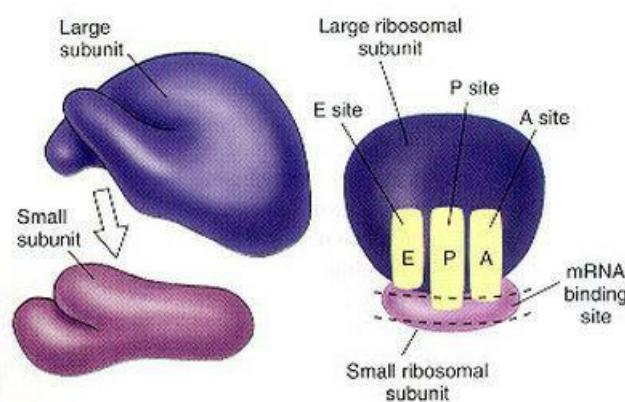


Figure 1.18: Golgi Apparatus

- It is made up of flat disc-shaped structures called cisternae.
- It is absent in red blood cells of humans and sieve cells of plants.
- They are arranged parallel and concentrically near the nucleus.
- It is an important site for the formation of glycoproteins and glycolipids.

Ribosomes



Ribosome Subunits

The smaller subunit fits into a depression on the surface of the larger one. The A, P, and E sites on the ribosome play key roles in protein synthesis.

Figure 1.19: Ribosome

80S ribosomes are present in the eukaryotic cell. 80S ribosomes have two subunits called the 60S (large) and 40 S (small) subunits. The A, P, and E sites on the ribosomes play key roles in protein synthesis.

A site: The aminoacyl binding Site holds the tRNA carrying the next amino acid to be added to the chain.

P site: Peptidyl Binding Site holds the tRNA carrying the growing polypeptide chain.

E site: Exit Site: the discharged tRNAs leave the ribosome from this site.

The main constituents of ribosomes are proteins and ribonucleic acids (rRNA). rRNA is also called ribozymes. Ribozymes act as an enzyme during protein synthesis. rRNA is the site where mRNA codon and tRNA anticodon couple.

Mitochondria

Mitochondria is a double membranous organelle. It is semi-autonomous in nature as it has its own DNA which can synthesize a few proteins.

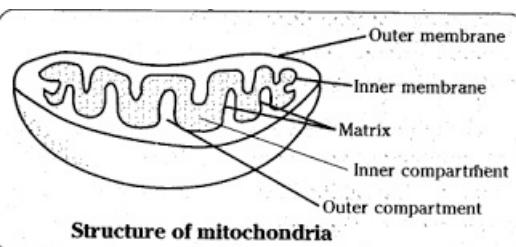


Figure 1.20: Mitochondria

- These are also known as the “powerhouse of cells” because they produce energy.
- It consists of an outer membrane and an inner membrane. The inner membrane is divided into folds called cristae.

- They help in the regulation of cell metabolism.

Lysosomes

They are known as “suicidal bags” because they possess hydrolytic enzymes to digest protein, lipids, carbohydrates, and nucleic acids.

Plastids

These are double-membraned structures and are found only in plant cells. These are of three types:

- Chloroplast that contains chlorophyll and is involved in photosynthesis. It is a double membranous structure. It is semi-autonomous organelle as it has its own DNA which can synthesize few proteins required by it.

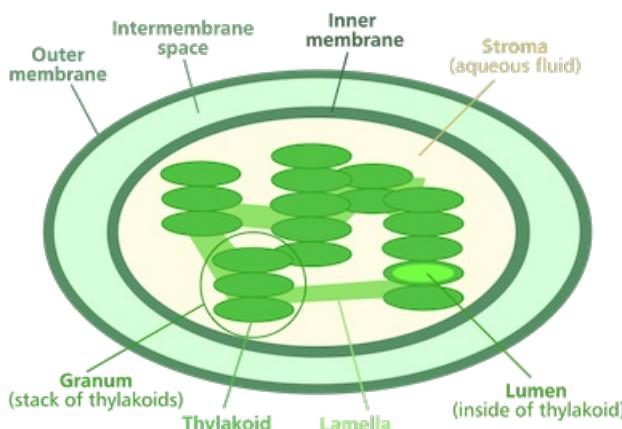


Figure 1.21: Chloroplast

- Chromoplast that contains a pigment called carotene that provides the plants yellow, red, or orange colours.
- Leucoplasts that are colourless and store oil, fats, carbohydrates, or proteins.

1. Transport Across the cell membrane

Plasma membranes must allow certain substances to enter and leave a cell, and prevent some harmful materials from entering and some essential materials from leaving. In other words, plasma membranes are selectively permeable—they allow some substances to pass through, but not others. Some cells require larger amounts of specific substances and must have a way of obtaining these materials from extracellular fluids. This may happen passively, as certain materials move back and forth, or the cell may have special mechanisms to facilitate transport. Some materials are so important to a cell that it spends some of its energy, hydrolyzing adenosine triphosphate (ATP), to obtain these materials. Interestingly, most cells spend a lot of their energy (approximately one third) on maintaining an imbalance of sodium and potassium ions between the cell's interior and exterior. The most direct forms of membrane transport are passive. Passive transport is a naturally occurring phenomenon and does not require the cell to exert any of its energy to accomplish the movement of substances from an area of higher concentration to an area of lower concentration.

Selective Permeability

Recall that plasma membranes are amphiphilic, containing hydrophilic and hydrophobic regions. This characteristic helps move some materials through the membrane and hinders the movement of others. Non-polar and lipid-soluble material with a low molecular weight can easily slip through the membrane's hydrophobic lipid core. Substances such as the fat-soluble vitamins A, D, E, and K readily pass through the plasma membranes in the digestive tract and other tissues. Fat-soluble drugs and hormones also gain easy entry into cells and readily transport themselves into the body's tissues and organs. Oxygen and carbon dioxide molecules have no charge and pass through membranes by simple diffusion.

Polar substances present problems for the membrane. While some polar molecules connect easily with the cell's outside, they cannot readily pass through the plasma membrane's hydrophobic lipid core. Additionally, while small ions could easily slip through the spaces in the membrane's mosaic, their charge prevents them from doing so. Ions such as sodium, potassium, calcium, and chloride must have special means of moving through plasma membranes. Simple sugars and amino acids also need the help of various transmembrane proteins to facilitate transport across plasma membranes.

Passive Transport: Diffusion, Osmosis, and Facilitated Diffusion

Diffusion

Diffusion is a passive process of transport where a single substance moves from a high concentration to a low concentration until the concentration is equal across space. You are familiar with the diffusion of substances through the air. For example, think about someone opening a bottle of perfume in a room filled with people. The perfume smell is at its highest concentration in the bottle. Its lowest concentration is at the room's edges. The perfume vapor will diffuse or spread away from the bottle, and gradually, increasingly more people will smell the perfume as it spreads. Materials move within the cell's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion (Figure 1.22). Diffusion expends no energy. The left part of Figure 1.22 shows a substance on one side of a membrane only in the extracellular fluid. The middle part shows that, after some time, some of the substance has diffused across the plasma membrane, from the extracellular fluid, and into the cytoplasm. The right part shows that, after more time, an equal amount of the substance is on each side of the membrane.

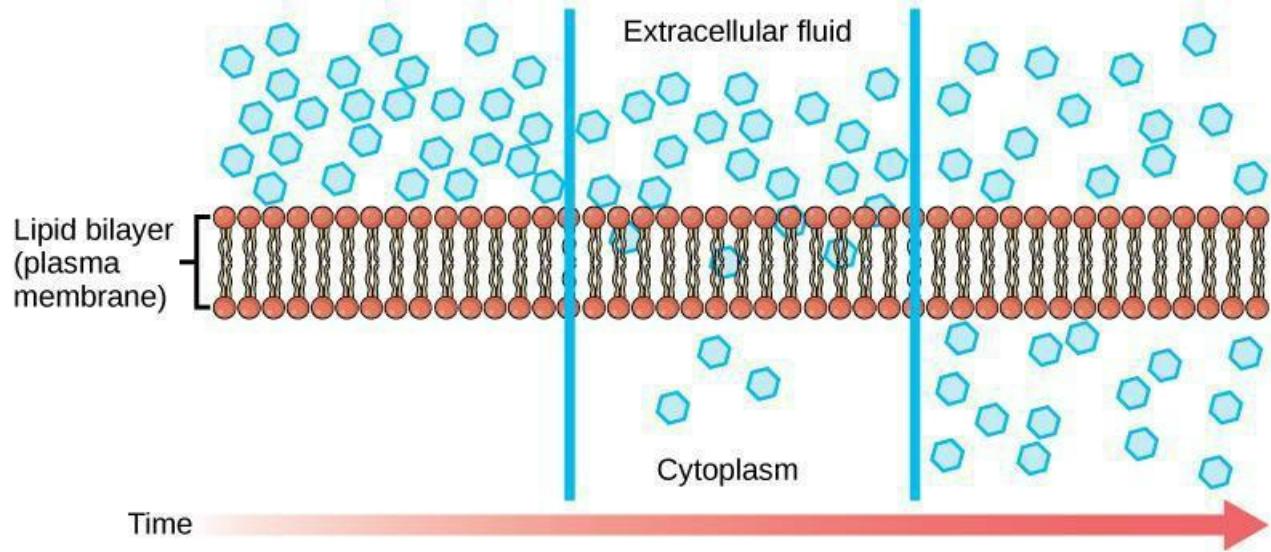


Figure 1.22: Diffusion. Diffusion through a permeable membrane moves a substance from a high concentration area (extracellular fluid, in this case) down its concentration gradient (into the cytoplasm).

Each separate substance in a medium, such as the extracellular fluid, has a unique concentration gradient, independent of other materials' concentration gradients. Each substance will diffuse, passively, according to that gradient. Within a system, there will be different diffusion rates of various substances in the medium.

Osmosis

Osmosis is the movement of water through a semipermeable membrane according to the water's concentration gradient across the membrane, which is inversely proportional to the solutes' concentration. While the term diffusion refers to the transport of material (other than water) across membranes and within cells, the term osmosis refers specifically to the transport only of water across a membrane. Not surprisingly, the aquaporins that facilitate water movement play a large role in osmosis, most prominently in red blood cells and the membranes of kidney tubules.

Osmosis is a special case of diffusion. Water, like other substances, moves from an area of high concentration to one of low concentration. Imagine a beaker with a semipermeable membrane separating the two sides or halves. On both sides of the membrane, the water level is the same, but there are different concentrations of solutes on each side of the membrane, and the different solutes cannot cross the membrane. In figure 1.23, there is a container whose contents are separated by a semipermeable membrane. Initially, there is a high concentration of solute on the right side of the membrane and a low concentration of the left. Over time, water diffuses across the membrane toward the side of the container that initially had a higher concentration of solute (lower concentration of water not bound to solute). As a result of osmosis, the water level is higher on this side of the membrane, and the solute concentration is the same on both sides.

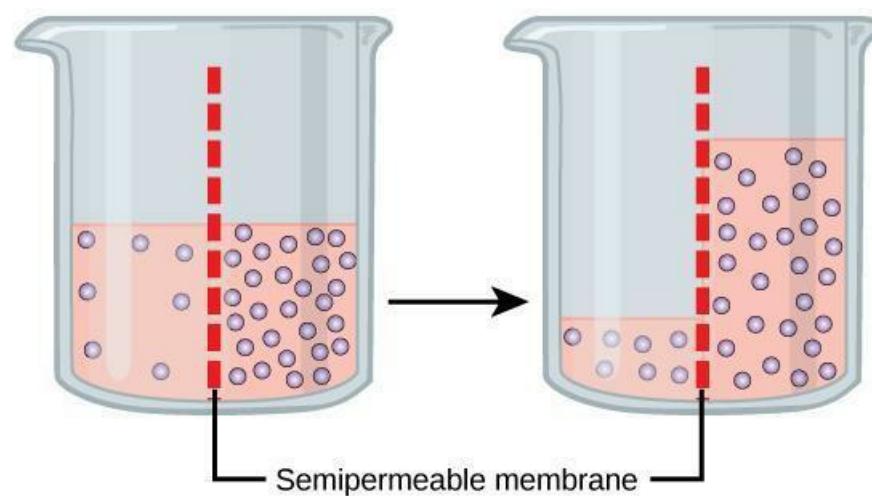


Figure 1.23: The movement of water through a semi-permeable membrane. In osmosis, water always moves from an area of higher water concentration to one of lower concentration. In the diagram, the solute cannot pass through the selectively permeable membrane, but the water can. Note at the beginning, the volume of water is the same, but the concentration of solute-unbound water is greater on the left because there is less solute. There are fewer solute-unbound water molecules on the right because there is so much more solute. Therefore, there is a higher concentration of "free" water molecules on the left than on the right of the membrane in the first beaker.

The beaker has a solute mixture on either side of the membrane. A principle of diffusion is that the molecules move around and will spread evenly throughout the medium if they can. However, only the material capable of getting through the membrane will diffuse through it. In this example, the solute cannot diffuse through the membrane, but the water can. Water has a concentration gradient in this system. Thus, water will diffuse down its concentration gradient, crossing the membrane to the side where it is less concentrated. This diffusion of water through the membrane—osmosis—will continue until the water's concentration gradient goes to zero or until the water's hydrostatic pressure balances the osmotic pressure. Osmosis constantly proceeds in living systems.

Facilitated Transport

In facilitated transport or facilitated diffusion, materials that cannot use simple diffusion, are transported passively across the plasma membrane with the help of membrane proteins. A concentration gradient exists that would allow these materials to diffuse into the cell without expending cellular energy. However, these

materials are polar molecules or ions that the cell membrane's hydrophobic parts repel. Facilitated transport proteins shield these materials from the membrane's repulsive force, allowing them to diffuse into the cell.

The transported material first attaches to protein or glycoprotein receptors on the plasma membrane's exterior surface. The substances then pass through specific integral proteins to move into the cell. Some of these integral proteins form a pore or channel through the phospholipid bilayer; others are carrier proteins that contain a binding site for a specific substance to aid its diffusion through the membrane.

Channels

The integral proteins involved in facilitated transport are types of transport proteins, and they function as either channels or carriers/transporters for the material. Channels are specific for the ions and molecules and have hydrophilic domains exposed to the intracellular and extracellular fluids with hydrophilic channels (or pathways) through their core that provide a hydrated opening through the membrane layers (Figure 1.24A). As such, channels are often described as "pores" in the membrane. Passage through the channel allows polar compounds to avoid the plasma membrane's nonpolar central layer that would otherwise slow or prevent their entry into the cell.

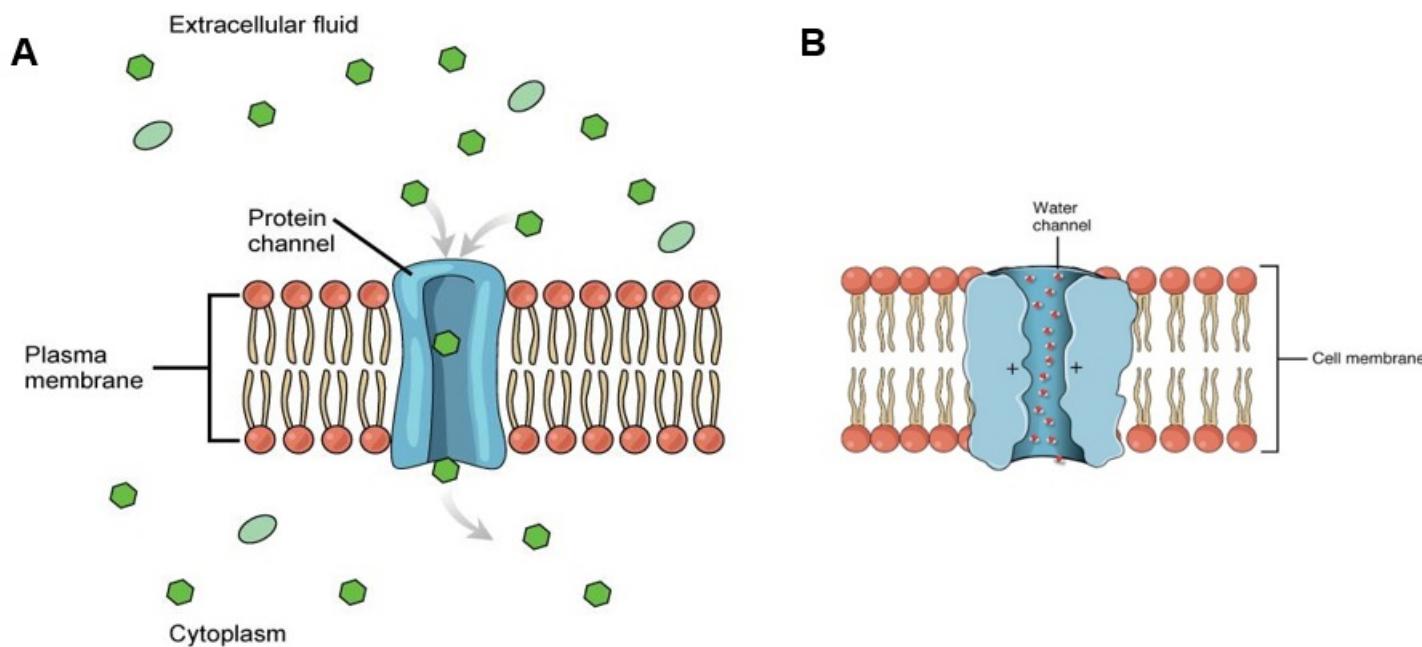


Figure 1.24: Channel proteins. (A) Facilitated transport moves substances down their concentration gradients. The substance may cross the plasma membrane with the aid of channel proteins. (B) View of aquaporin channel protein from the top. Aquaporins move water from the extracellular space to the cytoplasm in some cells of the body.

Channel proteins

These consist of two forms; one form is open at all times allowing substances to move with the gradient; the second form is "gated," which controls the channel's opening and closing. No matter the form, channel proteins will facilitate the passive diffusion of substances with the concentration gradient. Aquaporins are channel proteins that are opened at all times to allow water to pass through the membrane at a very high rate (Figure 1.24B). Alternatively, an example of a gated channel is when a particular ion attaches to the channel protein, and controls the opening, or other mechanisms or substances may be involved. In some tissues, sodium and chloride ions pass freely through open channels, whereas, in other tissues, a gate must open to allow passage. An example of this occurs in the kidney, where there are both channel forms in different parts of the renal tubules. Cells involved in transmitting electrical impulses, such as nerve and muscle cells, have gated channels for sodium, potassium, and calcium in their membranes. Opening and closing these channels changes the relative concentrations on opposing sides of the membrane of these ions, resulting in facilitating electrical transmission along membranes (in the case of nerve cells) or in muscle contraction (in the case of muscle cells).

1. **Aquaporins:** are channel proteins that allow water to cross the membrane very quickly, and they play important roles in plant cells, red blood cells, and certain parts of the kidney.
2. **Leaky Channels:** In neurons it maintains or controls resting potential of the nerve membrane. A. Potassium ion channel, B. Sodium ion channel
3. **Voltage gated ion channel:** It requires threshold potential to get open. Eg. In neurons, when the voltage reaches -50 to -55mV it opens to move positive sodium ions inside the nerve. It is very important for action potential.
4. **Ligand gated ion channel:** At neuromuscular junction when the neurons release the neurotransmitters like acetylcholine it causes opening of this channel as acetylcholine gets bound to this channel protein. Opening of this channel will allow the passage of sodium ions into the muscle leading to the muscle contraction.
5. **Mechanically gated ion channel:** Large amount of pressure stimulates this channel to open. Eg. Smashing of fingers causes mechanical stress. This mechanical stress will cause the pain receptors of sensory nerves to open and allow the movement of ions into the nerve.

Carrier/Transporter Proteins

Another type of protein embedded in the plasma membrane is a carrier/transporter protein. This aptly named protein binds a substance and triggers a change of its shape, moving the bound molecule from one side of the membrane to another, and can result in movement that can be with (passive) or against (active) the concentration gradient (Figure 1.25). Carrier proteins are typically specific for a single substance; this selectivity adds to the plasma membrane's overall selectivity.

Figure 1.25 shows a carrier/transporter protein embedded in the membrane with an opening that initially faces the extracellular surface. After a substance binds the

carrier, it changes shape so that the opening faces the cytoplasm, and the substance is released.

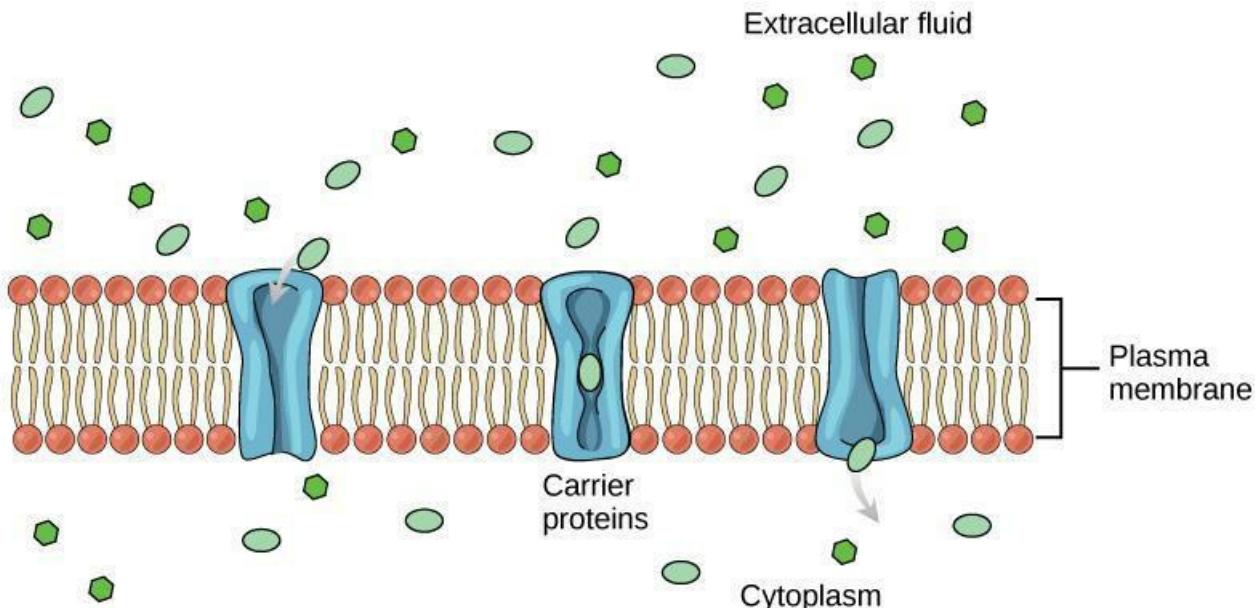


Figure 1.25: Carrier/Transporter proteins. Facilitated diffusion through a carrier/transporter protein. Some substances move down their concentration gradient across the plasma membrane with the aid of carrier proteins. Carrier proteins change shape as they move molecules across the membrane.

An example of this process occurs in the kidney. In one part, the kidney filters glucose, water, salts, ions, and amino acids that the body requires. This filtrate, which includes glucose, then reabsorbs in another part of the kidney. Because there are only a finite number of carrier proteins for glucose, if more glucose is present than the proteins can handle, the excess is not transported, and the body excretes this through urine. In a diabetic individual, the term is “spilling glucose into the urine.” A different group of carrier proteins, glucose transport proteins, or GLUTs, are involved in transporting glucose and other hexose sugars through plasma membranes within the body.

The rate of transport by channel and carrier proteins differs because of the way they physically interact with their substrates. Channel proteins facilitate diffusion at a rate of tens of millions of molecules per second; whereas, carrier proteins work at a rate of a thousand to a million molecules per second.

Direction of transport: Uniports, Antiports, and Symports

A protein involved in moving only one type of molecule across a membrane is called a uniport. Proteins that move two different types of molecules in the same direction across the membrane are called symporters. If two different types of molecules move in opposite directions across the bilayer, the protein is called an antiport (Figure 1.26).

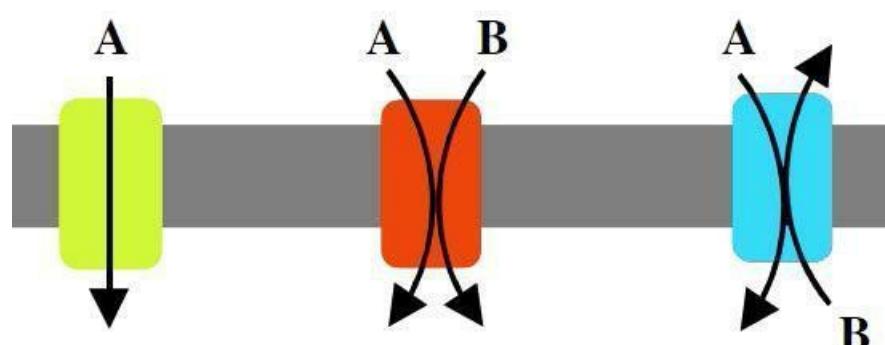


Figure 1.26: Direction of transport. A uniport (yellow), a symport (red), and an antiport (blue).

Active transport

In some instances, cells must move materials against a concentration gradient, and when this occurs, another source of energy is required. This process is known as active transport. Active transport can be used with all three types of proteins shown in Figure 1.26 if the molecules are moved against their concentration gradient with the use of energy to drive this transport.

A good definition of active transport is that at least one molecule is moved against a concentration gradient. A common, but not exclusive energy source is ATP; other energy sources can also be employed. For example, the sodium-glucose transporter uses a sodium gradient as an energy source for actively transporting glucose into a cell. Additionally, the prokaryotic protein bacteriorhodopsin utilizes light energy to actively pump protons across cell membranes. Thus, it is essential to know that not all active transport uses ATP energy.

Sodium Potassium Pump (Na^+/K^+ ATPase)

An essential, integral membrane transport protein, the Na^+/K^+ ATPase antiport (Na^+/K^+ pump) (Figures 1.26 and 1.27), moves three sodium ions out of the cell and two potassium ions into the cell with each cycle of action. In each case, the movement of both ions is against their concentration gradients. Additionally, the movement of three positive charges out of the cell and only two positive charges in; cause a membrane potential, which is vital in establishing homeostasis within cells.

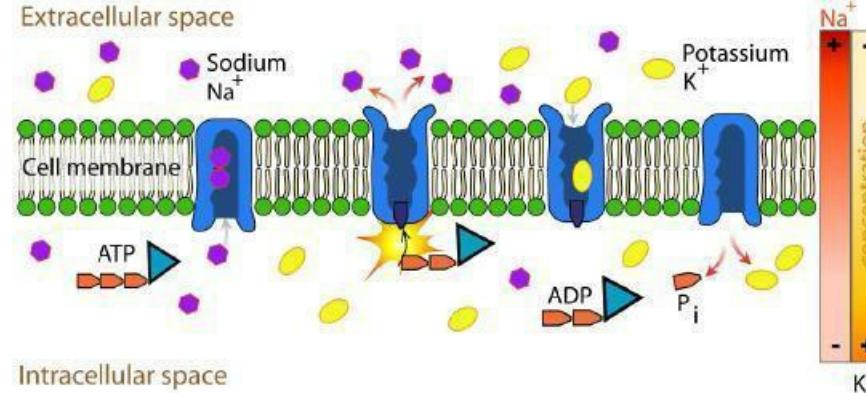


Figure 1.27: An overview of active transport by the Na^+K^+ ATPase. The protein transports three sodium out of the cell and two potassium into the cell each cycle. This cycle requires the use of one ATP molecule for energy as it is driving the ions against the concentration gradient (from low concentration to high) in both directions.

The Na^+K^+ pump uses the energy of ATP to create and maintain ion gradients that are important both in maintaining cellular osmotic pressure and (in nerve cells) for creating the sodium and potassium gradients necessary for signal transmission. Failure of the system to function results in swelling of the cell due to the movement of water into the cell through osmotic pressure. The transporter expends about one-third of the ATP energy of animal cells. The cycle of the action occurs as follows and as shown in Figure 1.27:

1. Pump binds three Na^+ ions from the cytoplasm of the cell, followed by one ATP molecule.
2. ATP hydrolysis results in phosphorylation of aspartate residue of the pump. ADP is released.
3. Phosphorylated pump undergoes a conformational change to expose Na^+ ions to the exterior of the cell. Na^+ ions are released.
4. Pump binds two extracellular K^+ ions.
5. Pump dephosphorylates, causing it to change conformation and release K^+ ions to the cytoplasm as the pump returns to the original shape.
6. Pump binds three Na^+ ions from the cytoplasm to restart the cycle.

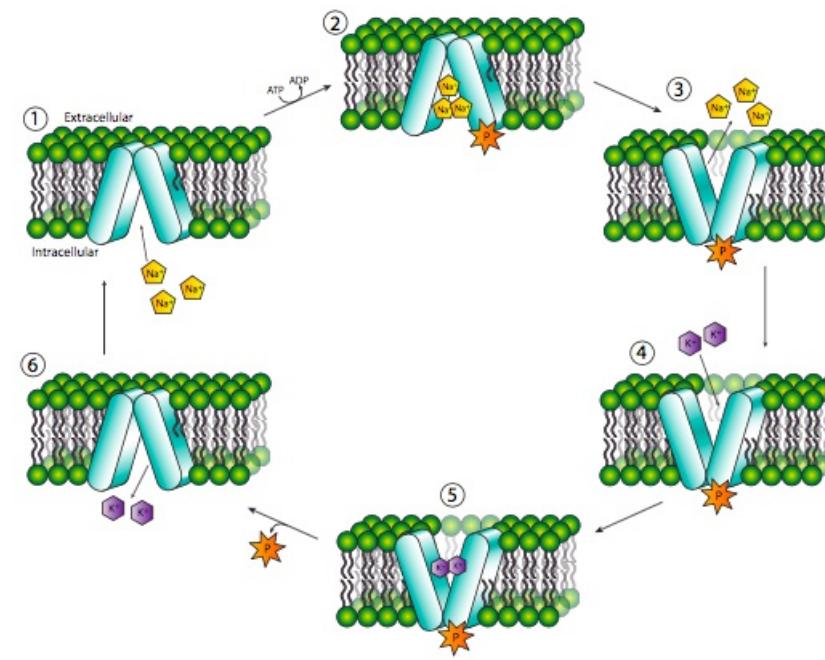


Figure 1.28: Active Transport by Na^+K^+ ATPase. This enzyme pushes three Na^+ ions out of the cell and two K^+ ions into the cell, going against the gradient in both directions and using energy from ATP hydrolysis.

Sodium/Glucose Transporter

Hydrolysis of ATP, while a common source of energy for many biological processes, is not the only source of energy for transport. Coupling the active transport of one solute against its gradient with the energy from passive transport of another solute down its gradient is also possible. Illustrated in Figure 1.28, is the sodium/glucose transporter, an example of a symport with both solutes crossing the membrane in the same physical direction. However, one solute is traveling down its gradient (sodium ions), and one solute traveling up or against its concentration gradient (glucose). The movement of Na^+ is the driving force behind this transport mechanism. The Na^+ gradient across the membrane is an extremely important source of energy for most animal cells. However, this is not universal for all cells, or even all eukaryotic cells. In most plant cells and unicellular organisms, the H^+ (proton) gradient plays the role that Na^+ does in animals.

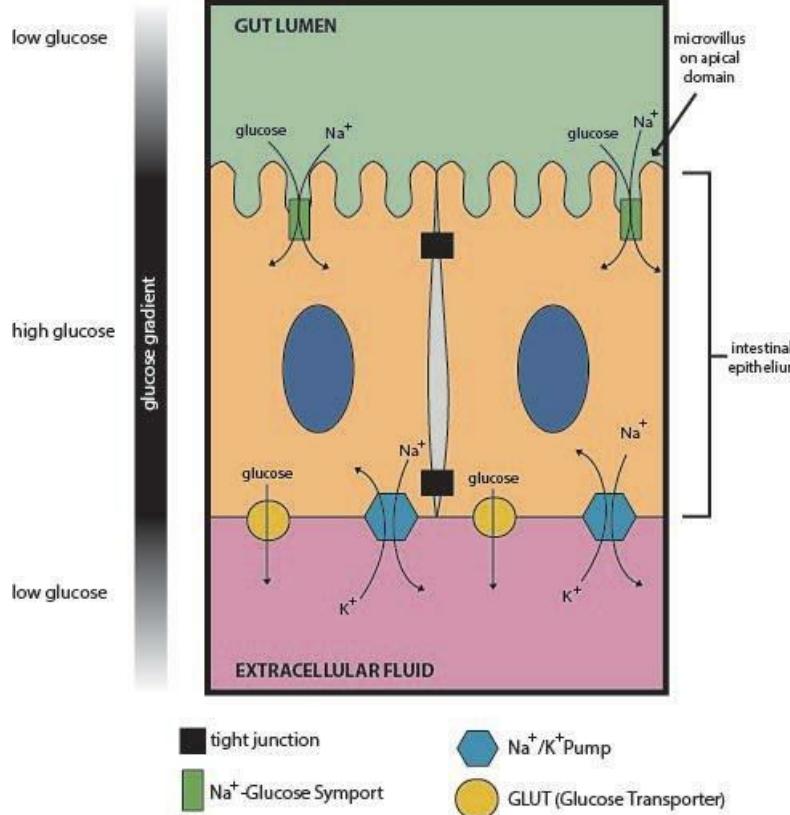


Figure 1.29: Transporters in the gut. In this symport, the energy release from passive transport of Na^+ into the cell actively transports glucose into the cell on the apical side (green protein). On the basal side of the cell, transport proteins work together. The Na^+/K^+ ATPase (blue) restores the normal concentration of sodium ions that entered passively through the sodium glucose transporter and glucose transporters (yellow) move the glucose from the cell to the bloodstream in the extracellular space.

Absorbing nutrients from the digestive system is necessary for animal life. The sodium/glucose transport protein is a symporter that moves glucose into intestinal cells. This transporter is located in cellular membranes of the intestinal mucosa and the proximal tubule of the nephron of the kidney. It functions in the latter to promote reabsorption of glucose. For intestinal mucosa, the pump transports glucose out of the gut and into the cells lining the lumen of the small intestine. Later, the glucose is exported out the other side of the gut cells to the interstitial space for use in the body. A glucose transporter performs the export of glucose from intestinal cells. This is a uniport protein that passively transports glucose due to the spontaneous opening and closing of the transport protein. This results in the movement of glucose from a high concentration within the intestinal cells to the bloodstream for transport to the cells of the body. The sodium concentration is then restored to homeostasis levels using a sodium-potassium pump (Figure 1.29).

Calcium pumps

Unlike Na^+ or K^+ , the Ca^{2+} gradient is not very important for the electrochemical membrane potential or the use of its energy. However, calcium ions are necessary for muscular contraction and play important roles as signaling molecules within cells. The Ca^{2+} concentration is kept very low in the cytoplasm as a result of the action of pumps. Pumps are in the plasma membrane, which pumps calcium outwards from the cytoplasm and into organelles, and the endoplasmic reticulum (sarcoplasmic reticulum of muscle cells), which pump calcium out of the cytoplasm and into these organelles.

The opening of calcium channels, then, increases calcium concentration quickly in the cytoplasm resulting in a quick response, whether the intention is signaling or contraction of a muscle. After the response, the calcium is pumped back out of the cytoplasm by the respective calcium pumps. Ca^{2+} pumps are uniport proteins that use the energy supplied by ATP hydrolysis to drive the transport of two Ca^{2+} molecules from the cytoplasm to the extracellular side of the plasma membrane.

Examples of Membrane transport in organisms

CFTR transporter and Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive genetic disorder arising from mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This transporter system, which moves chloride and thiocyanate ions across epithelial tissue membranes, exerts its effect mostly in the lungs. Still, the pancreas, liver, kidneys, and intestine are all also affected by it.

CFTR has roles in the production of sweat, mucus, and digestive fluids. Manifestations of the disease include breathing difficulty and overproduction of mucus in the lungs. When CFTR is functional, these fluids usually are thin, but when the gene is non-functional, they become much thicker and are points of infection (Figure 1.30).

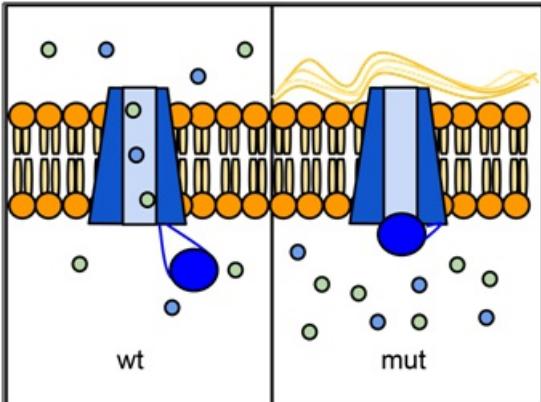


Figure 1.30: CFTR. Wild-type and mutant forms of CFTR in the cell membrane: In wild-type, the CFTR ion channel is gated; when activated by ATP, the channel opens and allows ions to move across the membrane. In some CFTR mutants, the channel does not open. This prevents the movement of ions and water and allows mucus to build up on the lung epithelium.

Glucose Transport proteins (GLUTs)

GLUTs (GLUcose Transport proteins) are uniport, type III integral membrane proteins that participate in the transport of glucose across membranes into cells. GLUTs are found in all phyla and are abundant in humans, with 12 GLUT genes. GLUT1, in erythrocytes, is well-studied. Through GLUT 1, glucose enters and passes through it via facilitated diffusion at a rate that is 50,000 higher than in its absence. The body contains many types of GLUTs found in different cells of the body. GLUT 1 levels in erythrocytes go up as glucose levels decrease and decrease when glucose levels go down via facilitated diffusion.

Regulated by insulin, GLUT 4 is found primarily in adipose and striated muscle tissue. Insulin alters intracellular trafficking pathways in response to increases in blood sugar to favour movement of various GLUT proteins (including GLUT 4) from intracellular vesicles to the cell membrane, thus stimulating the uptake of the glucose. Found in the hippocampus is GLUT 4, where the disruption of traffic may result in depressive behaviour and cognitive dysfunction.

The key to keeping the glucose in the cell is the phosphorylation of it by the glycolysis enzyme, hexokinase, in the cytoplasm. Phosphorylated glucose molecules cannot enter the active site of the various GLUTs and therefore don't have an easy means of exiting the cell. Essentially, phosphorylation traps glucose inside the cell.

Table: Glucose transporters. Glucose transporters (GLUTs) found in cells.

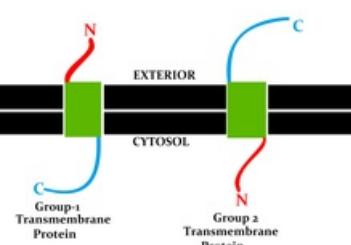
Glucose Transporters

Name	Tissue	K _M (μM)	Comments
GLUT1	All mammalian tissues	1	Basal glucose uptake
GLUT2	Liver and pancreatic β cells	15-20	Participates in insulin regulation in the pancreas; removes excess glucose from the blood in the liver
GLUT3	All mammalian tissues	1	Basal glucose uptake
GLUT4	Muscle and fat cells	5	Concentration in plasma membrane of muscles rises with endurance training
GLUT5	Small intestine	---	Fructose transport

*Additional Information:

a. Integral polytopic protein

The most common type of IMP is the transmembrane protein (TM), which spans the entire biological membrane. Single-pass membrane proteins cross the membrane only once, while multi-pass membrane proteins weave in and out, crossing several times. Single pass TM proteins can be categorized as Type I, which are positioned such that their carboxyl-terminus is towards the cytosol, or Type II, which have their amino-terminus towards the cytosol. Type III proteins have multiple transmembrane domains in a single polypeptide, while type IV consists of several different polypeptides assembled together in a channel through the membrane. Type V proteins are anchored to the lipid bilayer through covalently linked lipids. Finally Type VI proteins have both transmembrane domains and lipid anchors.^[4]



Group I and II transmembrane proteins have opposite orientations. Group I proteins have the N terminus on the far side and C terminus on the cytosolic side. Group II proteins have the C terminus on the far side and N terminus in the cytosol.

b. Integral monotopic proteins

Integral monotopic proteins are associated with the membrane from one side but do not span the lipid bilayer completely.

Bulk Transport

This mode of Transportation is needed when the particles are of larger size or to engulf the microorganism or whole cell. Plasma membrane folds or invaginate or form pseudopodia to internalise the large particle or fluids in large amounts.

Endocytosis

There are many functions and factors relating to cell membranes that don't fit into broad categories. Those items will be the focus of this section.

Besides transporter proteins and ion channels, another common way for materials to get into cells is by the process of endocytosis. Endocytosis is an alternate form of active transport for getting materials into cells. Some of these processes, such as phagocytosis, can import much larger particles than would be possible via a transporter protein.

As a result, the process usually involves the import of many different molecules each time it occurs. The list of compounds entering cells in this way includes LDLs and their lipid contents, but it also has things like iron (packaged in transferrin), vitamins, hormones, proteins, and even some viruses sneak in by this means. There are three types of endocytosis we will consider (Figure 1.31).

Endocytosis

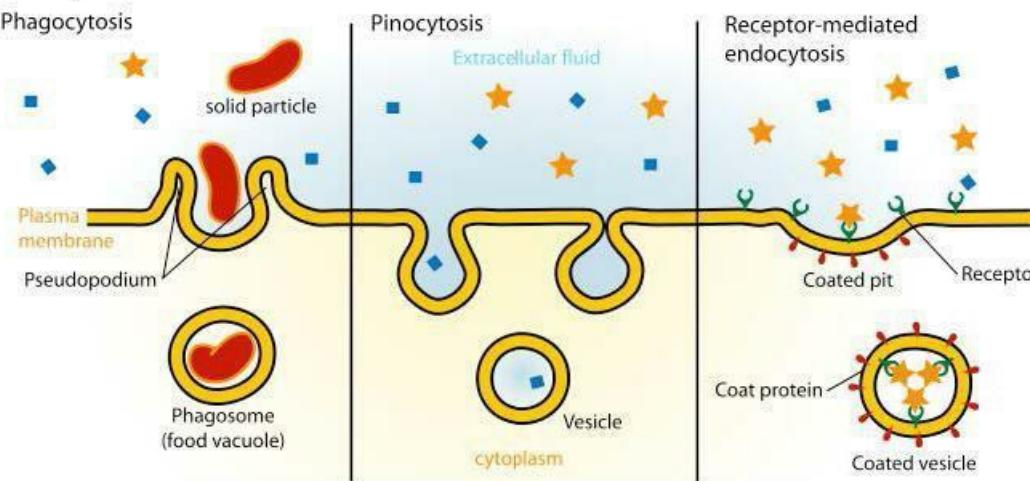


Figure 1.31: Three types of endocytosis. These include (left) phagocytosis- a nonspecific uptake of molecules, (middle) pinocytosis- a non-specific uptake of fluid, and (right) receptor-mediated endocytosis- the specific uptake of molecules after binding to a cell surface receptor.

Pinocytosis

A phenomenon known as “cell drinking,” pinocytosis, literally involves a cell “taking a gulp” of the extracellular fluid. It does this, as shown in Figure 1.32, by a simple invagination of the plasma membrane. A pocket results, which pinches off internally to create a vesicle containing extracellular fluid and dissolved molecules. Within the cytosol, this internalized vesicle will fuse with endosomes and lysosomes. The process is non-specific for materials internalized.

Pinocytosis

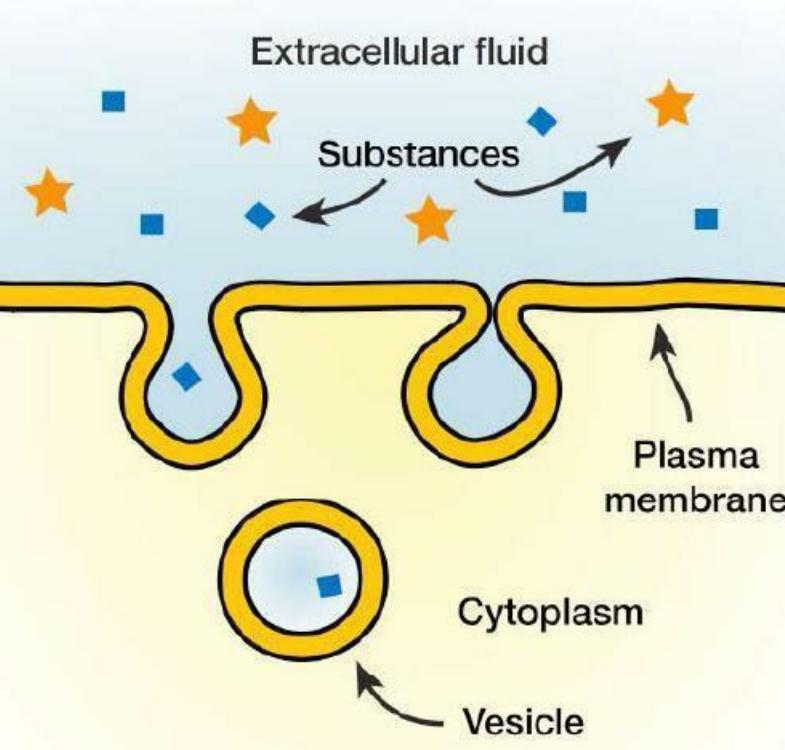


Figure 1.32: Pinocytosis. Pinocytosis, the uptake of fluid from the extracellular side of the membrane into a vesicle on the intracellular side.

Phagocytosis

Phagocytosis is a process whereby relatively large particles ($0.75 \mu\text{m}$ in diameter) are internalized. Cells of the immune system, such as neutrophils, macrophages, and others, use phagocytosis to internalize cell debris, apoptotic cells, and microorganisms.

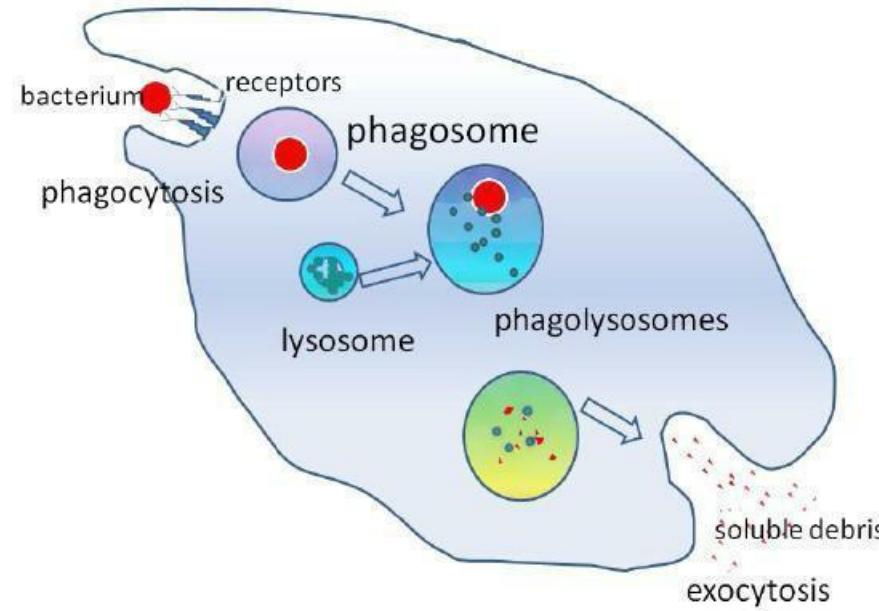
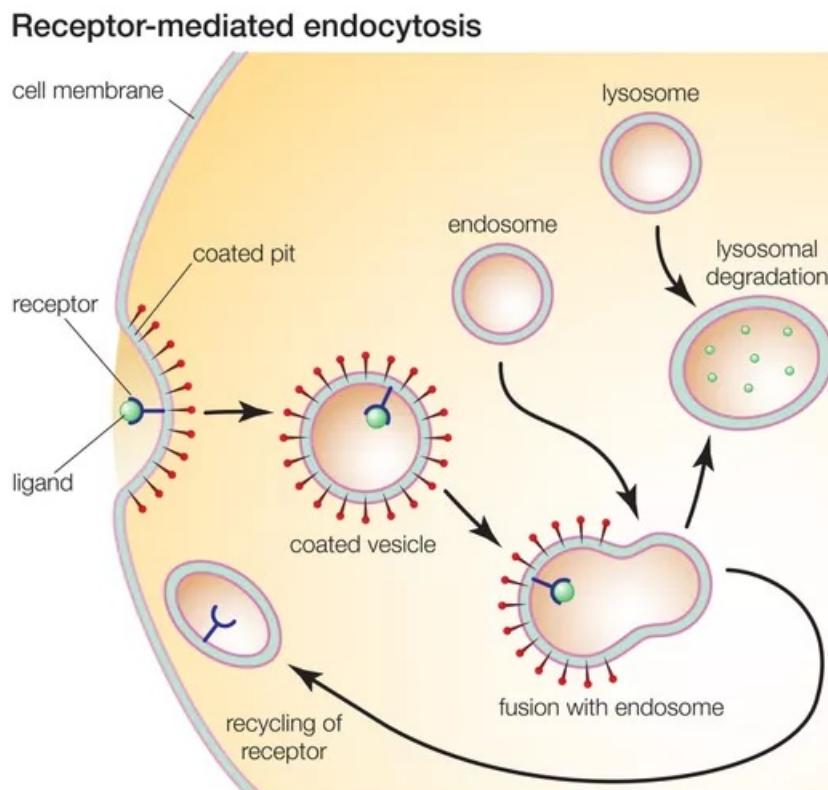


Figure 1.33: Generalized scheme for phagocytosis of a bacterium. The bacterium is taken up by phagocytosis into a phagosome that merges with the lysosome to create a phagolysosome.

The process operates through specific receptors on the surface of the cell, and the phagocytosing cell engulfs its target by growing around it. The internalized structure is known as a phagosome, which quickly merges with a lysosome to create a phagolysosome (Figure 1.33). The phagolysosome subjects the engulfed particle to toxic conditions to kill it, if it is a cell, and/or to digest it into smaller pieces. In some cases, soluble debris may be released by the phagocytosing cell.

Receptor-Mediated Endocytosis

In some instances, endocytosis is a specific mechanism to take up molecules that the cell needs to function. The molecule/ligand can bind to a specific receptor on the surface of the cell and this triggers the formation of a vesicle that is formed to import the molecule, via vesicle, into the cell. This process is called receptor mediated endocytosis. Cells in a human can take up LDL (low-density lipoprotein), through receptor mediated endocytosis, where the LDL binds specific LDL receptors (LDL-R) on the surface of the cell. The LDL is internalized into a vesicle called clathrin coated vesicle (a kind of endosome) which fuses with another type of endosome in the cytoplasm, during this time the LDL receptors are recycled to the surface of the cell. The endosome will then fuse with the lysosome where the LDL can then be broken down into monomers needed for cell function.



Endosomes provide an environment for material to be sorted before it reaches the degradative lysosome.

Clathrin is a protein that plays a major role in the formation of coated vesicles. Clathrin proteins help in pulling the membrane and forming the vesicles. It helps the vesicle to move inside the cell to the endosome.

Exocytosis

The process of exocytosis is used by cells to export molecules out of cells that would not otherwise pass easily through the plasma membrane. In the process, secretory vesicles fuse with the plasma membrane and release their contents extracellularly. Materials, such as proteins and lipids embedded in the membranes of the vesicles, become a part of the plasma membrane when fusion between it and the vesicles occurs.

Fusion is a membrane process where two distinct lipid bilayers merge their hydrophobic cores, producing one interconnected structure. Membrane fusion involving vesicles is the mechanism by which the processes of endocytosis and exocytosis occur.

Common processes involving membrane fusion include fertilization of an egg by a sperm, separation of membranes in cell division, transport of waste products, and neurotransmitter release (Figure 1.34). Artificial membranes such as liposomes can also fuse with cellular membranes. Fusion is also important for transporting lipids from the point of synthesis inside the cell to the membrane for use. The entry of pathogens can also be governed by fusion, as many bilayer-coated viruses use fusion proteins in entering host cells.

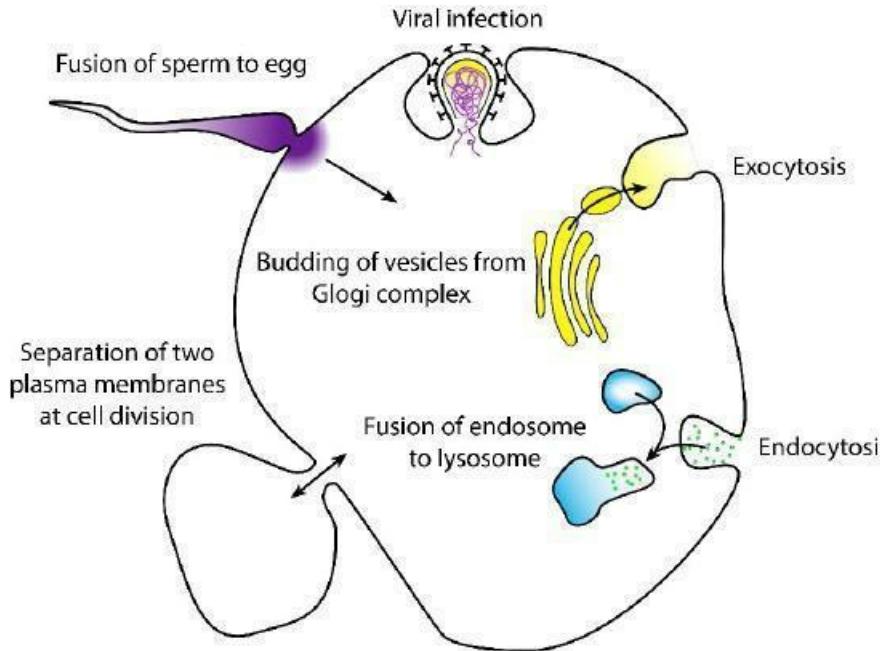


Figure 1.34: Membrane Fusion. Cell membrane fusion examples including endocytosis where the endosome fuses with the lysosome, when vesicles from the Golgi fuse with the plasma membrane, and a sperm fuses with an egg.

1. Cell Signaling:

Introduction

Think your cells are just simple building blocks, unconscious and static as bricks in a wall? If so, think again! Cells can detect what's going on around them, and they can respond in real time to cues from their neighbours and environment. At this very moment, your cells are sending and receiving millions of messages in the form of chemical signaling molecules!

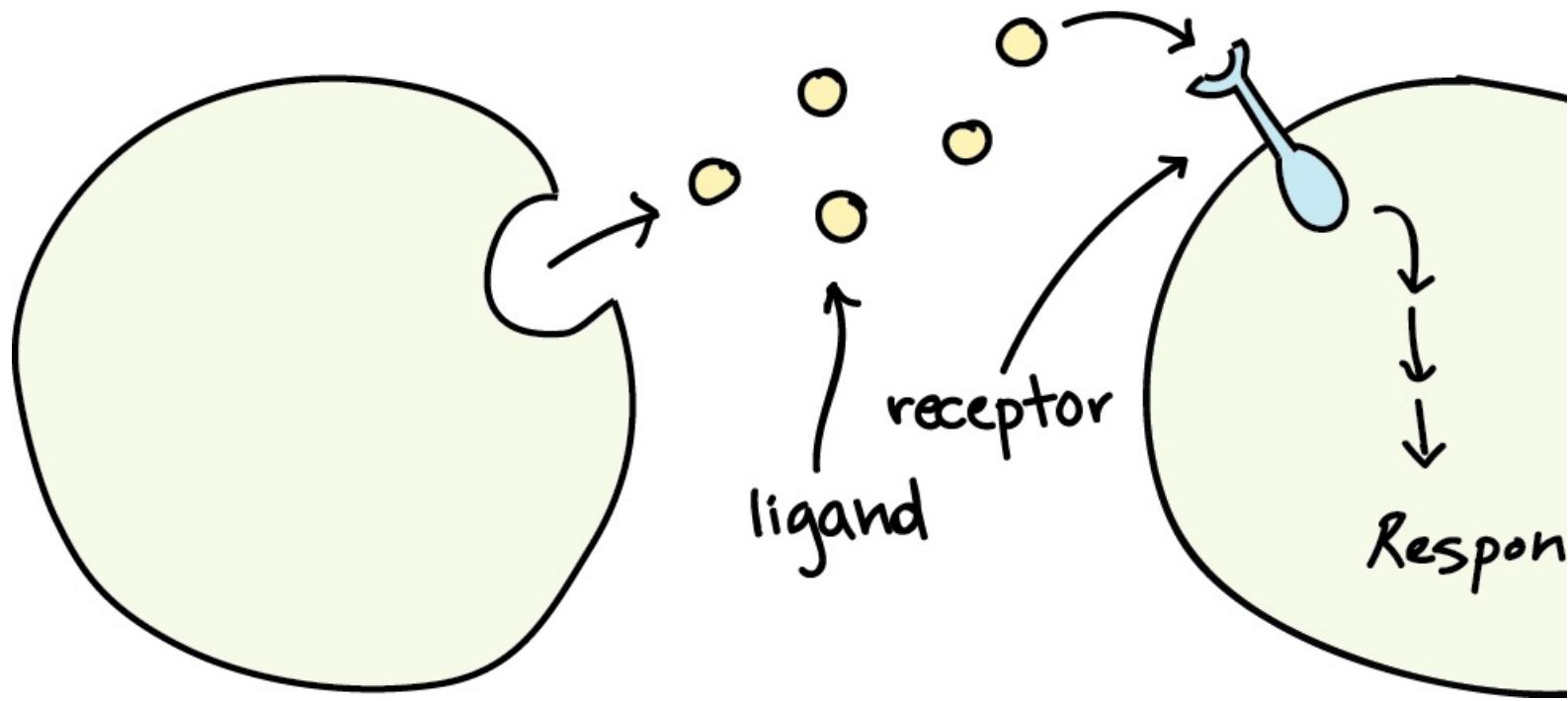
In this article, we'll examine the basic principles of how cells communicate with one another. We'll first look at how cell-cell signaling works, then consider different kinds of short- and long-range signaling that happen in our bodies.

Overview of cell signaling

Cells typically communicate using chemical signals. These chemical signals, which are proteins or other molecules produced by a sending cell, are often secreted from the cell and released into the extracellular space. There, they can float – like messages in a bottle – over to neighboring cells.

SENDING CELL

TARGET C



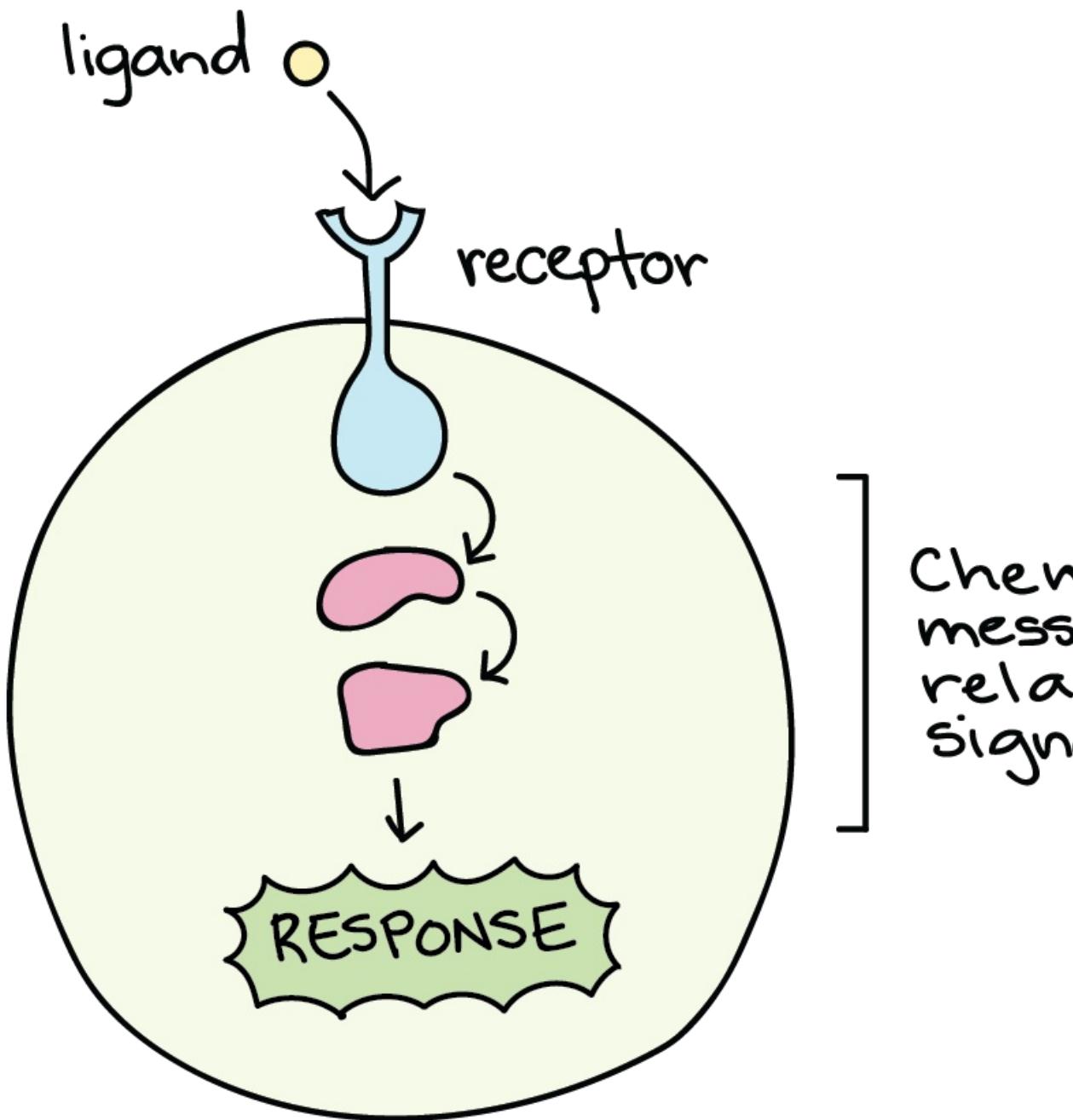
Sending cell: this cell secretes a ligand.

Target cell: this cell has a receptor that can bind the ligand. The ligand binds to the receptor and triggers a signaling cascade inside the cell, leading to a response.

Non Target cell: this cell does not have a receptor for the ligand (though it may have other kinds of receptors). The cell does not perceive the ligand and thus does not respond to it.

Not all cells can “hear” a particular chemical message. In order to detect a signal (that is, to be a target cell), a neighbour cell must have the right receptor for that signal. When a signaling molecule binds to its receptor, it alters the shape or activity of the receptor, triggering a change inside of the cell. Signaling molecules are often called ligands, a general term for molecules that bind specifically to other molecules (such as receptors).

The message carried by a ligand is often relayed through a chain of chemical messengers inside the cell. Ultimately, it leads to a change in the cell, such as alteration in the activity of a gene or even the induction of a whole process, such as cell division. Thus, the original intercellular (between-cells) signal is converted into an intracellular (within-cell) signal that triggers a response.



You can learn more about how this works in the articles on ligands and receptors, signal relay, and cellular responses.

Forms of signaling

Cell-cell signaling involves the transmission of a signal from a sending cell to a receiving cell. However, not all sending and receiving cells are next-door neighbours, nor do all cell pairs exchange signals in the same way.

There are four basic categories of chemical signaling found in multicellular organisms: paracrine signaling, autocrine signaling, endocrine signaling, and signaling by direct contact. The main difference between the different categories of signaling is the distance that the signal travels through the organism to reach the target cell.

Paracrine signaling

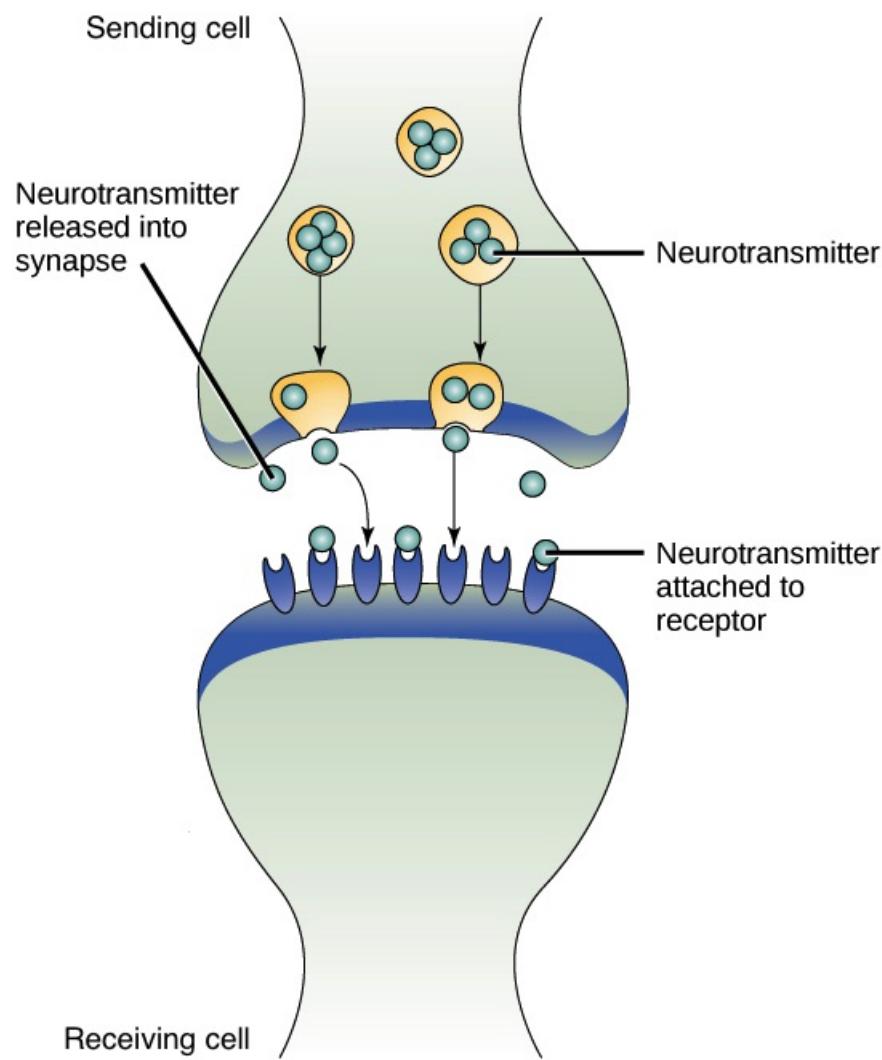
Often, cells that are near one another communicate through the release of chemical messengers (ligands that can diffuse through the space between the cells). This type of signaling, in which cells communicate over relatively short distances, is known as paracrine signaling.

Paracrine signaling allows cells to locally coordinate activities with their neighbours. Although they're used in many different tissues and contexts, paracrine signals are especially important during development, when they allow one group of cells to tell a neighbouring group of cells what cellular identity to take on.

Synaptic signaling

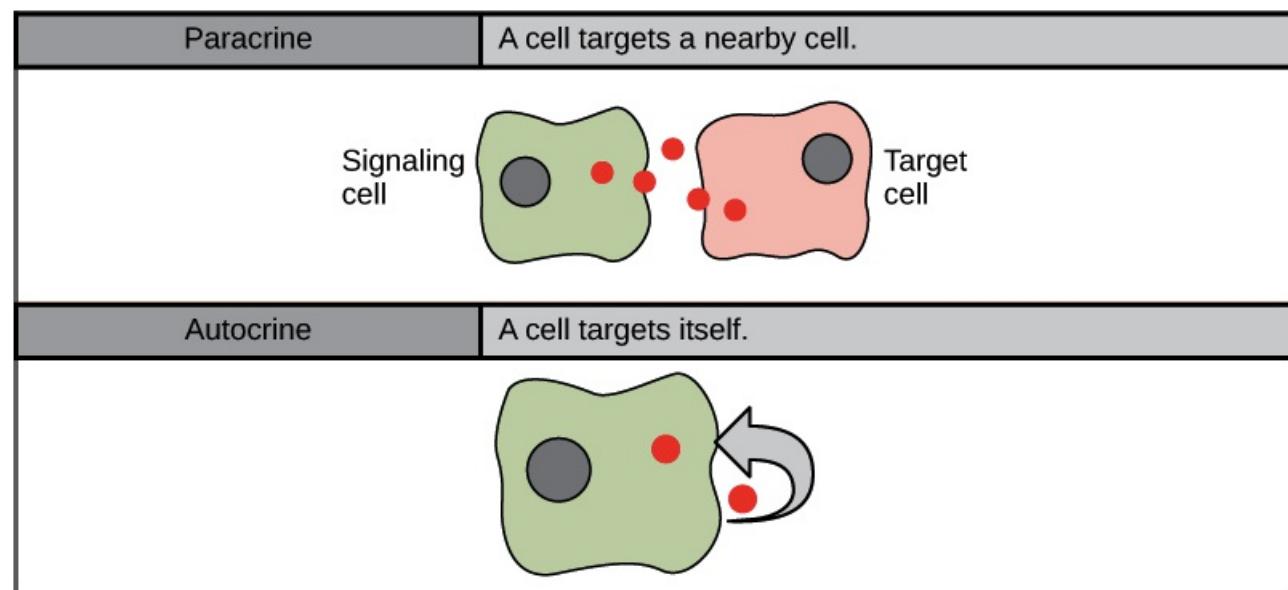
One unique example of paracrine signaling is synaptic signaling, in which nerve cells transmit signals. This process is named for the synapse, the junction between two nerve cells where signal transmission occurs.

When the sending neuron fires, an electrical impulse moves rapidly through the cell, traveling down a long, fibre-like extension called an axon. When the impulse reaches the synapse, it triggers the release of ligands called neurotransmitters, which quickly cross the small gap between the nerve cells. When the neurotransmitters arrive at the receiving cell, they bind to receptors and cause a chemical change inside of the cell (often, opening ion channels and changing the electrical potential across the membrane).



Synaptic signaling. Neurotransmitter is released from vesicles at the end of the axon of the sending cell. It diffuses across the small gap between sending and target neurons and binds to receptors on the target neuron.

The neurotransmitters that are released into the chemical synapse are quickly degraded or taken back up by the sending cell. This "resets" the system so they synapse is prepared to respond quickly to the next signal.



Paracrine signaling: a cell targets a nearby cell (one not attached by gap junctions). The image shows a signaling molecule produced by one cell diffusing a short distance to a neighbouring cell.

Autocrine signaling: a cell targets itself, releasing a signal that can bind to receptors on its own surface.

Autocrine signaling

In autocrine signaling, a cell signals to itself, releasing a ligand that binds to receptors on its own surface (or, depending on the type of signal, to receptors inside of the cell). This may seem like an odd thing for a cell to do, but autocrine signaling plays an important role in many processes.

For instance, autocrine signaling is important during development, helping cells take on and reinforce their correct identities. From a medical standpoint, autocrine signaling is important in cancer and is thought to play a key role in metastasis (the spread of cancer from its original site to other parts of the body). In many cases, a signal may have both autocrine and paracrine effects, binding to the sending cell as well as other similar cells in the area.

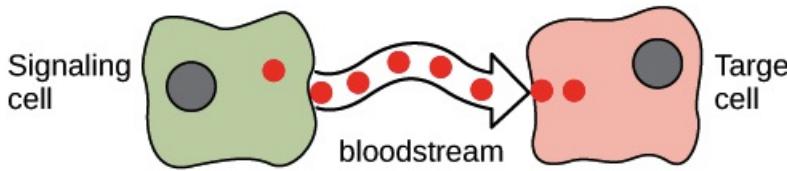
Endocrine signaling

When cells need to transmit signals over long distances, they often use the circulatory system as a distribution network for the messages they send. In long-distance endocrine signaling, signals are produced by specialized cells and released into the bloodstream, which carries them to target cells in distant parts of the body. Signals that are produced in one part of the body and travel through the circulation to reach far-away targets are known as hormones.

In humans, endocrine glands that release hormones include the thyroid, the hypothalamus, and the pituitary, as well as the gonads (testes and ovaries) and the pancreas. Each endocrine gland releases one or more types of hormones, many of which are master regulators of development and physiology.

For example, the pituitary releases growth hormone (GH), which promotes growth, particularly of the skeleton and cartilage. Like most hormones, GH affects many different types of cells throughout the body. However, cartilage cells provide one example of how GH functions: it binds to receptors on the surface of these cells and encourages them to divide.

Endocrine A cell targets a distant cell through the bloodstream.



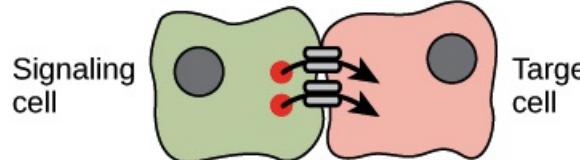
Endocrine signaling: a cell targets a distant cell through the bloodstream. A signaling molecule is released by one cell, then travels through the bloodstream to bind to receptors on a distant target cell elsewhere in the body.

Juxtacrine signaling or Signaling through cell-cell contact

Gap junctions in animals and plasmodesmata in plants are tiny channels that directly connect neighbouring cells. These water-filled channels allow small signaling molecules, called intracellular mediators, to diffuse between the two cells. Small molecules and ions are able to move between cells, but large molecules like proteins and DNA cannot fit through the channels without special assistance.

The transfer of signaling molecules transmits the current state of one cell to its neighbour. This allows a group of cells to coordinate their response to a signal that only one of them may have received. In plants, there are plasmodesmata between almost all cells, making the entire plant into one giant network.

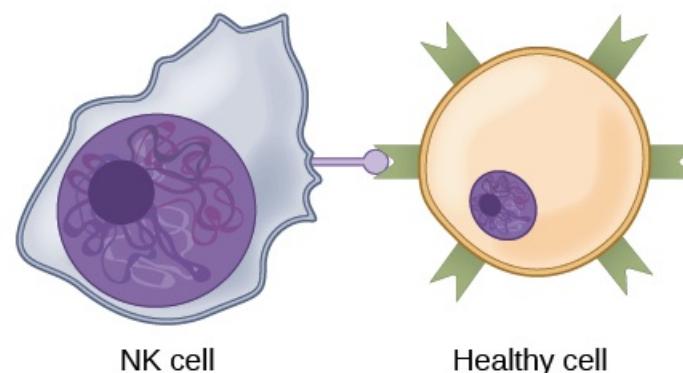
Signaling across gap junctions A cell targets a cell connected by gap junctions.



Signaling across gap junctions. A cell targets a neighboring cell connected via gap junctions. Signals travel from one cell to the other by passing through the gap junctions.

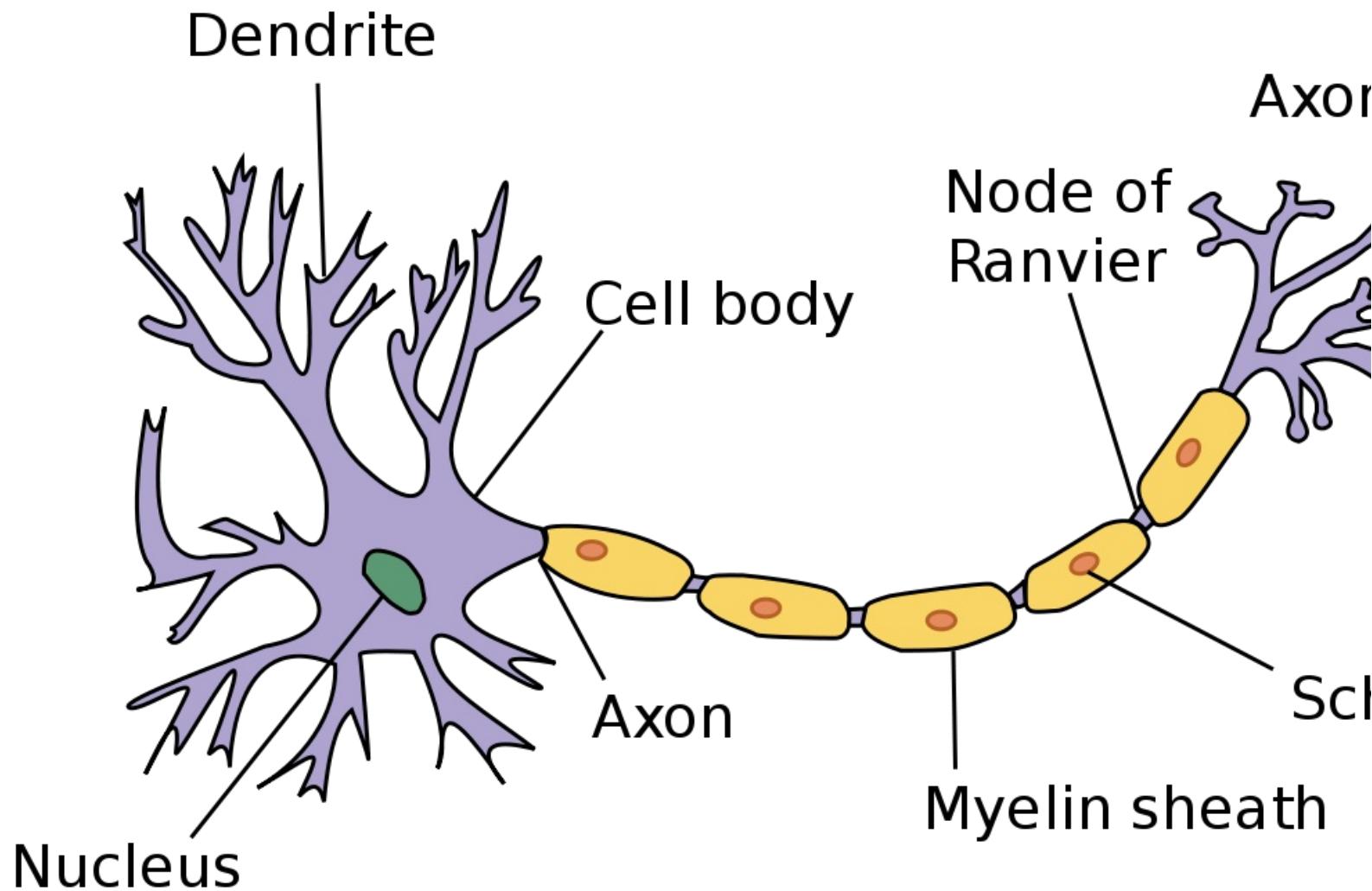
In another form of direct signaling, two cells may bind to one another because they carry complementary proteins on their surfaces. When the proteins bind to one another, this interaction changes the shape of one or both proteins, transmitting a signal. This kind of signaling is especially important in the immune system, where immune cells use cell-surface markers to recognize "self" cells (the body's own cells) and cells infected by pathogens.

A natural killer (NK) immune cell recognizes a healthy cell of the body by binding to a "self" marker on the cell's surface.



- Cell signaling involves the detection and binding of signal molecules to cell receptors in order to induce specific cellular responses, researchers have taken advantage of this mechanism to develop drugs and chemicals aimed at influencing given responses.
- In drug development, molecules are developed with the aim of identifying a given target on the cell surface or within the cell with the goal of influencing given responses. Therefore, signaling is one of the most important areas in drug discovery.
- Currently, more focus has been directed towards developing drugs that will take advantage of cell signaling to develop drugs for the treatment of cardiovascular diseases, Alzheimer's diseases as well as wound healing.
- Apart from drug development, different types of proteins are manufactured by using biotechnology. Here, cells are influenced to participate in protein synthesis as well as other molecules.
- Essentially, this involves taking advantage of the same mechanism used *in vivo*. This has proved to be particularly effective using different types of cells including bacteria and some protists.

1. Nerve Impulse Conduction



Axon – The long, thin structure in which action potentials are generated; the transmitting part of the neuron. After initiation, action potentials travel down axons to cause release of neurotransmitters.

Dendrite – The receiving part of the neuron. Dendrites receive synaptic inputs from axons, with the sum total of dendritic inputs determining whether the neuron will fire an action potential.

Spine – The small protrusions found on dendrites that are, for many synapses, the postsynaptic contact site.

Membrane potential – The electrical potential across the neuron's cell membrane, which arises due to different distributions of positively and negatively charged ions within and outside of the cell. The value inside of the cell is always stated relative to the outside: -70 mV means the inside is 70 mV more negative than the outside (which is given a value of 0 mV).

Action potential – Brief (~1 ms) electrical event typically generated in the axon that signals the neuron as 'active'. An action potential travels the length of the axon and causes release of neurotransmitters into the synapse. The action potential and consequent transmitter release allow the neuron to communicate with other neurons.

Neurotransmitter – A chemical released from a neuron following an action potential, example: Acetylcholine. The neurotransmitter travels across the synapse to excite or inhibit the target neuron. Different types of neurons use different neurotransmitters and therefore have different effects on their targets.

Synapse – The junction between the axon of one neuron and the dendrite of another, through which the two neurons communicate.

Resting Membrane Potential:

- **Membrane potential of a neuron, when it is not transmitting any signal, with respect to its immediate surrounding is called resting potential.**

Generally the value of resting potential is -70mV.

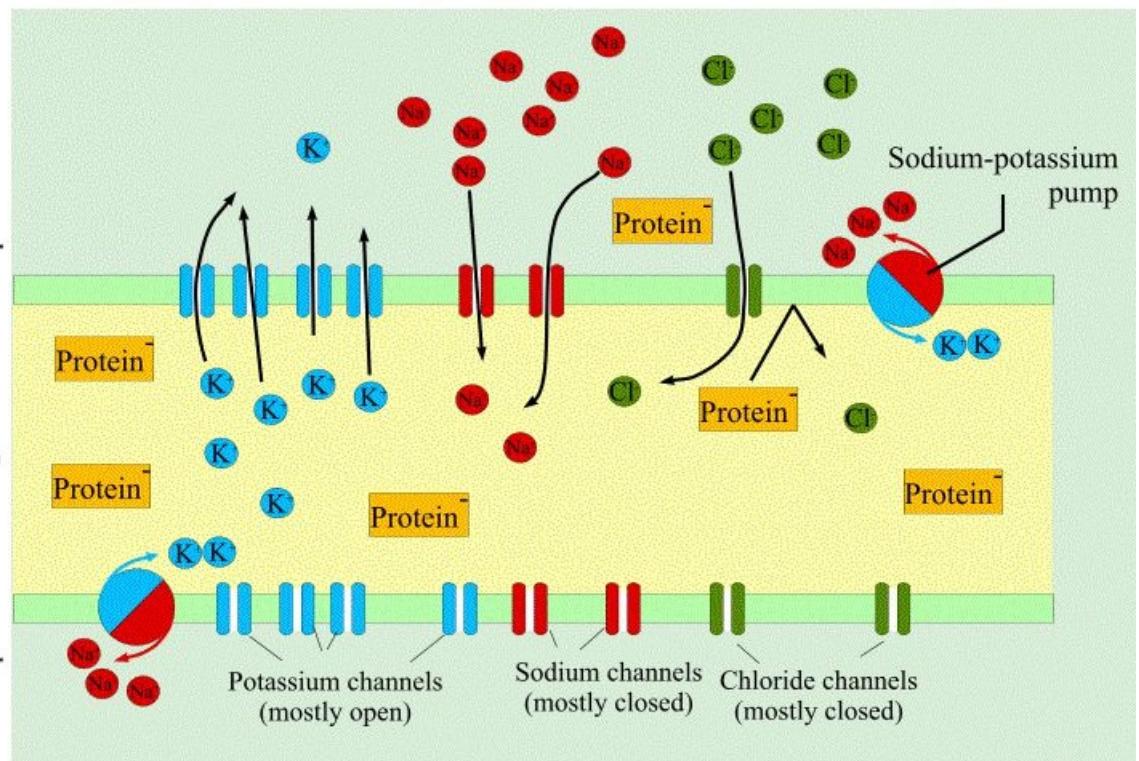
- Resting potential exists in all the cells.

Resting membrane potential is the potential across the axolemma of a neuron cell when there is no stimulus.

Resting potential

60 mV
+
-

The result of all of these differences in concentration and permeability is the **resting potential** of -60 mV.



Explanation:

Resting membrane potential is negative due to:

1. presence of large number of positive Na ions towards outside of membrane
2. presence of smaller number of positive K ions towards inside of membrane
3. zwitterionic protein molecules of cytoplasm behave as negative ions in presence of highly charged K
4. Na-K ion pumps continuously pump out three sodium ions while only two potassium ions are taken inside the cell.

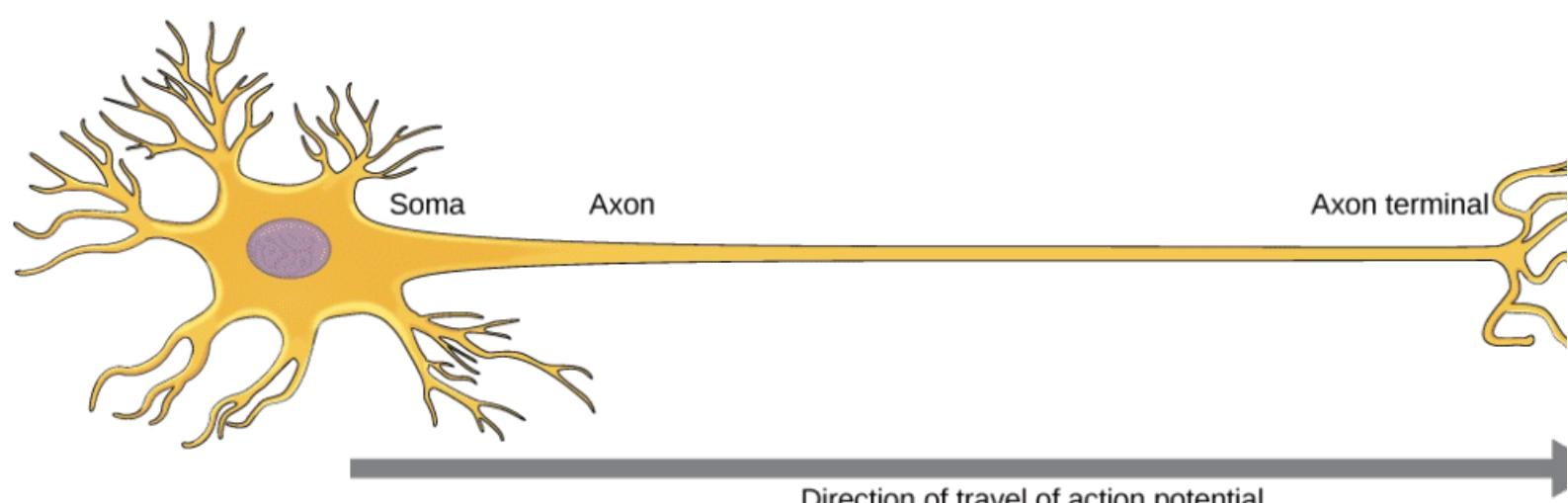
Once the neuron receives stimulus nerve impulse conduction starts.

Introduction

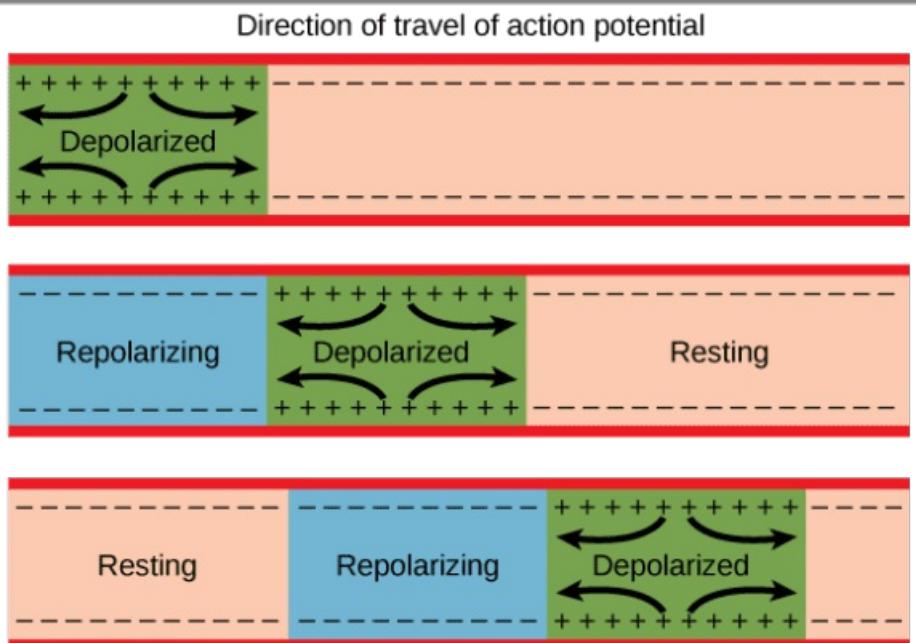
Nerve impulse was discovered by British Scientist Lord Adrian in the 1930s. Owing to the importance of this discovery, he was awarded the Nobel Prize in 1932. Nerve Impulse is a major mode of signal transmission for the Nervous system. Neurons sense the changes in the environment and as a result, generate nerve impulses to prepare the body against those changes.

Definition

Nerve Impulse is defined as a wave of electrical chemical changes across the neuron that helps in the generation of the action potential in response to the stimulus. This transmission of a nerve impulse across the neuron membrane as a result of a change in membrane potential is known as Nerve impulse conduction.



- a. In response to a signal, the soma end of the axon becomes depolarized.
- b. The depolarization spreads down the axon. Meanwhile, the first part of the membrane repolarizes. Because Na^+ channels are inactivated and additional K^+ channels have opened, the membrane cannot depolarize again.
- c. The action potential continues to travel down the axon.



It is a change in the resting state of the neuron. Due to nerve impulse, the resting potential is changed to an action potential to conduct signals to the target in response to a stimulus. The stimulus can be a chemical, electrical, or mechanical signal.

The action potential is a result of the movement of ions in and out of the cell. Particularly the ions included in this process are sodium and potassium ions. These ions are propagated inside and outside the cell through specific sodium and potassium pumps present in the neuron membrane. The transmission of a nerve impulse from one neuron to another neuron is achieved by a synaptic connection (synapse) between them. It is thus a mode of communication between different cells.

The speed of nerve impulse propagation varies in different types of cells. The rate of transmission and generation of nerve impulses depends upon the type of cell. Besides, Myelin Sheath also helps in accelerating the rate of signal conduction (about 20 times). Generally, the speed of nerve impulse is 0.1-100 m/s.

Mechanism of Nerve Impulse Conduction

Nerve impulse conduction is a major process occurring in the body responsible for organized functions of the body. So, for conduction of nerve impulse there are two mechanisms:

1. Continuous conduction
2. Saltatory conduction

Continuous Conduction

Continuous nerve impulse conduction occurs in non-myelinated axons. The action potential travels along the entire length of the axon. Hence, more time is taken in generating and then transmitting nerve impulses during an action potential.

Continuous conduction requires more energy to transmit impulses and is a slower process (approximately 0.1 m/s). It delays the process of conducting signals because it uses a higher number of ion channels to alter the resting state of the neuron.

Saltatory Conduction

Saltatory is faster than continuous conduction and occurs in myelinated neurons. In myelinated neurons, myelinated sheaths are present. Between these myelinated sheaths, unmyelinated gaps are presently known as the nodes of Ranvier. Nerve impulse propagates by jumping from one node of Ranvier to the next. This makes the process of nerve impulse faster as the nerve impulse does not travel the entire length of the axon (this happens in case of continuous conduction). The nerve impulse travels at a speed of 100 m/s in saltatory conduction.

The number of channels utilized in saltatory conduction is less than continuous conduction due to which delay of nerve impulse does not occur. This mode of nerve impulse transmission utilizes less energy as well.

If you consider the axon as an electrical wire or loop, nerve impulse that travels along the axon as current, and the charged particles (sodium and potassium ions) as the electron particles then the process can be understood quite easily. As the flow of current in a wire occurs at a specific voltage only, similarly the conduction of nerve impulse occurs when a stimulus has a maximum threshold value of -55 millivolts. This is essential for altering the resting membrane state to action membrane potential.

When the voltage has the required number of electron particles it conducts current. Similarly, in the case of nerve impulse conduction, the stimulus must have a threshold value for causing the movement of ions across the length of the axon (for conducting nerve impulse) by opening the voltage-gated ion channels.

Process of transmission of Nerve Impulse

For the transmission of a nerve impulse, the stages are below:

1. Polarization
2. Depolarization
3. Repolarization
4. Refractory Period
5. Synapse

Before going into the details of the process of nerve impulse transmission, let's first discuss action and resting potential states.

Resting Membrane Potential

The resting membrane potential refers to the non-excited state of the nerve cell at rest when no nerve impulse is being conducted. The resting membrane potential of the nerve cell is -70 mV. It is a static state and both the sodium and potassium channels are closed during this state, maintaining a high concentration of sodium ions outside and high potassium ions concentration inside the cell.

Action Potential

An action potential occurs when the nerve cell is in an excited state while conducting nerve impulses. In this situation, sodium channels open and potassium channels are closed. This results in a huge influx of sodium ions inside the cells which trigger the nerve impulse conduction. The action potential is +40 mV.

Polarization

Polarization is the situation in which the membrane is electrically charged but non-conductive. It means it doesn't conduct nerve impulses in this state. During polarization, the membrane is in a resting potential state. The concentration of sodium ions is about 16 times more outside the axon than inside. In contrast, the concentration of potassium ions is 25 times more inside the axon than outside.

The polarization state is also known as the "Unstimulated or non-conductive state". Due to the difference in the concentration of ions inside and outside the membrane, a potential gradient is established ranging between -20-200mV (in the case of humans, the potential gradient in the polarized state is nearly -70mV). In the polarized state, the axon membrane is more permeable to potassium ions instead of sodium ions and as a result, it causes rapid diffusion of potassium ions.

In the resting state, the membrane potential becomes electro-negatively charged due to the movement of positively charged potassium ions outside the cell and the presence of electro-negative proteins in the intracellular space.

Depolarization

It refers to a graded potential state because a threshold stimulus of about -55mV causes a change in the membrane potential. The threshold stimulus must be strong enough to change the resting membrane potential into action membrane potential.

This results in the alteration in the electro-negativity of the membrane because the stimulus causes the influx of sodium ions (electropositive ions) by 10 times more than in the resting state. For this, sodium voltage-gated channels open. The action potential state is based on the "All or none" method and has two possibilities:

If the stimulus is not more than the threshold value, then there will be no action potential state across the length of the axon.

If the stimulus is more than the threshold value, then it will generate a nerve impulse that will travel across the entire length of the axon.

Repolarization

It is a condition during which the electrical balance is restored inside and outside the axon membrane. Due to the high concentration of sodium ions inside the axoplasm, the potassium channels will open. During the repolarization state, efflux of potassium ions through the potassium channel occurs. As a result of the opening of potassium voltage-gated channels, sodium voltage-gated channels will be closed. Thus, no sodium ions will move inside the membrane. Therefore, repolarization helps in maintaining or restoring the original membrane potential state.

Until potassium channels close, the number of potassium ions that have moved across the membrane is enough to restore the initial polarized potential state. As a result of this, the membrane becomes hyperpolarized and have a potential difference of -90 mV.

Refractory Period

The refractory phase is a brief period after the successful transmission of a nerve impulse. During this period, the membrane prepares itself for the conduction of the second stimulus after restoring the original resting state. It persists for only 2 milliseconds.

During this, the sodium ATPase pump allows the re-establishment of the original distribution of sodium and potassium ions. The sodium and potassium ATPase pump, driven by using ATP, helps to restore the resting membrane state for the conduction of a second nerve impulse in response to the other stimulus. It causes the movement of ions both against the concentration gradient. For every two potassium ions that move inside the cell, three sodium ions are transported outside.

This process requires ATP because the movement of ions is against the concentration gradient of both ions.

Synapses

The process of transmission of a nerve impulse from one neuron to the other, after reaching the axon's synaptic terminal, is known as synapses. This transmission of the nerve impulse by synapses involves the interaction between the axon ending of one neuron (Presynaptic neuron) to the dendrite of another neuron (Postsynaptic neuron). There is space between the pre-synaptic neuron and post-synaptic neuron which is known as a synaptic cleft or synaptic gap.

After transmitting from one neuron to another, the nerve impulse generates a particular response after reaching the target site. If somehow the synaptic gap doesn't allow the passage of nerve impulse, the transmission of nerve impulse will not occur and consequently required response too.

Types of synapses

There are two types of synapses:

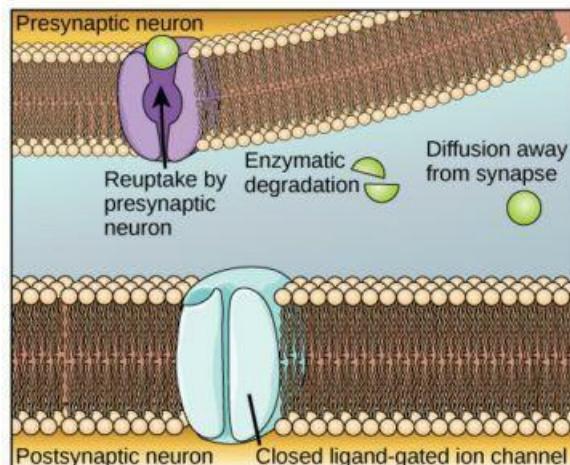
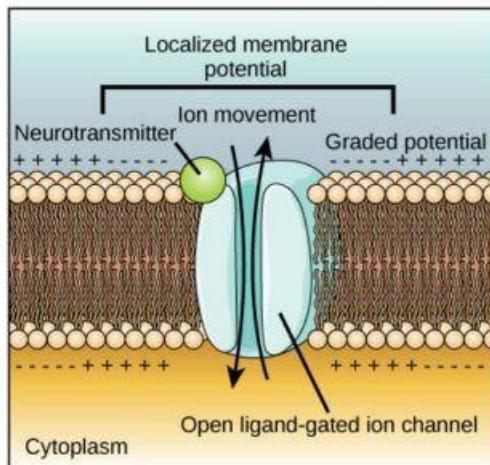
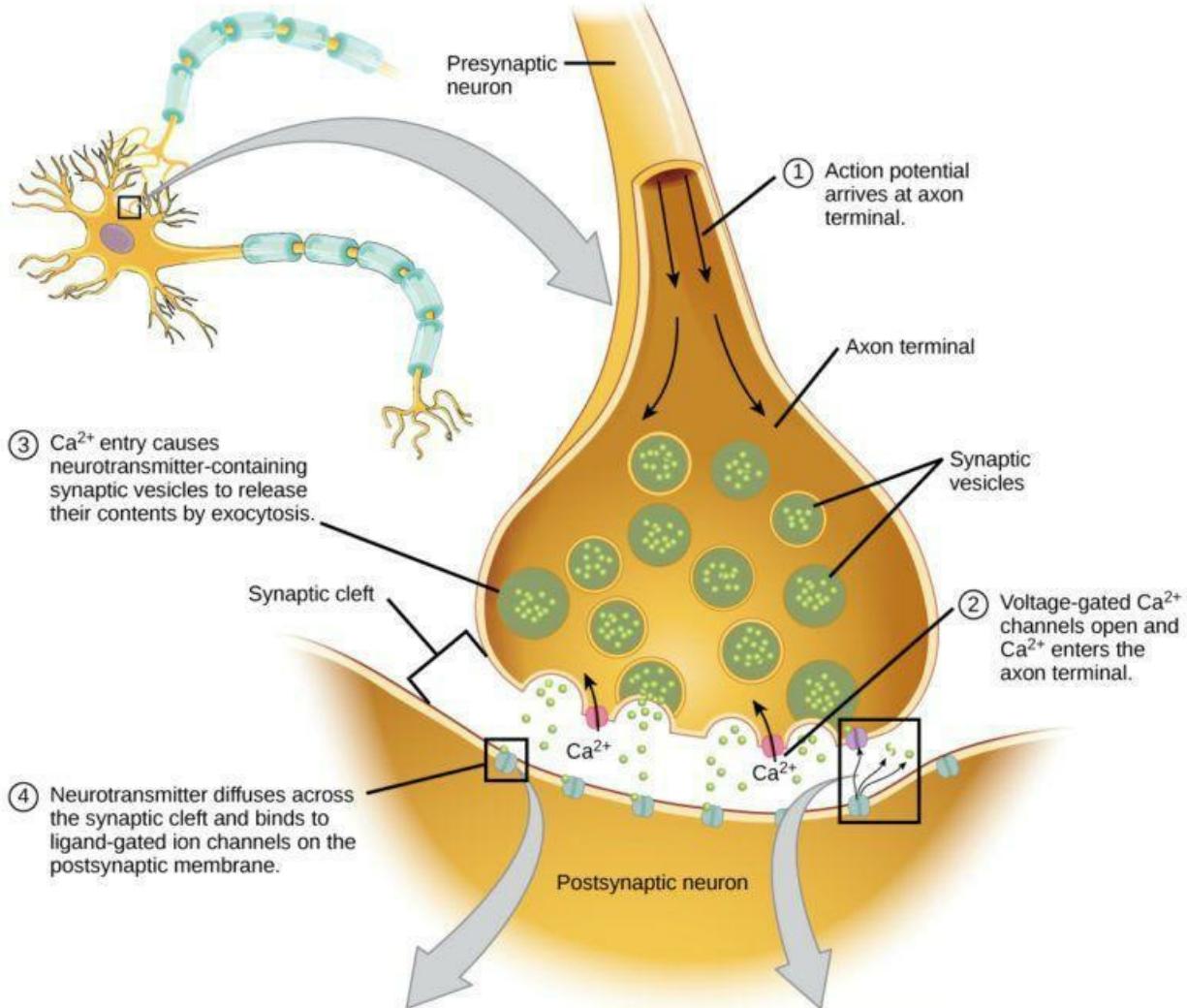
1. Electrical synapses
2. Chemical synapses

Electrical synapses

In electrical synapses, two neurons are connected through channel proteins for transmitting a nerve impulse. The nerve impulse travels across the membrane of the axon in the form of an electrical signal. The signal is transmitted in the form of ions and therefore it is much faster than chemical synapses.

In electrical synapses, the synaptic gap is about 0.2nm which also favors faster nerve impulse conduction.

Chemical synapses



⑤ Binding of neurotransmitter opens ligand-gated ion channels, resulting in graded potentials.

⑥ Reuptake by the presynaptic neuron, enzymatic degradation, and diffusion reduce neurotransmitter levels, terminating the signal.

In chemical synapses, the conduction of nerve impulse occurs through chemical signals. These chemical signals are neurotransmitters. In this type of nerve impulse conduction, the synaptic gap is more than electrical synapses and is about 10-20 nm. Due to this, the transmission of nerve impulses is slower than electrical synapses.

CNS and Nerve Impulse

Neurons help in transmitting signals in the form of a nerve impulse from the Central nervous system to the peripheral body parts. Neurons are a complex network of fibres that transmit information from the axon ending of one neuron to the dendrite of another neuron. The signal finally reaches the target cell where it shows a response.

In conducting nerve impulse, the following play a major role:

- Axon- Helps in the propagation of nerve impulses to the target cell.
- Dendrites- Receive the signals from the axon ends.
- Axon Ending- Acts as a transmitter of signals.

Axon plays a major role in the process by transmitting signals in the form of nerve impulses via synapses to the target cells. The neuron is responsible for transferring signals to three target cells:

- Muscle
- Gland
- Another neuron

And this results in the contraction of muscle, secretion by glands and helps neurons to transmit action potential.

Factors Affecting the Speed of Nerve Impulse

Following are some major factors that affect the speed of nerve impulses:

1. Myelin Sheath
2. Axon Diameter
3. Temperature

Myelin Sheath

Myelin sheath is present around the neuron and functions as an electrical insulator. Due to this sheath, an action potential is not formed on the surface of the neuron. This Myelin sheath has regular gaps, where it is not present, called nodes of Ranvier. An action potential can form at these gaps and impulse will jump from node to node by saltatory conduction. This can be a factor for increasing the speed of nerve impulse from about 3 folds.

Axon Diameter

As the axon diameter increases, the speed of nerve impulses increases as well. This is because a larger axon diminishes the ion-leakage out of the axon. This helps in maintaining the membrane potential and thus favours faster nerve impulses.

Temperature

Temperature causes changes in the rate of diffusion of ions across the neuron membrane. Temperature directly correlates with the transmission of nerve impulses. If the temperature is higher, the rate of diffusion of sodium and potassium ions will be high and the axon will become depolarized quickly which will cause a faster nerve impulse conduction.

A nerve impulse is thus an important signal transduction mode for triggering a response in major body parts due to a strong stimulus. Any distraction in this process can have drastic effects on the body.

Module 2

1. Exothermic and endothermic versus endergonic and exergonic reactions; Concept of K_{eq} and its relation to standard free energy
2. Spontaneity; ATP as an energy currency
3. Aerobic Respiration (Glycolysis and Krebs cycle)
4. Synthesis of glucose (Photosynthesis), Energy yielding and energy consuming reactions
5. Concept of energy charge, Morphology of chromosomes; The cell cycle and phases
6. Mitosis and meiosis

Metabolism

Metabolism: It is the sum total of all the biochemical reactions which take place inside living organisms to keep them alive. The biochemical reactions occur due to interactions among different types of molecules within the cell.

- It is also the sequence of chemical reactions which forms the metabolic pathways in the living system.
- Each step of the chemical reactions forming the metabolic pathways in the living organisms is catalysed by a specific enzyme (protein). The product formed in each step is called Metabolite.
- Metabolic pathways are of 2 types:

1. Anabolic Pathway: Synthesis of complex compounds from simple substances. It requires energy. Eg. a. Acetic acid \rightarrow Cholesterol b. Glucose \rightarrow Starch
2. Catabolic Pathway: Complex molecules are broken down to simpler forms.

Example: Starch \rightarrow Glucose

Hence, metabolism occurs either by absorbing or releasing energy. Some complex molecules are formed whereas some complex molecules are broken down to be utilized by the cell for various functions. In doing so, energy flows and transforms. Hence, bioenergetics is also important to understand while studying metabolism. Bioenergetics deals with the energy flow in the living systems.

Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and functions of the chemical processes underlying these transductions. Biological energy transformations obey the Laws of Thermodynamics.

In the living system, all cells work continuously in order to stay alive, grow and reproduce. To perform these functions the cell requires energy. Cells can derive energy either from the Sun (plants derive energy from sun) or nutrients (animals get energy from food or nutrients). By using nutrients cells perform functions such as:

- Formation of cellular components
- Generation of potential gradients
- Nerve Impulses

- Muscle contraction
- Transport of ions and molecules across the cell membrane

Bioenergetics gives all the information such as-

- energy transformation that occurs in the living cells.
- the nature and function of chemical processes underlying the energy transformation that occurs in the cell.

When we come to the term energy, we should know the “laws of thermodynamics”.

- 1. First law of thermodynamics: It is also known as Law of Conservation of Energy. It states that energy can neither be created nor be destroyed but it can be converted from one form to another form.

Cells convert light energy into nutrient molecules or chemical components. These nutrient molecules are converted into chemical energy, mechanical energy, electrical energy, etc. Thus, cells have the ability to transform one form of energy to another. Hence, 1st law of thermodynamics is satisfied.

- 1. Second Law of Thermodynamics: Entropy or randomness of the universe always tends to increase.

Living organisms receive energy from their surroundings and synthesize nutrient molecules, perform several cellular functions and return this energy to the environment in the form of heat or in the form of entropy in equal amounts. Also, cells of the living organisms are highly organized, in order to maintain highly ordered form, it creates randomness in their surroundings. Hence, the second law of thermodynamics is also satisfied.

Energy Transformation in living systems occurs in three stages:

1. Photosynthesis

2. Respiration

3. Utilization of Energy

1. Photosynthesis (in plants):

Solar Energy

Green Plants →→→ Chemical Energy (solar energy is converted into chemical energy)

↓

Nutrient molecules are synthesized

(Carbohydrates, proteins, lipids by utilizing CO₂ and H₂O)

Energy is stored in nutrient molecules. These nutrient molecules will be converted into other forms of energy during respiration.

2. Respiration (in animals):

Oxidation

Nutrient molecules →→ Chemical energy (utilized to perform various cellular activities)

3. Utilization of energy:

Cells → Utilize chemical energy for various functions → Rest energy will be dissipated to the surroundings in the form of heat.

• Some part of the energy produced during respiration will be dissipated to the surroundings in the form of heat. These are unavailable energy in the cells.

Why is all energy not utilized?

Example-

Take Machine as an example to explain:

The machine converts heat into other forms of energy. In order to do so, the machine will transfer heat from the region of higher temperature to lower temperature.

This is not possible in the case of biological systems because biological systems are isothermal. Here, the temperature remains constant throughout the body/tissues/cells. There is no difference in temperature in any two regions of the biological systems.

Considering the human body, all cellular processes occur at a constant temperature. No two positions/regions of a cell/tissue/whole body have a different average temperature. So, heat cannot be transformed completely and used up directly by the living system. Here, solar energy has to get converted into chemical energy and chemical energy into nutrient molecules. These nutrient molecules are taken as food by the animals where oxidations of these nutrients occur. Oxidations of these nutrient molecules lead to the production of energy that is utilized by the cells to perform various functions of life processes.

All the amount of energy produced during respiration is not completely available and utilized by the cells to perform various activities.

Biological systems (plants or animals) are the mechanical engine that works under isothermal conditions i.e. at constant temperature and pressure.

The energy available for the cells at constant temperature and pressure to perform various cellular activities is called FREE ENERGY (denoted by G). It is also known as Gibb's free energy.

To measure the exact value of free energy is very difficult so, a change in free energy is measured i.e (ΔG).

For a reaction:

$A \rightleftharpoons B$; Where, A is the reactant, B is the product (Reaction is reversible. It means reaction is favoured in both directions; A is formed from the B and B is formed by the A)

$$\Delta G = G_B - G_A$$

G_B is the free energy of product B

G_A is the free energy of reactant A

ΔG is the change in free energy in the system.

Also,

$$\Delta G = \Delta H - T\Delta S; H \text{ is enthalpy, } S \text{ is entropy or randomness/disorder in the system}$$

Where, ΔG is the available free energy for the living system to do work.

ΔH is the total heat content of the system or total energy of the system.

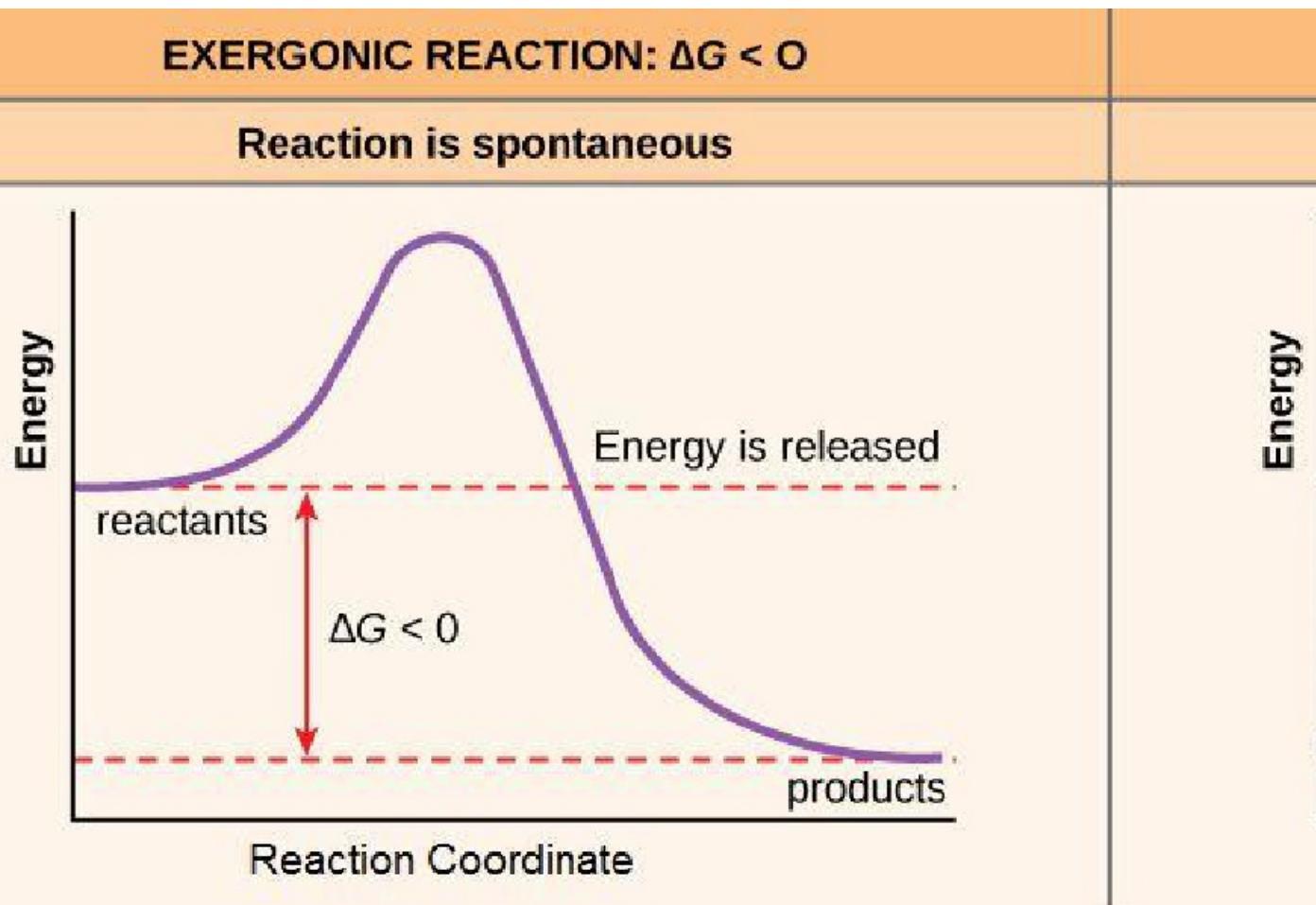
T is the temperature. ΔS is the entropy of the system.

The term $T\Delta S$ is the dissipated energy to the surroundings or unavailable energy to the living system to perform life processes.

By knowing the value of ΔG we can know:

1. Direction of the reactions that are going to take place.
2. Evaluate the amount of useful work that can be done at constant temperature and pressure.
3. Whether the reaction is spontaneous or not.

ΔG can have positive, 2. Negative and 3. Zero values ($\Delta G = +ve$, $\Delta G = -ve$, $\Delta G = 0$)



ΔG is negative. It has less value than 0.

- Products have lower free energy than reactants.
- Reaction proceeds in forward direction i.e. reaction is thermodynamically more favourable.
- Energy is liberated during the reaction and it is known as exergonic reaction.
- It is a spontaneous reaction.

ΔG is positive. It has more value than 0.

- Products have higher free energy than reactants.
- Backward reaction is favoured and thermodynamically unfavorable.

$A \xrightarrow{x} B$ (Forward reaction is unfavourable)

- Energy is required for the reaction to occur in forward direction; hence it is regarded as an endergonic reaction. Here, energy is absorbed from the surroundings.
- It is a non-spontaneous reaction.

1. When $\Delta G=0$

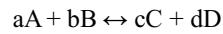
$A \leftrightarrow B$; Reaction is in equilibrium.

- Reaction is favoured in both directions.
- Rate of formation of products and rate of formation of reactants are equal.

Relationship between standard free energy (G^0) and equilibrium constant (K_{eq})

Biological systems operate in isothermal condition i.e at constant temperature (298 K) and pressure (1atm)

If two reactants and products are taken into consideration:



Here, ΔG is related to ΔG^0 and reaction coefficient Q as,

$$\Delta G = \Delta G^0 + RT \ln Q \quad (R = \text{universal gas constant}; T = \text{Temperature in Kelvin})$$

When the reaction is not at equilibrium:

$$Q = [C]^c \cdot [D]^d / [A]^a \cdot [B]^b$$

So,

$$\Delta G = \Delta G^0 + RT \ln [C]^c \cdot [D]^d / [A]^a \cdot [B]^b$$

At equilibrium:

$$Q = K_{eq} \quad (\text{equilibrium constant})$$

When equilibrium is attained, there is no free energy change. i.e. $\Delta G = 0$ and $Q = K_{eq}$

Hence, the above equation becomes

$$\Delta G = \Delta G^0 + RT \ln K_{eq}$$

$$0 = \Delta G^0 + RT \ln K_{eq}$$

$$\Delta G^0 = -RT \ln K_{eq}$$

$$\Delta G^0 = -2.303 RT \log_{10} K_{eq}$$

$$\Delta G_0 = -2.303 RT \log_{10} K_{eq}$$

If $K_{eq}=1$ $\Delta G^0 = 0$; at equilibrium

$K_{eq} > 1$ ΔG^0 = negative; reaction will proceed in forward direction

$K_{eq} < 1$ ΔG^0 = positive; reaction favoured in backward direction

Example: 1. Glucose-6-phosphate + H₂O → Glucose + Pi ($\Delta G^0 = -13.8$ KJ/mol) So, it is a spontaneous reaction.

2. Glycylglycine + H₂O → 2 Glycine ($\Delta G^0 = -9.2 \text{ KJ/mol}$) So, it is also a spontaneous reaction but it is less favourable than the 1st reaction because it has less negative free energy value.

3. Glucose + 6 O₂ → 6 CO₂ + 6 H₂O ($\Delta G^0 = -2840 \text{ KJ/mol}$) It is the most favourable reaction in the living systems.

Living cells and organisms in contrast to the reacting system in thermodynamics are open systems, they exchange both material and energy with the surroundings.

Enthalpy (H)

Enthalpy is the heat content of the reacting system.

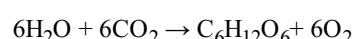
ΔH is the change in enthalpy or heat energy during a chemical reaction.

$\Delta H = H_p - H_R$ (H_p is the heat content or enthalpy of the product formed and H_R is the enthalpy of the reactant)

ΔH is related to ΔG in the following way:

$$\Delta G = \Delta H - T\Delta S$$

- If ΔH is positive, the reaction is an endothermic reaction. Endothermic reactions absorb or require energy in order to proceed. Example: Photosynthesis.



- If ΔH is negative, the reaction is an exothermic reaction. Exothermic reactions release energy in the form of heat or light or sound. Example: Respiration, Neutralisation reaction, etc.

Exothermic and Endothermic Vs Exergonic and Endergonic Reactions:

$$\Delta G = \Delta H - T\Delta S$$

Exothermic reactions:

Exo means release and therm means heat.

$\Delta H < 0$ (negative), (Releases heat energy or enthalpy)

1. $\Delta S > 0$ (positive), $\Delta G < 0$ (negative), $\Delta H < 0$ (negative)

- Products formed in the reaction will have less energy than the reactants.**
 - It means energy has been released.**

Exergonic reactions

- Energy that has been released comes from the breaking bonds. Here, electrons are going to lower energy levels to gain stability and new bond formation (product formation).**

$\Delta G < 0$ (negative)

(Releases free work energy)

- Also, the energy released by the electrons while moving to the lower energy level from higher energy level, is being transferred to the molecules that have been formed, hence increasing the disorderness or randomness or entropy (S) of the system.**

Hence,

Reactions will be spontaneous under such condition

Endergonic reactions

2. $\Delta G > 0$ (positive),

$\Delta S < 0$ (negative), $\Delta H < 0$ (negative), High Temperature (T)

- It is a non-spontaneous reaction because the entropy of the system gets reduced. And here entropy matters because temperature is high.**

$\Delta G > 0$ (positive)

Work energy is needed for the reaction.

Endothermic reactions:

Endo means absorb and therm means heat

$\Delta H > 0$ (positive), (absorb heat energy or enthalpy)

3. $\Delta G < 0$ (negative),

$\Delta S > 0$ (positive), $\Delta H > 0$ (positive), High Temperature (T)

- Absorb heat. Still, it is a spontaneous reaction under such conditions at high temperature.**
- At high temperature, reacting molecules have higher kinetic energy.**
- The system is absorbing heat energy. But it is still exergonic in nature.**
- This happens due to the high temperature. The reactants will collide with each other very fast to form other smaller products. Here, the net entropy increased.**

Hence,

Reactions will be spontaneous under such conditions also.

4. $\Delta G > 0$ (positive),

$\Delta S < 0$ (negative), $\Delta H > 0$ (positive)

- Reactions will be non-spontaneous under such conditions.**

The above table also explains the spontaneity of the reactions under various conditions.

ATP as an energy currency

Energy is the ability to do work. The body is a complex system, and as such, it takes energy to maintain proper functioning.

How does our body store and produce energy?

Our body produces and stores energy in the form of energy molecules such as adenosine triphosphate (ATP), guanosine triphosphate (GTP), nicotinamide adenine dinucleotide (NADP), flavin adenine dinucleotide (FADH), etc.

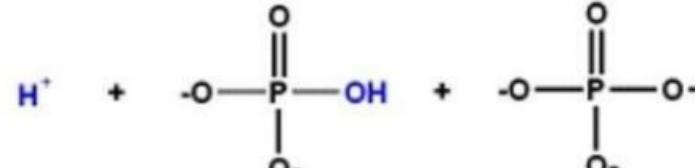
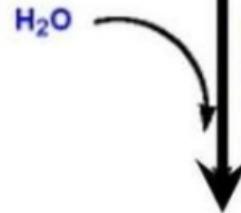
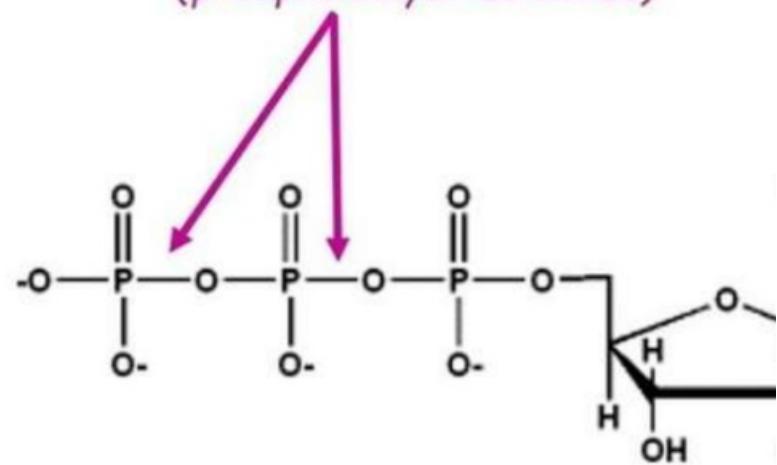
NADH and FADH are high-energy electron carriers that can be compared to the check book of your bank account. We can not immediately spend it to get energy. These two molecules help in generating ATP during aerobic respiration.

Adenosine triphosphate (ATP) is the source of energy for use and storage at the cellular level. The structure of ATP is a nucleoside triphosphate, consisting of a nitrogenous base (adenine), a ribose sugar, and three serially bonded phosphate groups. ATP is known as the “energy currency”, because it has readily releasable energy in the bond between the second and third phosphate groups. In addition to providing energy, the breakdown of ATP through hydrolysis serves a broad range of cell functions, including signaling and DNA/RNA synthesis. ATP synthesis utilizes energy obtained from multiple catabolic mechanisms, including cellular respiration, beta-oxidation, and ketosis. The majority of ATP synthesis occurs in cellular respiration within the mitochondrial matrix: generating approximately thirty-two ATP molecules per molecule of glucose that is oxidized. ATP is consumed for energy in processes including ion transport, muscle contraction, nerve impulse propagation, substrate phosphorylation, and chemical synthesis. These processes, as well as others, create a high demand for ATP. As a result, cells within the human body depend upon the hydrolysis of 100 to 150 moles of ATP per day to ensure proper functioning.

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Two high energy bonds
(phosphoanhydride bonds)



(won't be doubly protonated at neutral pH)

Inorganic phosphate



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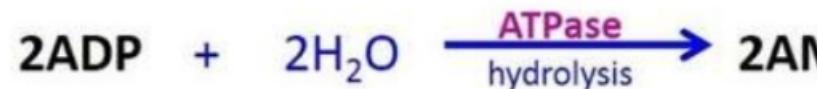
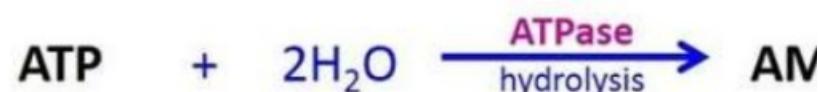
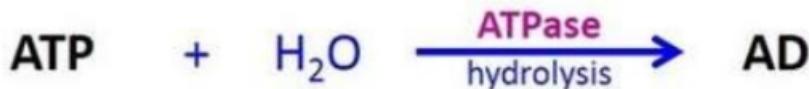
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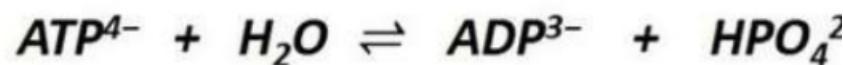
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At pH 7 (e.g. inside cells):



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Respiration

Respiration is an oxidative process, in which free energy released from organic compounds is used in the formation of ATP.

- The compounds that are oxidized during the process of respiration are known as respiratory substrates.
- Respiration is of two types:
 - The complete oxidation of respiratory substrates in the presence of oxygen is termed as aerobic respiration.
 - The incomplete oxidation of respiratory substrates occurring under absence of oxygen is called anaerobic respiration.

The aerobic respiration can be grouped into three major processes:

1. Glycolysis
2. Pyruvate oxidation

3. Citric acid cycle
4. Electron transport chain

In eukaryotes,

Glycolysis occurs in cytosol, pyruvate decarboxylation and citric acid cycle in the mitochondrial matrix and oxidative phosphorylation in the inner mitochondrial membrane.

In prokaryotes, Glycolysis and citric acid cycle occurs in cytosol and oxidative phosphorylation in the mesosome.

Glycolysis

'Glycos' means sugar and 'lysis' means splitting. It refers to a series of reactions by which hexose sugars undergo partial hydrolysis to form pyruvic acid or pyruvate. It is also known as the 'Embden-Meyerhof-Parnas (EMP) pathway'. Two molecules of Pyruvate, two molecules of NADH and two molecules of ATP are produced. It takes place in cytosol. It consists of a sequence of 10 enzymatic reactions in which one molecule of glucose is converted to two molecules of the three-carbon compound pyruvic acid.

Two stages in glycolysis:

1. Stage 1: Investment phase/Preparatory Phase: Glucose is phosphorylated and cleaved to form two molecules of triose phosphate. Two ATP molecules are used up during this stage. It can also be called the investment phase.

Following are the steps:

Step 1: Phosphorylation:

Glucose is phosphorylated by ATP to form a glucose-6-phosphate. This irreversible reaction is catalysed by hexokinase.

Step 2: Isomerization:

It involves the formation of a ketose sugar from an aldose sugar, i.e. isomerization of glucose-6-phosphate to fructose-6-phosphate.

Step 3: Phosphorylation:

Fructose-6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate.

Step 4: Cleavage:

The fructose 1,6-bisphosphate is cleaved to produce two 3C molecules: glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Hexokinase requires metal ions such as Mg^{2+}

Step 5: Isomerization

Dihydroxyacetone phosphate is isomerized to form glyceraldehyde-3-phosphate.

The first five steps of glycolysis constitute the preparatory phase. In this phase, energy is consumed as glucose is phosphorylated twice and, converted to FBP. For both phosphorylations, ATP is the phosphoryl group donor.

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Preparatory phase

Step-1

Glucose (G)

Hexokinase



Glucose – 6 – phosphate

Step-2

Phosphoglucoisomerase

Fructose - 6 - phosphate



Step-3

Phosphofructokinase

Fructose -1,6 - bisphosphate



Step-4

Aldolase

Glyceraldehyde – 3-
(G3P/3PGA)

Step-5

Triose phosphate isomerase

Dihydroxyacetone phosphate



Step-6

Page 24 / 45

- Stage 2: Payoff Phase: Two molecules of pyruvic acid are formed from triose phosphates. Four molecules of ATP are formed in this process.

Step 6:

The two molecules of glyceraldehyde-3-phosphate are oxidized. Here two molecules of G3P are converted into two molecules of 1,3-bisphosphoglycerate.

Step 7:

In this step a high-energy phosphate group is transferred from 1,3-bisphosphoglycerate to ADP. The formation of ATP is referred to as substrate-level phosphorylation, because the phosphate donor BPG is a substrate with high phosphoryl transfer potential.

Step 8:

The phosphate ester linkage in 3-phosphoglycerate is moved from C-3 to C-2 to form 2-phosphoglycerate.

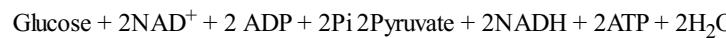
Step 9:

The removal of water from 2-phosphoglycerate forms phosphoenolpyruvate.

Step 10:

The transfer of a high-energy phosphate group to ADP forms ATP. It is the last step of glycolysis which is irreversible and forms two molecules of pyruvate ultimately.

Net reaction:



The last five steps of glycolysis constitute the payoff phase. The energy gain comes in the payoff phase.

At step 7, the high-energy phosphate on C1 of 1,3-bisphosphoglycerate is donated to ADP to produce ATP. This synthesis of ATP is substrate-level phosphorylation.

synthesis of ATP is called substrate-level phosphorylation

Payoff phase

Step-6	2	Glyceraldehyde – 3 – phosphate dehydrogenase	1K
Step-7	2	1,3- bisphosphoglycerate kinase	1K
Step-8	2	3- phosphoglycerate mutase	1L
Step-9	2	2- phosphoglycerate Enolase	1L
Step-10	2	Phosphoenolpyruvate Pyruvate kinase	Pyruvate

Page 27 / 45

Energy yield from glycolysis (in the form of ATP)

Energy yield from one molecule of glucose in glycolysis = $2\text{ATP} + 2\text{NADH}$

In the presence of oxygen, one NADH oxidized to yield = 3 ATP

Hence, glycolysis in the presence of oxygen yields = $2 \times 3\text{ATP} + 2\text{ATP} = 8\text{ATP}$

Glycolysis in the absence of oxygen (anaerobic glycolysis) produces only 2ATP as a result of substrate-level phosphorylation.

Fermentation is a general term for the anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP.

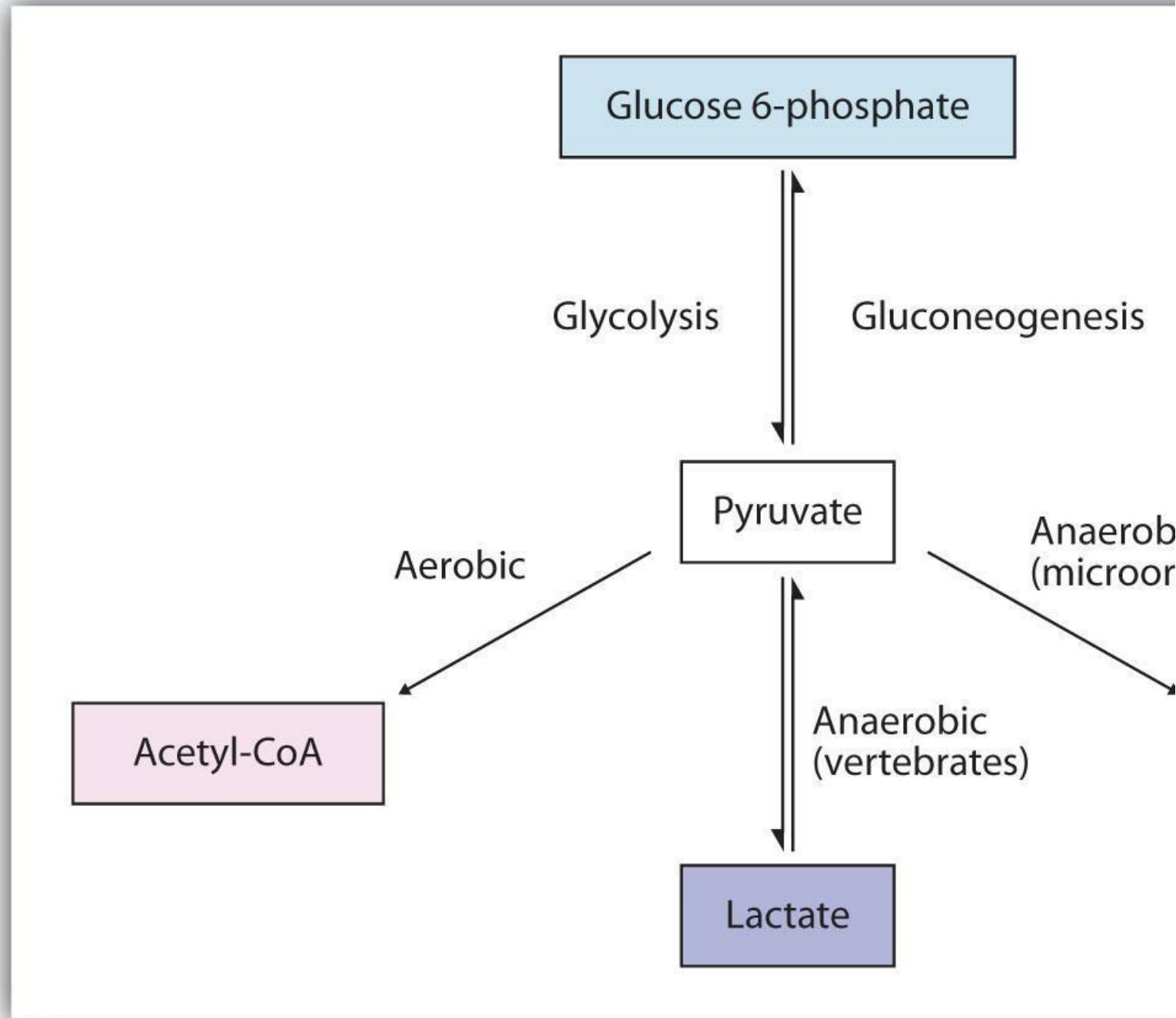
Significance of glycolysis

Glycolysis serves two major functions in the cell:

- 1. This set of reactions generate ATP.
 2. It provides the building block for biosynthesis of various biomolecules.

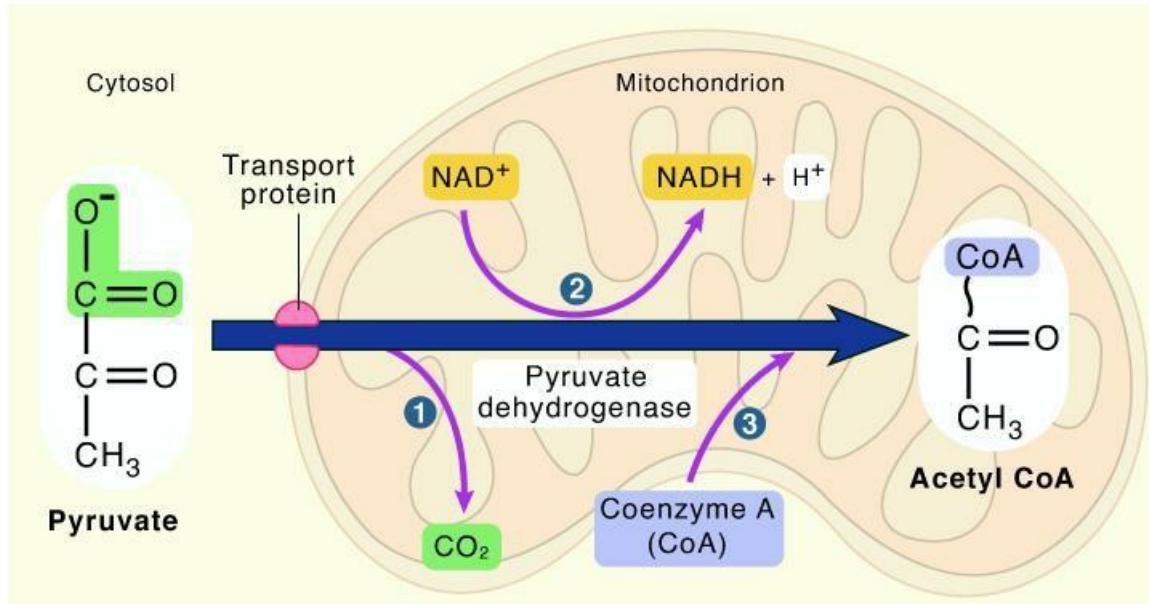
This process results in intermediate compounds, which may be used at various steps for other cellular processes. The breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, sperm, etc.)

Three fates of pyruvate:



In the presence of oxygen, further oxidation of pyruvate occurs in the mitochondrial matrix. The pyruvate oxidizes into acetyl-CoA.

Pyruvate Oxidation



1 Carboxyl group gets removed, forming CO₂

2 NAD⁺ gets reduced to NADH

3 Coenzyme A gets attached to acetate, forming acetyl CoA

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO₂ and the two remaining carbons become the acetyl group of acetyl-CoA.

Conversion of pyruvate to acetyl-CoA is the junction between glycolysis and citric acid cycle. Pyruvate is a charged molecule, so in eukaryotic cells, it must enter the mitochondria with the help of a transport protein. This is highly exergonic reaction ($G^0 = 33.4\text{KJ/mol}$).

Kreb's Cycle

Kreb's cycle is also known as the citric acid cycle or tricarboxylic acid (TCA) cycle. It was discovered by H.A. Kreb, a British biochemist in 1953. The name citric acid cycle is derived from the first product generated by the sequence of conversions, i.e., citric acid. It is a set of enzyme catalysed reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins to ATP and CO₂. It involves 8 steps:

Step 1: Condensation:

The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, catalysed by citrate synthase.

Step 2a and 2b: Dehydration and Hydration:

It is an isomerization reaction that involves 1st removal of water and then addition of water to form isocitrate from citrate. The enzyme catalysing this step is aconitase.

Step 3: Oxidative decarboxylation:

In this step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form α -ketoglutarate.

Step 4: Oxidative decarboxylation:

In this step α -ketoglutarate is converted to succinyl-CoA and CO₂ by the action of the α -ketoglutarate dehydrogenase complex. NAD⁺ serves as electron acceptor and CoA as the carrier of the succinyl group.

Step 5: Phosphorylation:

The cleavage of thioester bond of succinyl CoA is coupled phosphorylation of an ADP or a GDP (substrate level phosphorylation). Succinate is formed in the process. The enzyme that catalyzes this reversible reaction is called succinyl-CoA synthetase or succinic thiokinase.

Step 6: Dehydrogenation:

In this step, FAD removes two hydrogen atoms from succinate to form fumarate. The enzyme catalyzing this step is succinate dehydrogenase.

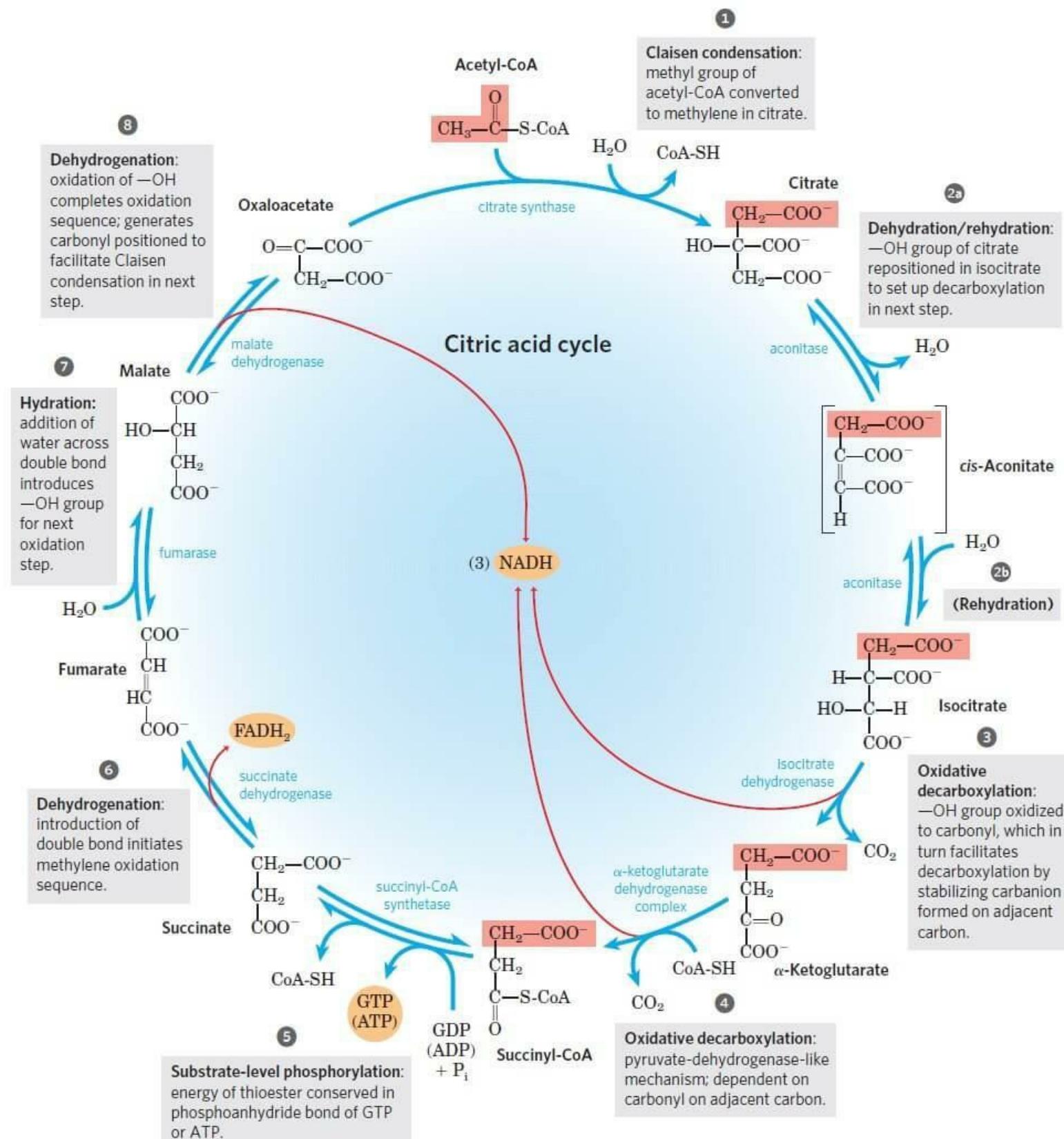
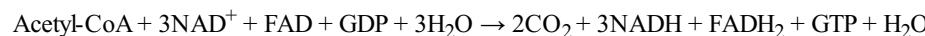
Step 7: Hydration:

The addition of water to fumarate places a hydroxyl group to carbonyl carbon, forming malate. The enzyme catalyzing this step is fumarase.

Step 8: Dehydrogenation:

The carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1. This process is catalyzed by the enzyme malate dehydrogenase.

Overall reaction:



Overview of Kreb's cycle

In eight step reaction sequence, the acetyl group of acetyl-CoA is oxidised into two molecules of CO₂. These reactions are catalysed by eight different enzymes. Eight electrons were removed from the acetyl group and transferred to the coenzymes NAD⁺ and FAD, which are reduced to NADH and FADH₂. They are therefore called electron carrier coenzymes and are used to transport electrons from the Krebs cycle to the respiratory chain. Through a series of molecules, the reduced coenzymes NADH and FADH₂ are oxidised and the released electrons are used to reduce CO₂. Finally, the electrons that are released in the Krebs cycle are transported to the respiratory chain and are used to produce ATP from ADP and Pi.



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Glucose

2 Pyruvate

2 Acetyl- CoA

Krebs
cycle

Net ATP gain from a



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Net ATP gain from a

	ATP	NADH
In glycolysis	2	2 (6 ATP in)
In link reaction		2 (6 ATP in)
In citric acid cycle	2	6 (18 ATP ETC)
Total		

- total 3 ATPs can be gained from 1 NADH reaction
- Total 2 ATPs can be gained from 1 FADH₂ reaction



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Significance of Kreb's cycle

- It supplies energy for the Electron transport chain.
- It acts as intermediate for other important reactions like the biosynthesis of glucose, fatty acids and amino acids.
- Due to the many functions of the citric acid cycle is also considered to be the "central hub of metabolism". This is because, as most of the absorbed nutrients, the fuel molecules are oxidized ultimately within the Krebs Cycle.

Photosynthesis

Photosynthesis is a physicochemical process by which plants use light energy and synthesize carbohydrates from carbon dioxide and water in the presence of chlorophyll.

On the basis of generation of oxygen during photosynthesis, organisms may be categorized as oxygenic or anoxygenic.

- Oxygenic photosynthesis: Oxygen is released at the end of photosynthesis. Example- Eukaryotes: Plants, algae, photosynthetic protists, Prokaryotes- cyanobacteria
- Anoxygenic photosynthesis: Oxygen is not released during photosynthesis. Example: prokaryotes (green and purple photosynthetic bacteria)

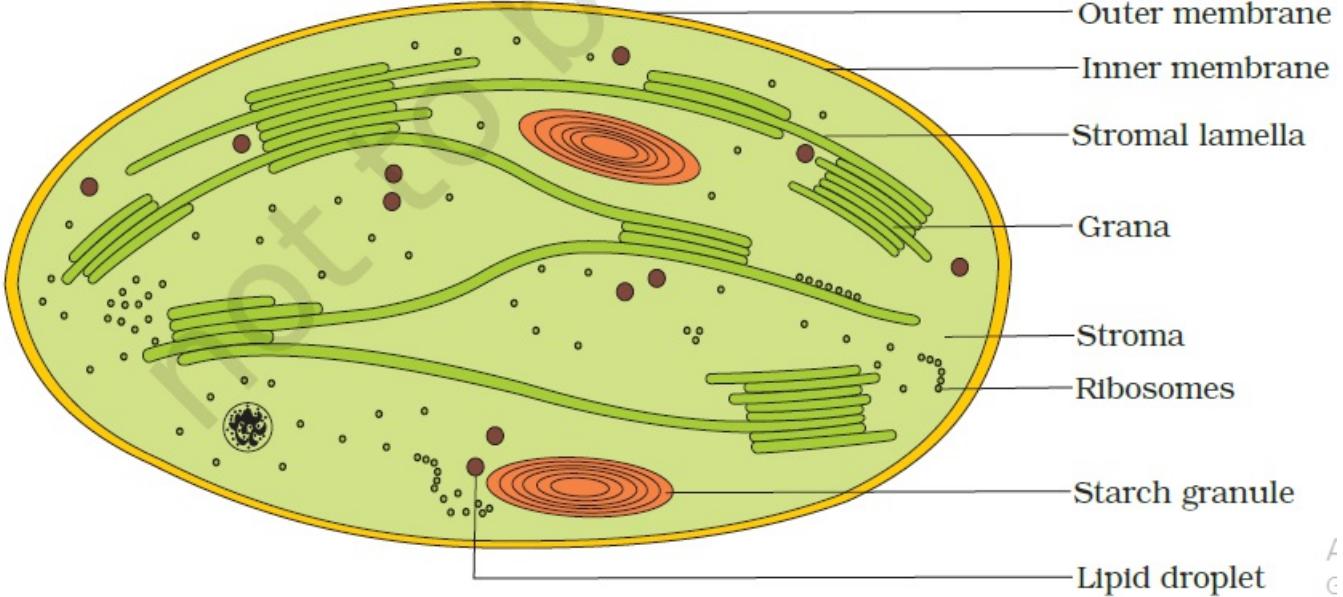
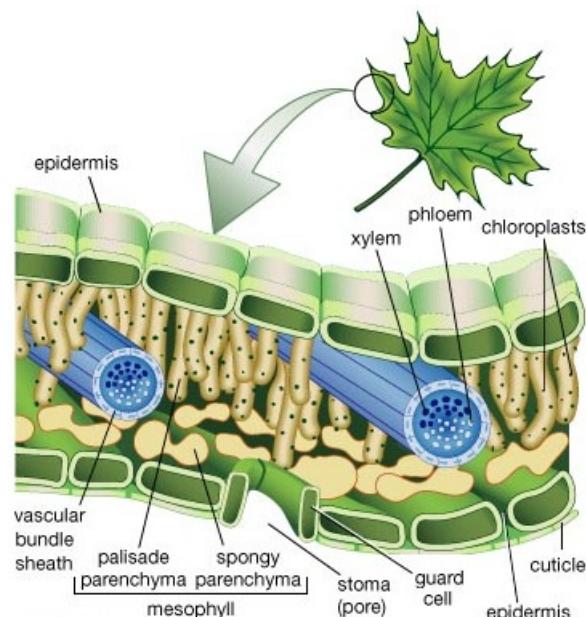


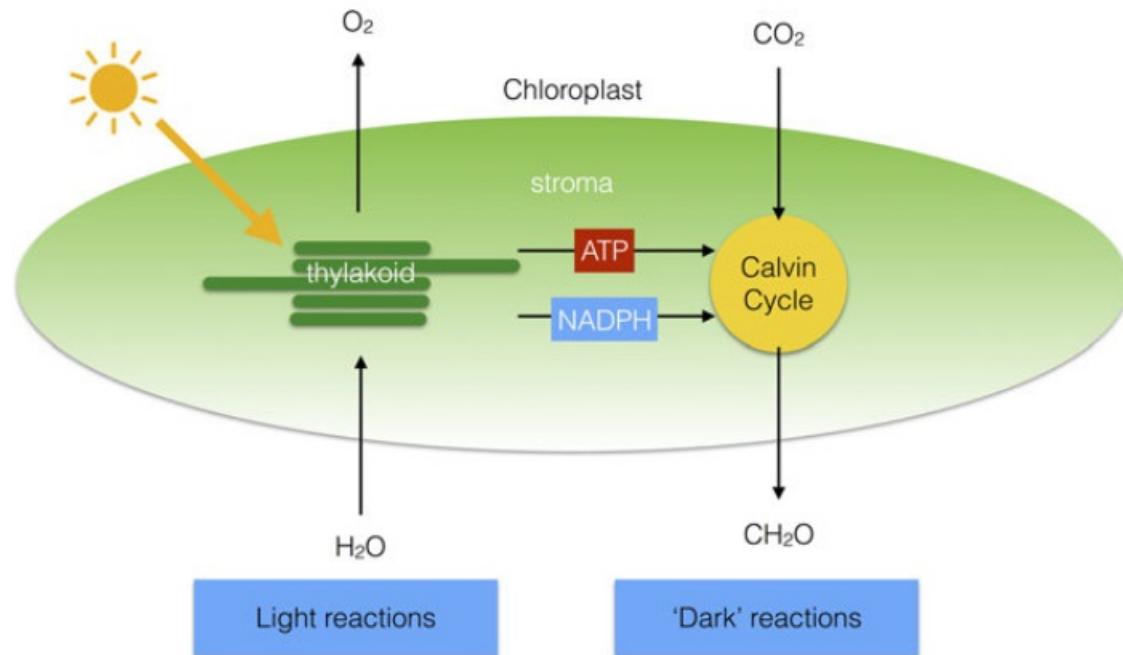
Figure: Chloroplast

Where does photosynthesis take place?

- The mesophyll cells in the leaves, have a large number of chloroplasts.
- Usually, the chloroplasts align themselves along the walls of the mesophyll cells, such that they get the optimum quantity of the incident light.



Raw materials for the process are sunlight, carbon dioxide, water and green pigments and a few enzymes.



The pigments involved in the process are chlorophyll a, chlorophyll b, xanthophyll, carotenes.

Stages of photosynthesis

Photosynthesis is a two stage process: one stage is dependent on the light and another is independent of it.

-

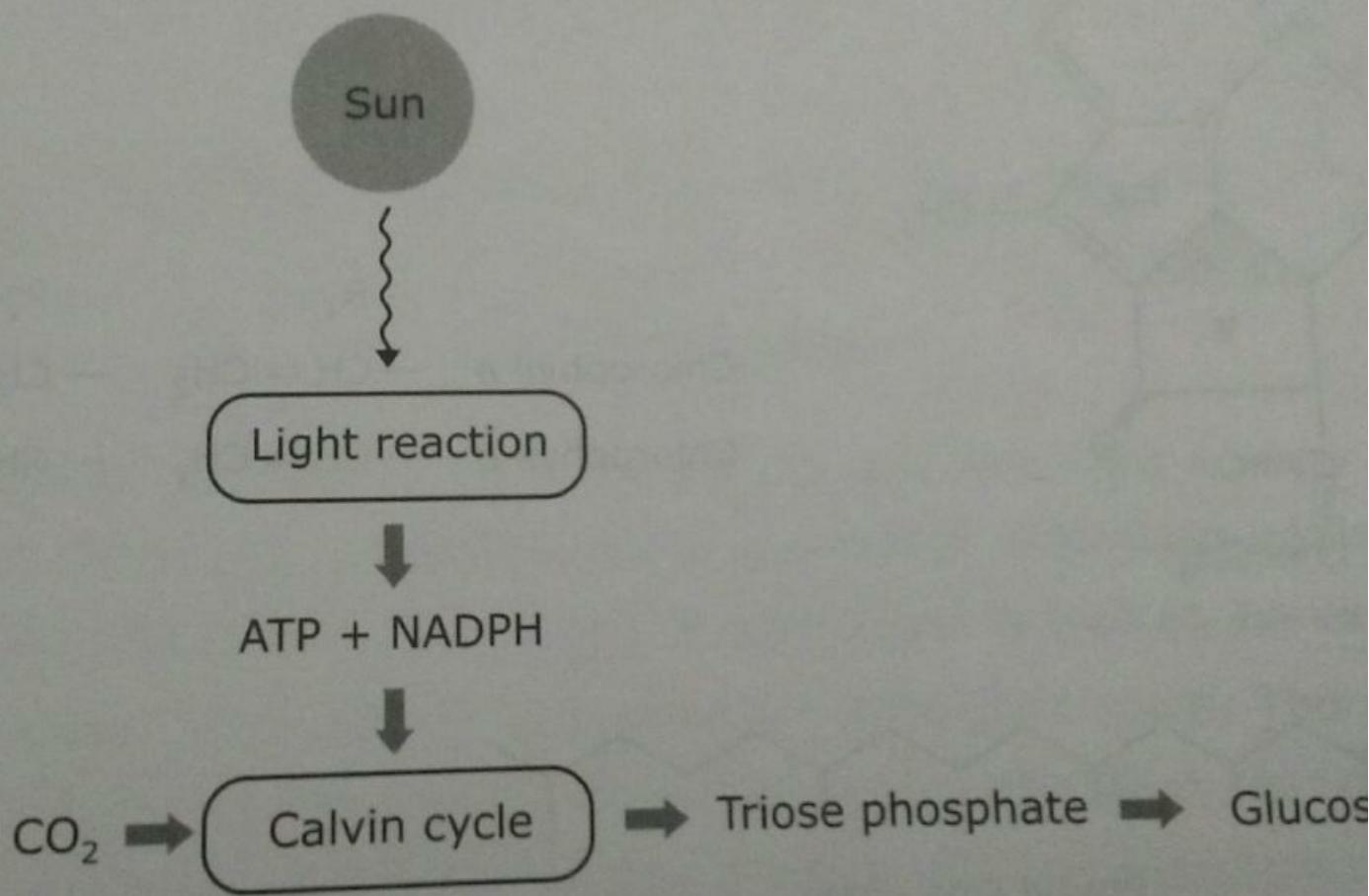
1. Light reaction:

It is light-dependent reaction, which occurs in the grana of chloroplast. It requires the direct energy of light to make NADPH and ATP that are used in dark reaction. [NADPH: nicotinamide adenine dinucleotide hydrogen]. The process of formation of ATP from ADP and inorganic phosphate by utilizing light energy is called photophosphorylation.

-

1. Dark reaction:

It is a light-independent reaction, which occurs in the stroma of the chloroplasts. Here the products of the light reaction i.e. ATP, NADPH are utilized in the dark reaction.



NADPH

- Nicotinamide adenine dinucleotide, or NAD, is in all living cells, where it functions as a coenzyme.
- It exists in either an oxidized form, NAD^+ , which can accept a hydrogen atom (i.e., a proton), or a reduced form, NADH , which can donate a hydrogen atom.
- The oxidized form is NADP^+ , while the reduced form is NADPH.

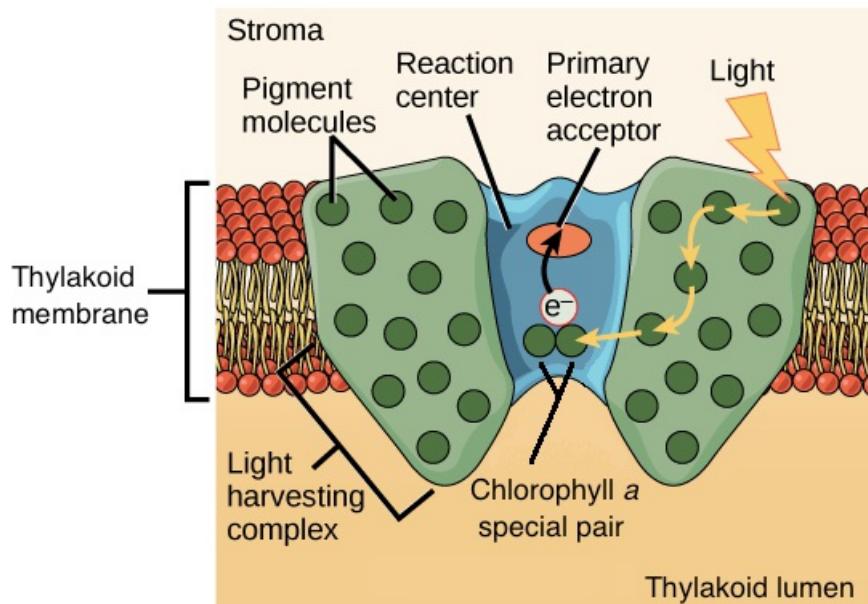
Photosynthetic pigment

- Pigments are substances that have an ability to absorb light, at specific wavelengths.
- The major photosynthetic pigment is chlorophyll.
- Chlorophyll is a light-absorbing green pigment.
- Oxygenic photosynthetic organisms contain different types of chlorophyll molecules like Chl a, Chl b, Chl c and Chl d.
- Besides chlorophyll molecules, there are two groups of accessory pigments: carotenoids & phycobilins.
- Carotenoids are again subdivided into 2 classes:
 -
 - Carotenes (responsible orange colour)
 - Xanthophylls- imparts yellow colour
- Most of the photosynthesis takes place in the blue and red regions of the spectrum (400 – 700 nm).

Concept of pigment system

- Photosynthesis involves two photosystems:
 1. One driven by light of long wavelength ($>680 \text{ nm}$)
 2. Other driven by light of short wavelength ($\leq 680 \text{ nm}$)
- In all natural photosynthetic systems, pigment molecules are bound to proteins forming pigment-protein complexes called pigment system (or photosystem).
- Pigment system have two components:
 1. Photochemical reaction centre: Chl a
 2. Antenna complex or Light harvesting complex
- In oxygenic photosynthetic organisms, two types of photosystems are present.
 1. PS-I
 2. PS-II

Photosystem



- The LHC is made up of hundreds of pigment molecules bound to proteins.
- Each photosystem has all the pigments (except two molecules of chlorophyll *a*) forming a light-harvesting system also called antennae.
- These pigments help to make photosynthesis more efficient by absorbing different wavelengths of light.
- A pair of chlorophyll *a* molecule forms the reaction centre.

Photosystem-I and Photosystem-II

Photosystem-I

PS-I comes 2nd in the path of electron flow.

The pigments in the photosystem-I absorb longer wavelengths of light which is 700 nm (P700).

The primary electron acceptor of PS-I is a modified chlorophyll molecule A_0 .

PS-I gains electrons from the PS II through an electron transport chain.

No photolysis occurs.

It is located on the non-appressed part (outer surface) of the thylakoid membrane.

Photosystem-II

PS-II comes 1st in the path of electron flow.

The pigments in the photosystem-II absorb shorter wavelengths of light which is 680 nm (P680).

The primary electron acceptor of PS-II is pheophytin, an organic molecule that resembles chlorophyll.

The PS-II reaction centre gets electrons from water.

Photolysis occurs in this system.

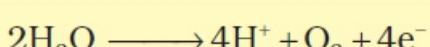
It is located on the (appressed part) inner surface of the thylakoid membrane.

1. Light reaction:

- It is also known as the ‘Photochemical’ phase.
- It includes light absorption, water splitting, oxygen release, and the formation of high-energy chemical intermediates, ATP and NADPH.
- It includes both cyclic and non-cyclic processes.
- The non-cyclic process is known as z-scheme, because of its overall Z-like form. This process involves both the photosystems.

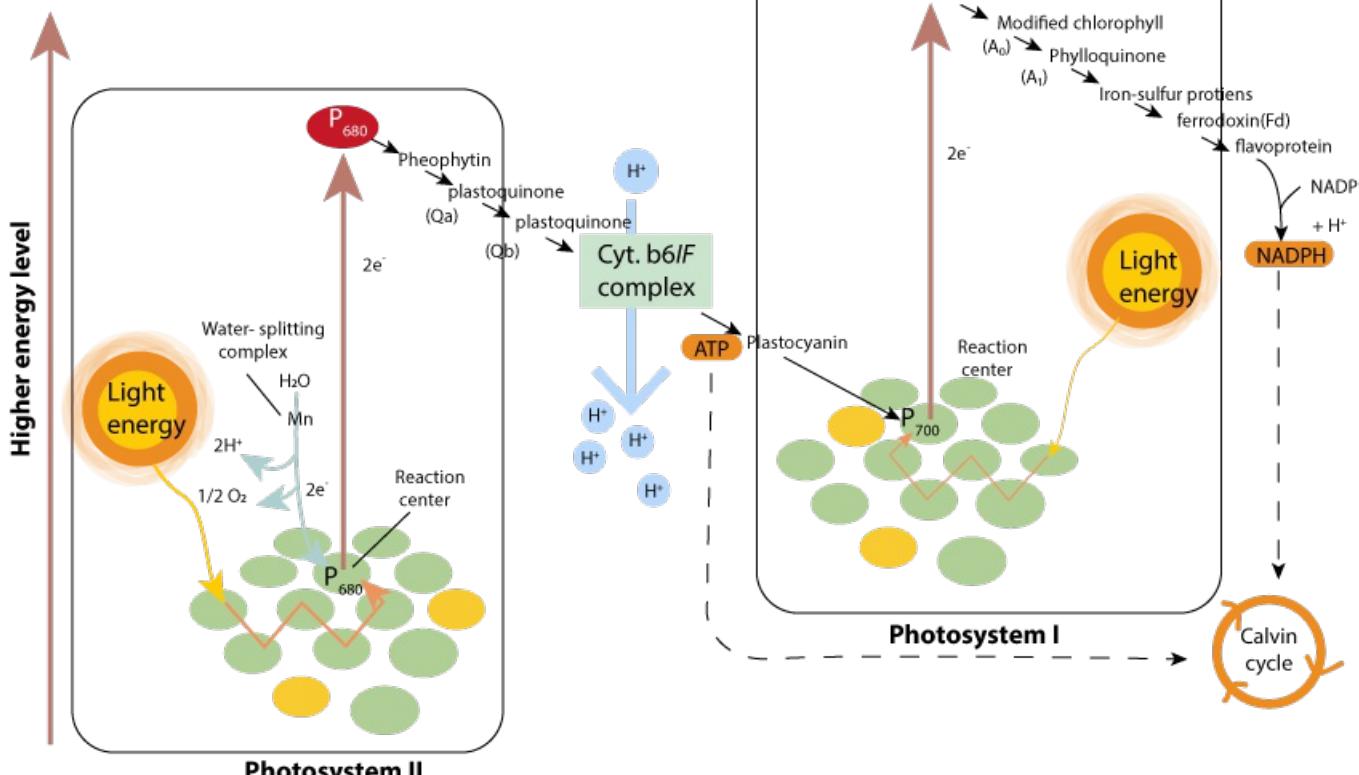
Z-scheme

- It begins with the absorption of a photon by PS II.
- Upon absorption of a photon, P680 is excited to P680*, which rapidly transforms an e- to a nearby pheophytin and changes to P680⁺.
- Pheophytin is a modified chlorophyll molecule.
- The positively charged P680⁺, a strong oxidizing agent, attracts an e- from an e- donor to regenerate the original P680.
- Here H₂O acts as an e- donor.
- The splitting of water is associated with the PS II; water is split into 2H⁺, [O] and electrons.

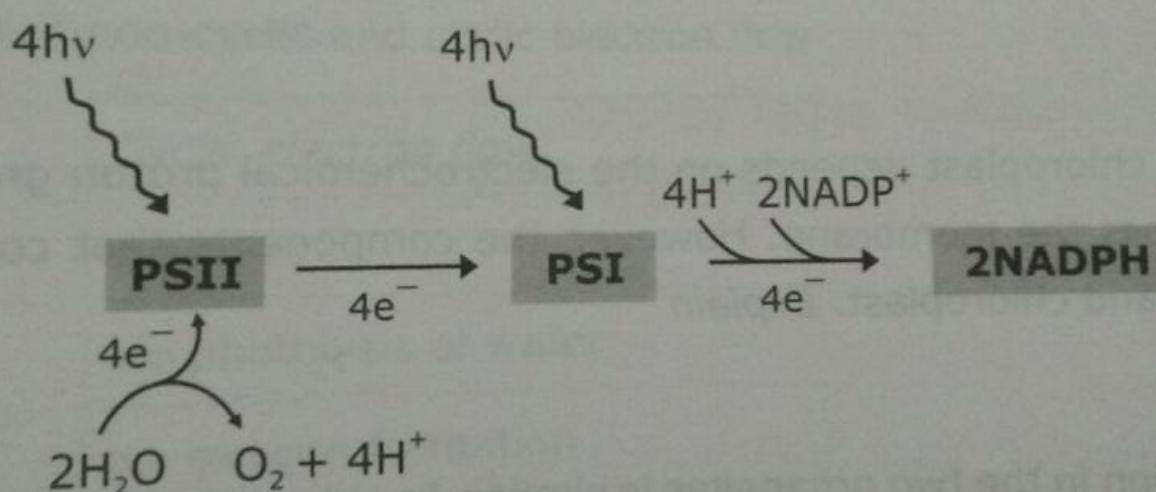


- This creates oxygen, one of the net products of photosynthesis.
- This process of splitting of water molecules in the presence of light into proton, electron and oxygen is called photolysis of water.
- Reduced pheophytin transfers e- to plastoquinone (Q_A & Q_B).
- Plastoquinone transfers the e- to Cytochrome b₆f (cytochrome complex).
- The function of Cytochrome b₆f is to pump proton from the stroma to thylakoid lumen.

- Cytochrome f then transfers an e- to blue-coloured protein plastocyanin.

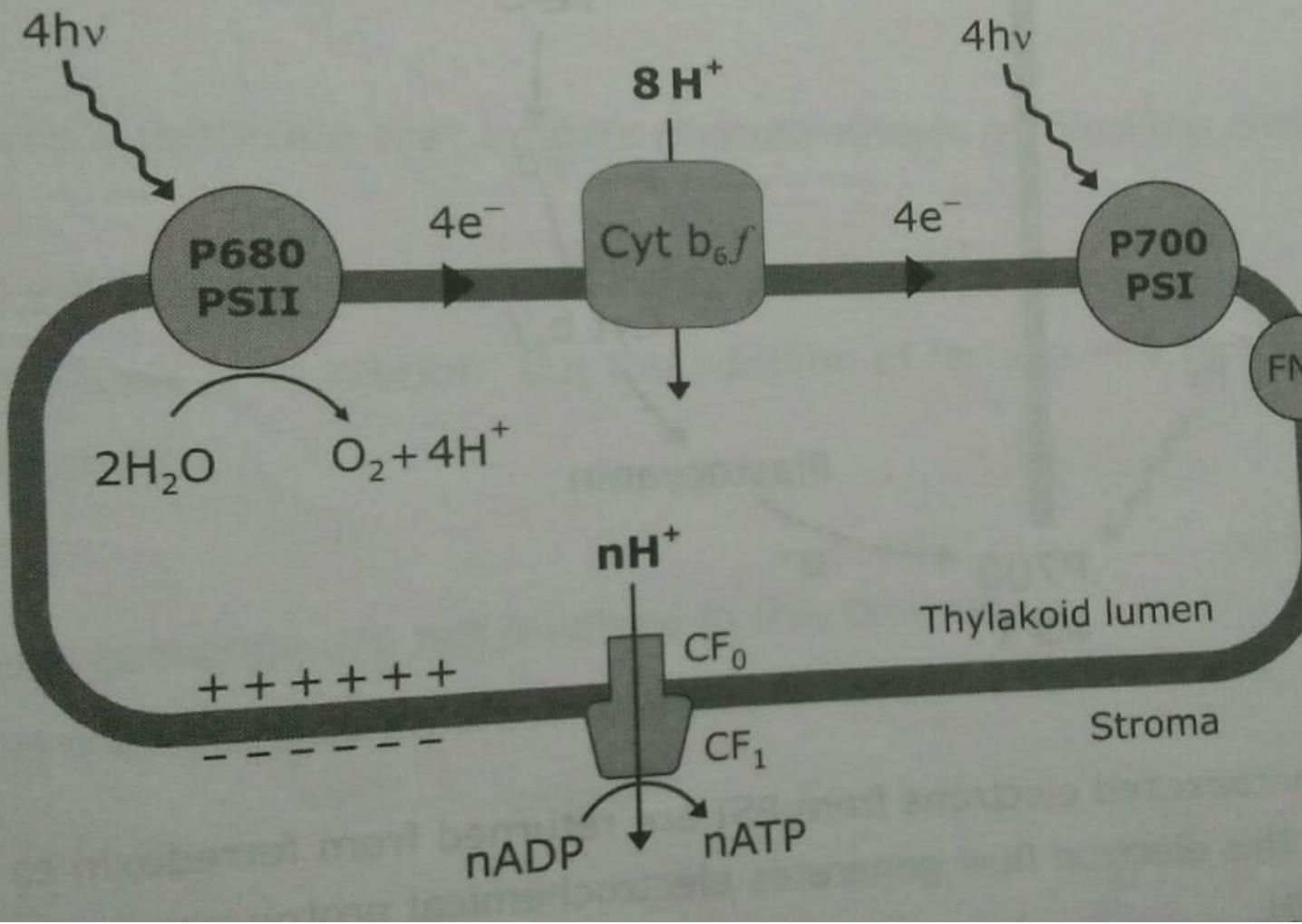


- Upon absorption of a photon, P700 is excited to P700*.
- The electrons needed to replace those removed from photosystem I are provided by photosystem II.
- The photoexcited e- is then transferred to PS I's primary e- acceptor, A₀, which is a modified chlorophyll molecule.
- The resulting oxidized P700⁺ now act as e- acceptor, accepting an e- from plastocyanin.
- Reduced A₀ transfers e- to A₁ (phylloquinone), then to a series of Fe-S centers.
- The e- is then transferred to ferredoxin.
- The FNR (ferredoxin- NADP⁺ reductase) catalyzes the transfer of electrons from ferredoxin to NADP⁺.
- For each e- transferred from H₂O to NADP⁺, two photons are absorbed, one by each photosystem.
- To form one molecule of O₂, which requires transfer of 4 electrons from 2 H₂O to 2 NADP⁺, a total of 8 photons must be absorbed, 4 by each photosystem.



ATP synthesis

- As the electrons are transported down the electron transport system, some energy released is used to pump proton across the thylakoid membrane from stroma to lumen, producing an electrochemical proton gradient.
- Electrochemical proton gradient generates proton motive force.
- The accumulating protons (H⁺) in the thylakoid membrane pass back to stroma through ATP synthase.
- ATP synthase catalyzes ATP synthesis as protons flow back along electrochemical proton gradient.
- Around ~4 ATPs per molecule of O₂ is evolved.



1. Dark reaction

Dark reaction is so called because it is independent of light. The sequence of these reactions was determined in Chlorella and Scenedesmus by Calvin, Benson, and Bassham using radioactive carbon 14 and techniques like chromatography and autoradiography. Therefore, it is also known as the Calvin cycle.

- The dark reaction of the photosynthesis involves reduction & fixation of carbon.
- In all oxygenic photosynthetic organisms, CO₂ reduction and fixation occur through the carbon reduction cycle called the Calvin cycle.
- The Calvin cycle is also known as the reductive pentose phosphate cycle or C₃ cycle.
- It is a series of biochemical reactions which reduce CO₂ to three-carbon sugar.
- It was discovered by Melvin Calvin & Andy Benson.
- The carbohydrate produced directly from Calvin cycle is actually not glucose, but a 3-C sugar: glyceraldehyde-3-phosphate.

Phases of Calvin cycle:

- The reactions of Calvin cycle can be divided into 3 phases: Carboxylation, reduction and regeneration of the ribulose-1,5-bisphosphate (RuBP).

Carboxylation:

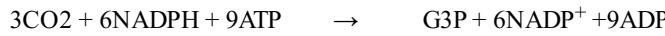
- In this phase, CO₂ is incorporated into a 5-C compound ribulose-1,5-bisphosphate.
- The enzyme which catalyzes the 1st step is Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo).
- The product of the reaction is a 6-C compound which immediately splits to form two molecules of 3-phosphoglycerate.
- Thus, for every three molecule of CO₂ that enter the cycle, there are six molecule of 3-phosphoglycerate formed.
- RuBisCo is known as the pacemaker enzyme of the Calvin cycle. It is the most abundant protein in chloroplast and is also said to be the most abundant protein on the earth.

Reduction:

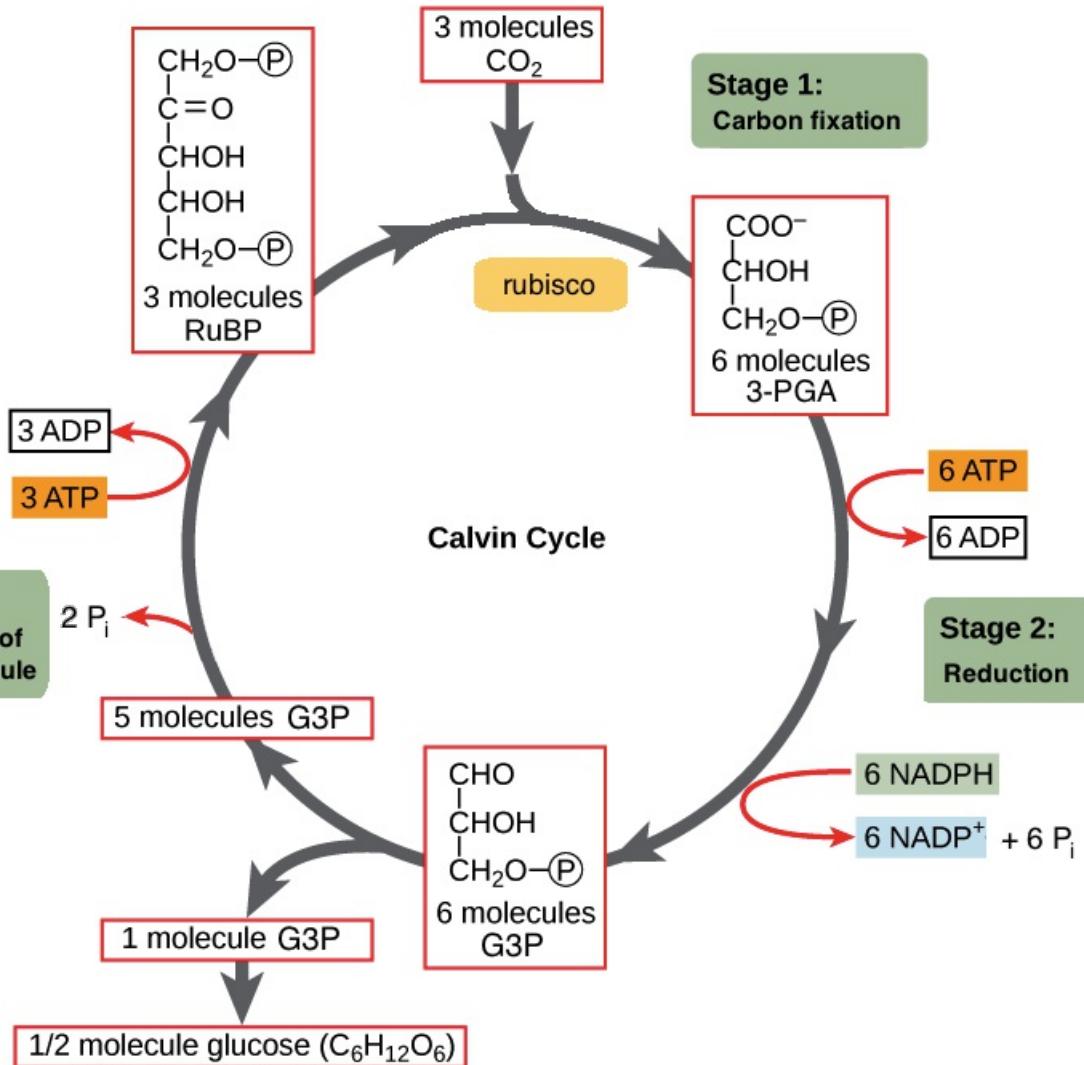
- This phase involves the reduction of 3-phosphoglycerate into glyceraldehyde-3-phosphate.
- 1st 3-phosphoglycerate is converted into 1,3-bisphosphoglycerate at the expense of 1 ATP. This step is catalyzed by phosphoglycerate kinase.
- At the 2nd step 1,3-bisphosphoglycerate is reduced to form one molecule of glyceraldehyde-3-phosphate at the expense of one NADPH. This step is catalyzed by phosphate dehydrogenase.
- Total 6 molecules of ATP are utilized in phosphorylating 6 molecules of 3-phosphoglycerate to 1,3-bisphosphoglycerate, and 6 molecules of NADPH are consumed in reducing 6 molecules of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate (G3P).

Regeneration:

- Regeneration of the CO₂ acceptor molecule RuBP is crucial if the cycle is to continue uninterrupted. The regeneration steps require one ATP for phosphorylation to form RuBP.
- Out of 6 G3P, 1 molecule G3P comes out of the cycle as a byproduct. The rest 5 G3P are processed in the cycle to regenerate 3 molecules of RuBP.
- Net reaction:



- For 3 molecules of RuBP (total 15 carbons) that are carboxylated, cleaved, phosphorylated, 6 molecules of G3P are produced (total 18 carbons).



Glucose formation

- Six rounds of Calvin cycle are required for synthesis of one glucose molecule, because one carbon is reduced in each round.
- 12 molecules of ATP are expended in phosphorylating 12 molecules of 3-phosphoglycerate to 1,3-bisphosphoglycerate, and 12 molecules of NADPH are consumed in reducing 12 molecules of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate.
- An additional 6 molecules of ATP are spent in regenerating RuBP.
- Hence, total 18 ATP and 12 NADPH are required for synthesis of a glucose molecule.

In	Out
Six CO ₂	One glucose
18 ATP	18 ADP
12 NADPH	12 NADP

Comparison between light and dark reaction

Light reaction

Light-dependent phase

It occurs in the grana of chloroplast

Dark reaction

Light-independent phase

ATP and NADPH are synthesized here

Oxidation of H₂O occurs

Reduction of CO₂ occurs

These are photochemical reactions

These are chemical reactions

Concept of Energy Charge

ATP is the energy currency of the cell which provides energy to the cell to perform various activities like:

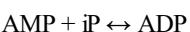
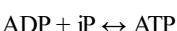
- Transport of ions and molecules across the cell membrane
- Conduction of nerve impulse
- Contraction and relaxation of muscle fibers
- Cell Division
- Provides energy to the endergonic reactions to proceed by coupling.



ATP is spent while performing the above functions. It should get regenerated immediately to maintain its optimum amount in a cell.

ATP is produced by two methods:

1. Substrate level phosphorylation: Approximately 10% ATP is produced by “substrate level phosphorylation” reactions (ADP phosphorylation by 1,3-bisphosphoglycerate, phosphoenolpyruvate, phosphocreatine), by the succinate CoA, phosphoenolpyruvate carboxykinase, and by adenylate kinase (an enzyme that maintains the three adenine nucleotides in equilibrium).
2. Oxidative phosphorylation: More than 90% of the ATP is synthesized by phosphorylation of ADP by the enzyme ATP synthase.



The adenylate energy charge is an index used to measure the energy status of biological cells.

The energy charge is related to ATP, ADP, and AMP concentrations. It was first defined by Atkinson and Walton who found that it is necessary to take into account the concentration of all three nucleotides (ATP, ADP, and AMP), rather than just ATP and ADP, to account for the energy status of a cell.

Since, adenylate kinase maintains two ADP molecules in equilibrium with one ATP, Atkinson defined the adenylate energy charge as:

$$\text{Energy charge} = [\text{ATP}] + \frac{1}{2} [\text{ADP}] / [\text{ATP}] + [\text{ADP}] + [\text{AMP}]$$

The energy charge of most cells varies between 0.75 and 0.90.

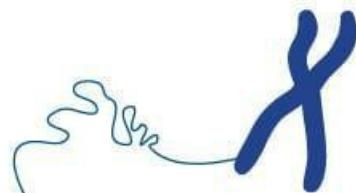
Life depends on an adequate energy charge. If ATP synthesis is momentarily insufficient to maintain an adequate energy charge, AMP can be converted to uric acid. This helps to buffer the adenylate energy charge by decreasing the total ([ATP] + [ADP] + [AMP]) concentration.

Morphology of chromosomes

Chromosomes are the rod-shaped, filamentous bodies present in the nucleus, which become visible during cell division. These are carriers of the genes. Chromosomes are not visible in active nucleus due to their high water content, but are clearly seen during cell division. Chromosomes were first described by Strasburger in 1875. The term ‘chromosome’, however was first used by Waldeyer in 1888. They were given the name chromosome (chromo=colour; soma=body) due to their marked affinity for basic dyes. Chromosomes are composed of thin chromatin threads called chromatin fibres. These fibres undergo folding, coiling, and super coiling during prophase so that the chromosome become progressively thicker and small. Therefore, chromosomes become readily observable under light microscope. At the end of cell division, on the other hand, the fibres uncoil and extend as fine chromatin threads, which are not visible at light microscope.

Each species has a definite chromosome number, represented by $2n$. Somatic cells contains two copies of each chromosome, which are identical in morphology, gene content and gene order and they are known as homologous chromosomes.

Gametic chromosome number is precisely one half of the somatic number, is represented by ‘ n ’ and zygote is produced by fusion of one male and one female gamete ($n+n=2n$).



Chromosome



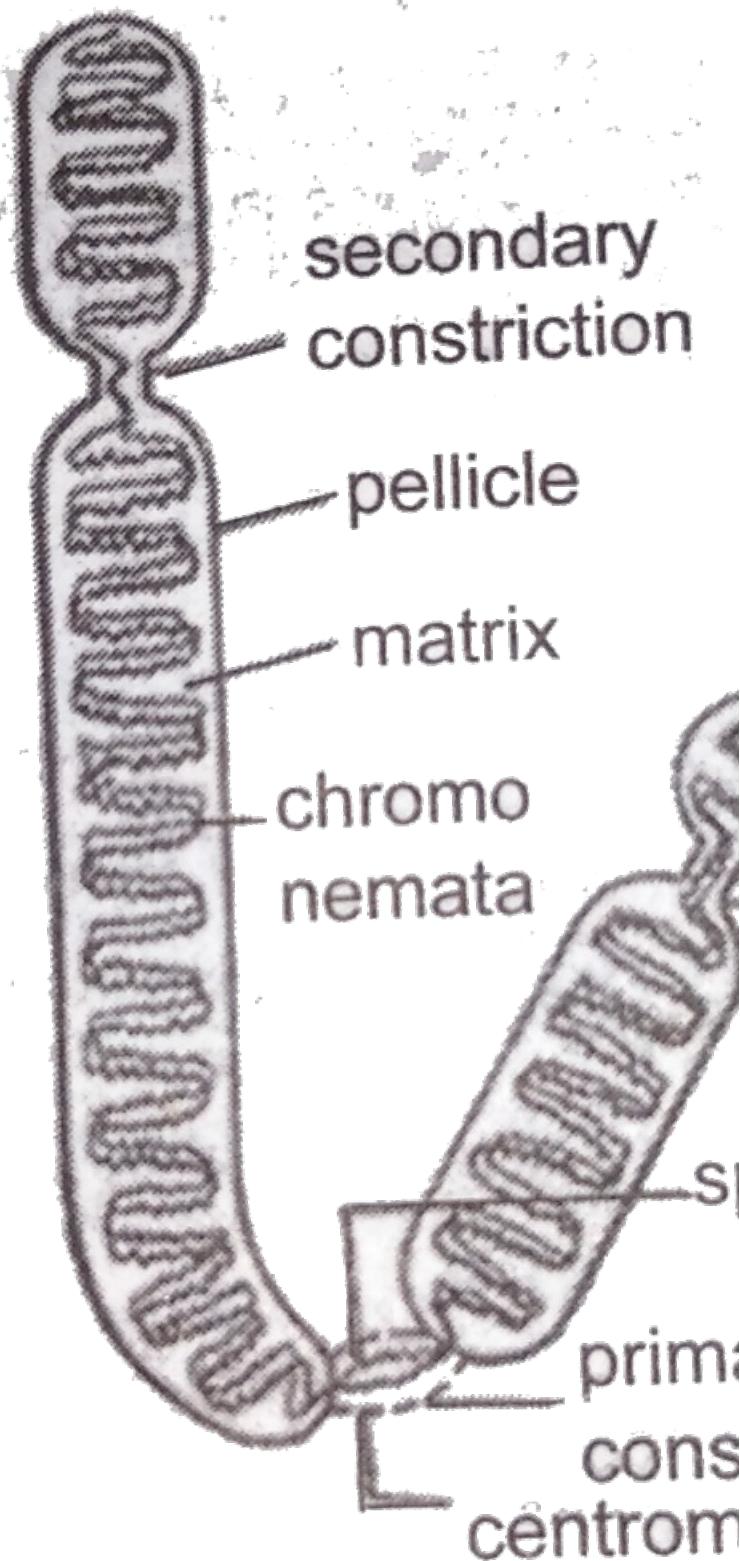
Chromatin fiber

Histones

"Beads on a string"
DNA wound on
nucleosomes



Double helix



Structure of Chromosome

The chromosomes are thin, long, and thread-like structures, with two identical strands called chromatids. They are held together by a centromere. The chromatid is made up of a spirally coiled, thin structure called chromonema, which has a number of bead-like structures along its length called chromatids.

A chromosome consists of the following regions:

(a) Primary constriction:

The two arms of a chromosome meet at a point called primary constriction or centromere. The centromere is the region, where spindle fibres attach to the chromosome during cell division. Centromere is surrounded or covered by kinetochore.

(b) Secondary constriction:

Some chromosomes have secondary constriction at any point of the chromosome, called the nuclear zone or nucleolar organizer. (Formation of nucleolus in the nucleus).

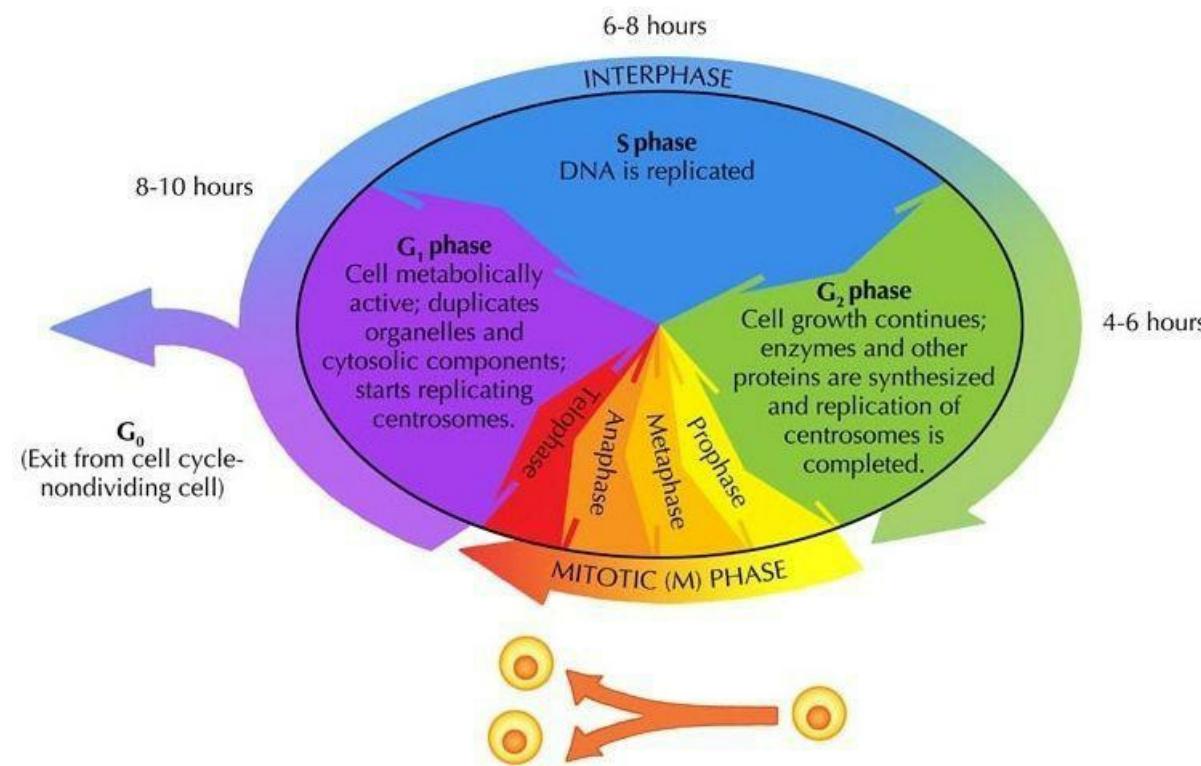
(c) Telomere:

The end of the chromosome is called telomere. Each extremity of the chromosome has a polarity and prevents it from joining the adjacent chromosome. It maintains and provides stability to the chromosome.

(d) Satellite:

Some of the chromosomes have an elongated knob-like appendage at one end of the chromosome, known as satellite. The chromosomes with satellites are called as sat-chromosomes.

Cell Cycle



Interphase

Interphase is the longest phase of the cell cycle. During this phase the cell grows to its maximum size, performs its normal cellular functions, replicates its DNA, and prepares for cell division. This stage is divided into three parts: G₁, G₂ and S phases.

G₁ phase: Occurs just after the two daughter cells have split and the cells have only one copy of their DNA. Cells in this stage synthesise proteins and increase in size. Cells can remain in this stage for a long time.

S phase: Is the stage during which DNA replication occurs. The cell makes an identical copy of each of its chromosomes. Chromosomes are found inside the nucleus of the cell and consist of long strands of DNA that contain the genetic information of the cell.

G₂ phase: Occurs after the DNA had been duplicated in S phase. During this phase the cell may continue to grow and undergo normal cellular functions. Towards the end of this phase the cell will start to replicate its organelles in preparation for mitosis.

Note: Some cells no longer need to divide and exit the cell cycle. These cells may exit the cell cycle permanently, such as neurons, or they may exit the cell cycle temporarily. These cells are said to be in G₀. G₀ is not a stage of the cell cycle.

In cells without a nucleus (prokaryotic cells e.g. bacteria), there are many copies of the DNA held in the nucleoid region of the cell. The prokaryotic cell cycle occurs through a process termed binary fission. In cells with a nucleus (eukaryotes) all the DNA is inside the nucleus and so a more complicated cell cycle is required for replication.

M phase: It stands for either mitosis or meiosis. Mitosis is division of somatic cells. Meiosis is the division of germ cells which leads to the formation of gametes.

Mitosis or Mitotic Phase

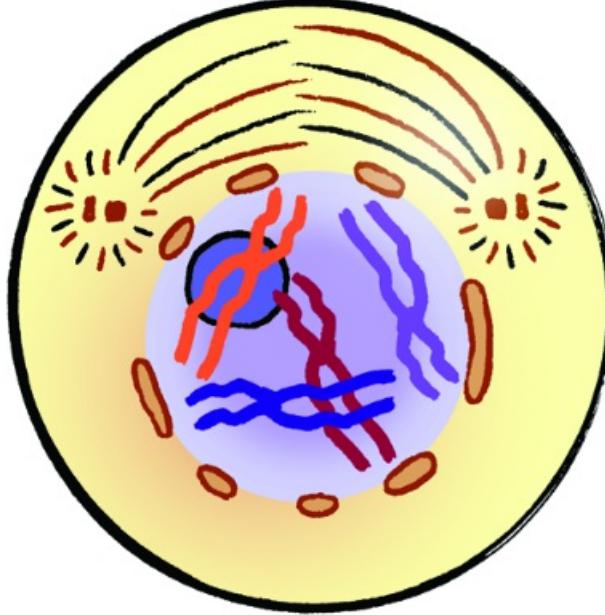
The mitotic phase (M phase) is composed of two tightly coupled processes: mitosis and cytokinesis. During mitosis the chromosomes in the cell nucleus separate into two identical sets in two nuclei. This is followed by cytokinesis in which the cytoplasm, organelles and cell membrane split into two cells containing roughly equal shares of these cellular components. We will now describe what takes place during the stages of M-phase, which includes the four broad phases of mitosis

(prophase, metaphase, anaphase, telophase) and the fifth phase of cytokinesis:

1. prophase
2. metaphase
3. anaphase
4. telophase
5. cytokinesis

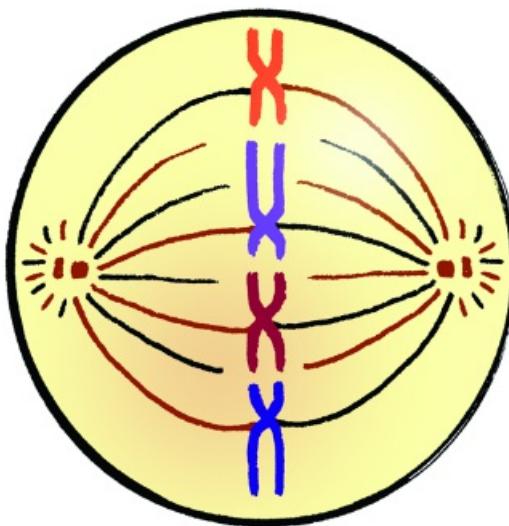
1. Prophase

During prophase, the chromatin material shortens and thickens into individual chromosomes which are visible under the light microscope. Each chromosome consists of two strands or chromatids joined by a centromere. As prophase progresses, the nuclear membrane and nucleolus disintegrates. In animal cells the centrioles separate and move to opposite poles. The centrioles give rise to the spindle fibres which form between the poles.



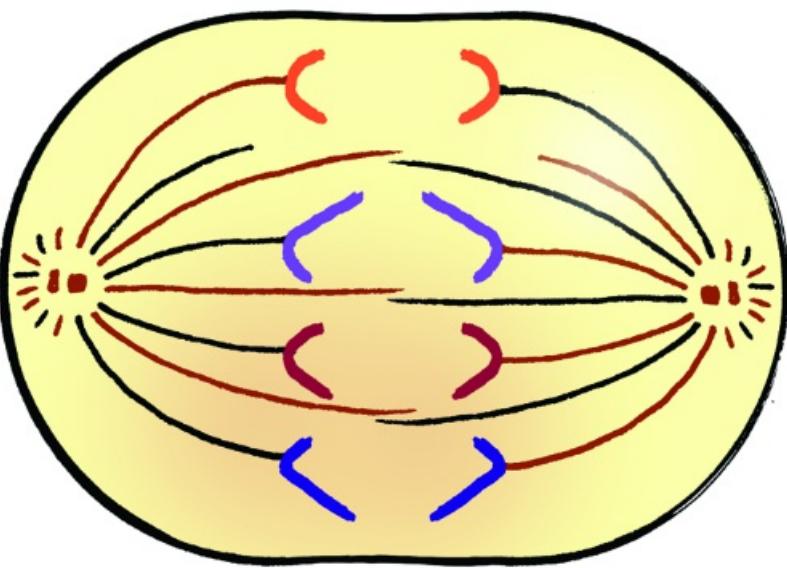
2. Metaphase

During metaphase, chromosomes line up on the equator of the cell. The chromosomes appear in a straight line across the middle of the cell. Each chromosome is attached to the spindle fibres by its centromere.



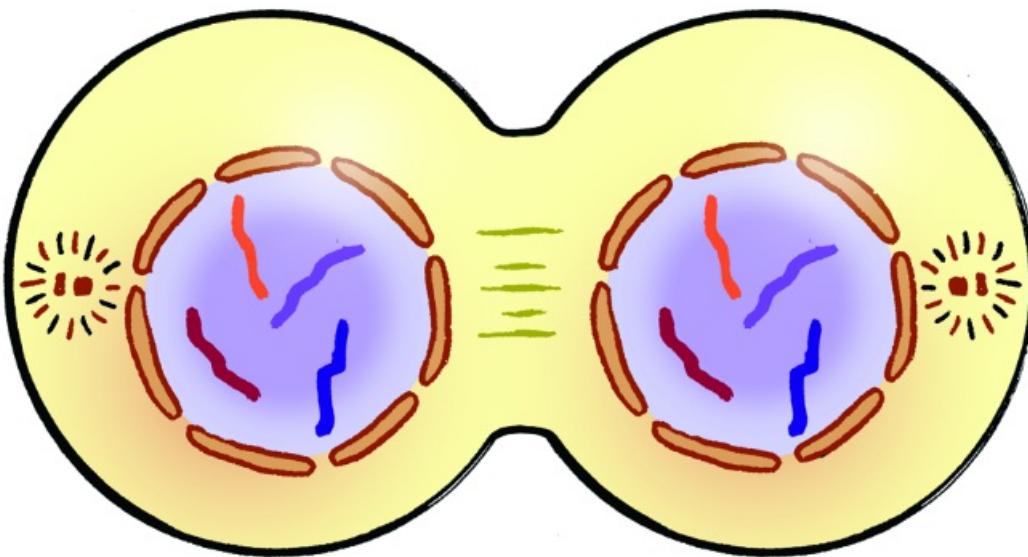
3. Anaphase

During anaphase the chromatids are pulled to opposite poles of the cell by the shortening of the spindle fibres. The chromatids are now called daughter chromosomes.



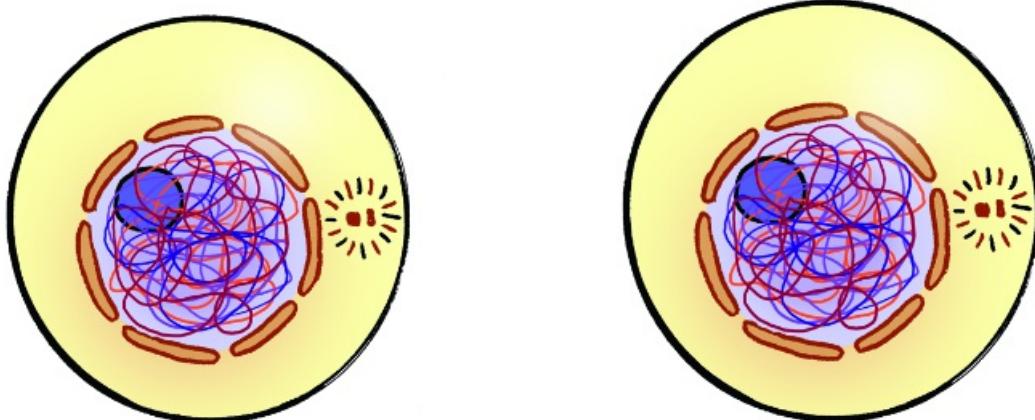
4. Telophase

During telophase, a nuclear membrane reforms around the daughter chromosomes that have gathered at each of the poles. The daughter chromosomes uncoil to form chromatin once again. The nuclear membrane reforms.



5. Cytokinesis

The cytoplasm then divides during a process called cytokinesis. Cytokinesis is not a stage of mitosis but the process of the cytoplasm splitting into two. In an animal cell the cell membrane constricts. This invagination or in-folding of the cytoplasm divides the cell in two. In a plant cell a cross wall is formed by the cell plate dividing the cytoplasm in two.



There are now two genetically identical daughter cells which are identical to the parent cell and to each other.

Sexual reproduction in organisms takes place through the fusion of male and female gametes, the sperm and the egg respectively. Gametes are haploid in nature, i.e., they contain only half the number of chromosomes. This genetic content makes them different from other body cells. Meiosis leads to the formation of haploid cells.

The different stages and phases of meiosis 1.

Meiosis 1

Mitotic cell division is equational in nature while meiosis is a reduction division. The salient features of meiotic division that make it different from mitosis are as follows:-

1. It occurs in two stages of the nuclear and cellular division as Meiosis I and Meiosis II. DNA replication occurs, however, only once.
2. It involves the pairing of homologous chromosomes and recombination between them.
3. Four haploid daughter cells are produced at the end, unlike two diploid daughter cells in mitosis.

Meiosis 1 separates the pair of homologous chromosomes and reduces the diploid cell to haploid. It is divided into several stages that include, prophase, metaphase, anaphase and telophase.

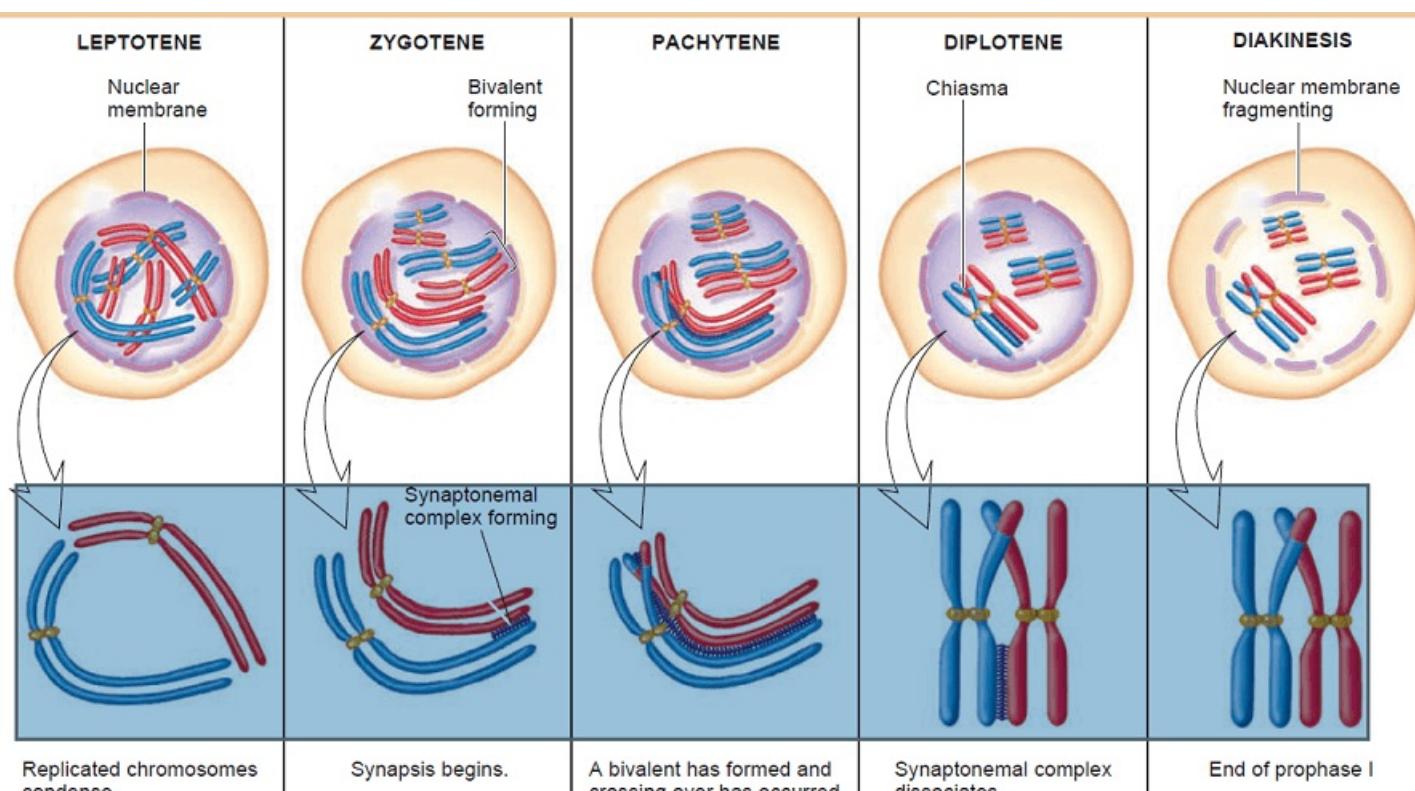
Meiosis 1 Stages

The different stages of meiosis 1 can be explained by the following phases:

- Prophase 1
- Metaphase 1
- Anaphase 1
- Telophase

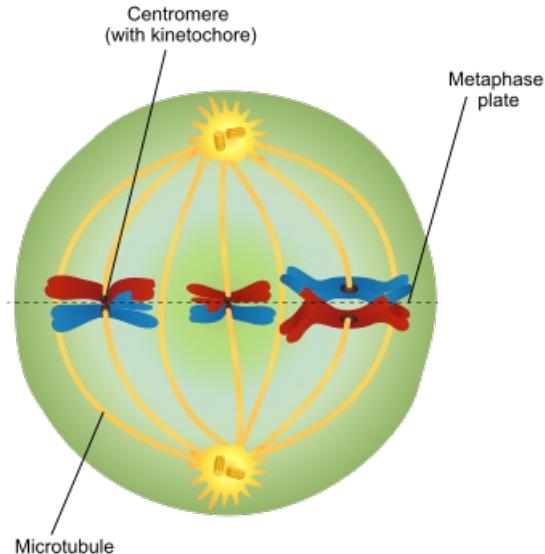
Phases of Meiosis 1

Prophase 1



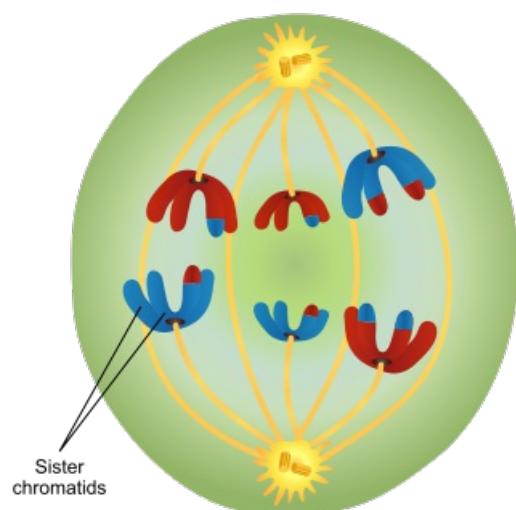
- Prophase I is longer than the mitotic prophase and is further subdivided into 5 substages,
 - leptotene
 - zygotene
 - pachytene
 - diplotene
 - diakinesis
- The chromosomes begin to condense and attain a compact structure during leptotene.
- In zygotene, the pairing of homologous chromosomes starts a process known as chromosomal synapsis, accompanied by the formation of a complex structure called synaptonemal complex. A pair of synapsed homologous chromosomes forms a complex known as bivalent or tetrad.
- At pachytene stage, crossing over of non-sister chromatids of homologous chromosomes occurs at the recombination nodules. The chromosomes remain linked at the sites of crossing over.
- Diplotene marks the dissolution of the synaptonemal complex and separation of the homologous chromosomes of the bivalents except at the sites of cross-over. The X-shaped structures formed during separation are known as chiasmata.
- Diakinesis is marked by the termination of chiasmata and assembly of the meiotic spindle to separate the homologous chromosomes. The nucleolus disappears and the nuclear envelope breaks down.

Metaphase 1



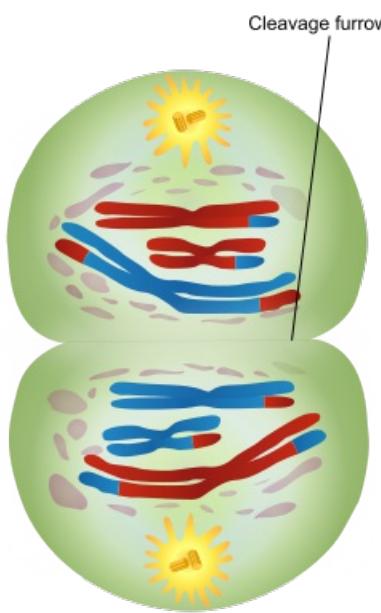
The bivalents align at the equatorial plate and microtubules from the opposite poles attach to the pairs of homologous chromosomes.

Anaphase 1



The two chromosomes of each bivalent separate and move to the opposite ends of the cells. The sister chromatids are attached to each other.

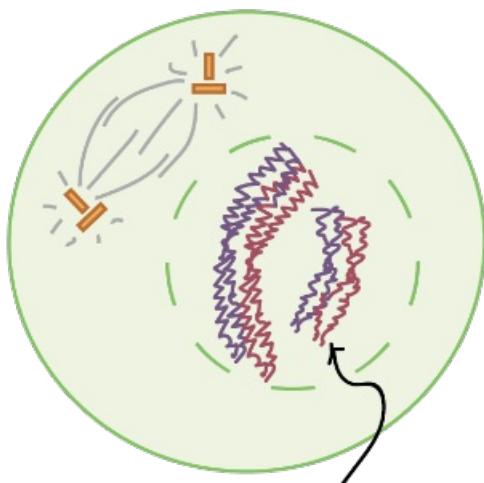
Telophase 1



The nuclear membrane reappears and is followed by cytokinesis. This gives rise to two haploid cells.

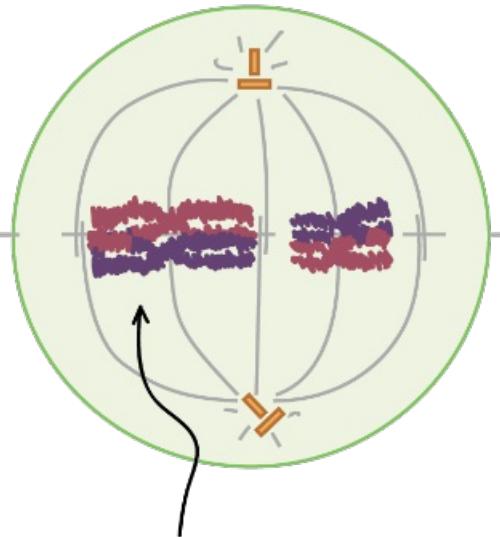
PHASES OF MEIOSIS I

Prophase I



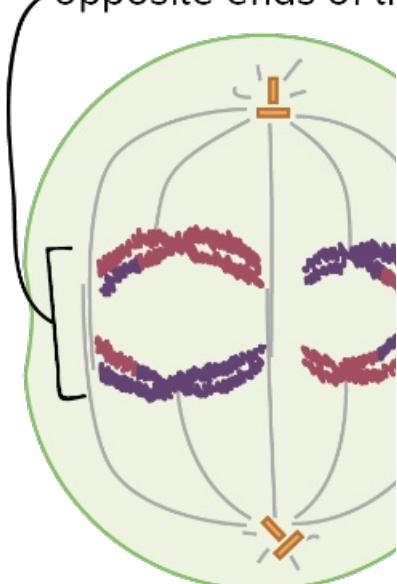
homologous chromosomes pair up and exchange fragments (crossing over)

Metaphase I



homologue pairs line up at the metaphase plate

Anaphase I



homologues separate at opposite ends of the cell

sister chromatids stay together

- Prophase II – It immediately sets off after the cytokinesis when the daughter cells are formed. The chromosomes begin to condense accompanied by the dissolution of the nuclear membrane and the disappearance of the Golgi apparatus and ER complex.
- Metaphase II – The chromosomes are connected to the centriole poles at the kinetochores of sister chromatids through the microtubules. They also get aligned at the equator to form the metaphase plate.
- Anaphase II – In this phase of meiosis II, there is a simultaneous splitting of the centromere of each chromosome and the sister chromatids are pulled away towards the opposite poles. As the chromatids move towards the poles, the kinetochore is at the leading edge with the chromosomal arms trailing.
- Telophase II – The chromosomes dissolve again into an undifferentiated lump and a nuclear envelope develops around it. Followed by cytokinesis, telophase II marks the end of meiosis. Four haploid daughter cells are formed as a result.

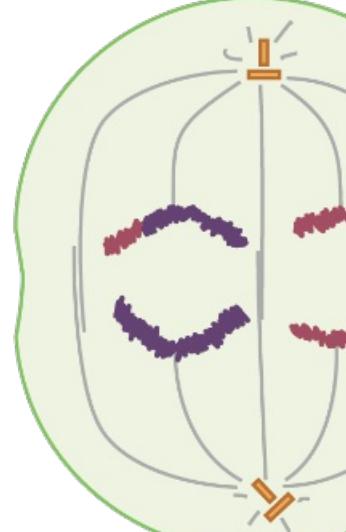
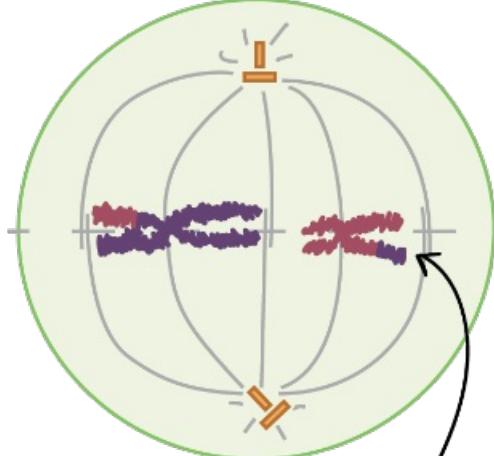
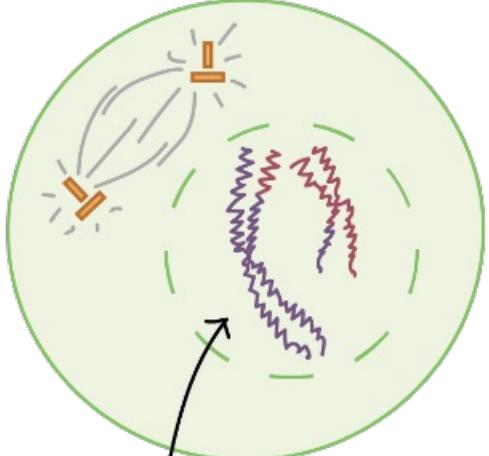
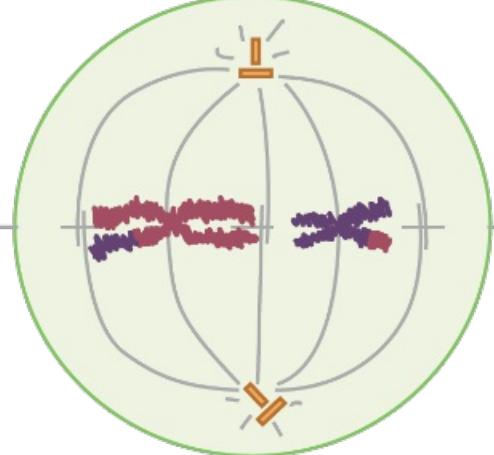
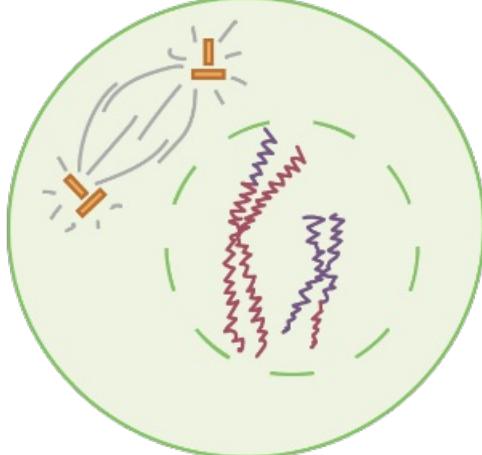
PHASES OF MEIOSIS II

Prophase II

Metaphase II

Anaphase

starting cells are the haploid cells made in meiosis I



chromosomes condense

chromosomes line up at metaphase plate

sister chromatids

Significance of Meiosis

Reproduction in animals takes place through the fusion of gametes i.e. two cells fuse together with their genetic material to develop a zygote. If germ cells, which give rise to gametes, also maintains their ploidy during division like the somatic cells, the zygote will have an accumulation of chromosomes in its nucleus. This accumulation will keep on increasing with every subsequent generation. Meiosis offers a very smart solution to this problem as it reduces the number of chromosomes in the gametes to half of their parent germ cells. Moreover, prophase I of meiosis allows recombination of homologous chromosomes.

This recombination is essential for the variation to be introduced in the genetic makeup of the gametes as this variation only holds the key to evolution through sexual reproduction.

Module 3

1. Laws of heredity: Mendelian and Non-Mendelian
2. Molecular genetics: Structure of DNA & RNA
3. Mutations- Cause, types, and effects on Species
4. Origin of life- Haldane and Oparin's concepts
5. Evolution: Modern concept of natural selection and speciation- Lamarckism, Darwinism/ Neo-Darwinism
6. Bioinformatics- brief idea

Genetics

Genetics is the branch of lifescience where we study about the genes, genetic variations and heredity of characters in the living organisms.

“Geno” is a Greek word that means “give birth”. The term Genetics has been derived from the word Geno. The scientific study of heredity, variations and environmental factors responsible for these, is known as Genetics. The word genetics was first suggested by British scientist William Bateson. Gregor Johann Mendel is known as the father of genetics. He was an Austrian mathematician appointed as monk who worked on the pea plants to study the pattern of inheritance and postulated laws of inheritance. In 1856, he published the results of his works titled ‘Experiments on Plant Hybrids’ in a journal ‘The proceedings of Brunn Society of Natural History’ and postulated the principles of inheritance popularly known as Mendel’s laws of inheritance. Mendel did a statistical study. But his works remained unrecognised until 1900, the work was independently rediscovered by three biologists- Hugo de Vries of Holland, Carl Correns of Germany and Erich Tschermark of Austria. Mendel discovered that individual traits are inherited as discrete factors which retain their physical identity in a hybrid. Later, these factors came to be known as genes. A gene is defined as a unit of heredity that may influence the outcome of an organism’s trait. The term was coined by Danish Botanist Wilhelm Johannsen in 1909.

Important Terms

Allele

Each gene may exist in alternative forms known as alleles, which code for different versions of a particular inherited character. We may also define alleles as genes occupying corresponding positions on homologous chromosomes and controlling the same characteristic (e.g. height of plant) but producing different effects (tall or short).

Homozygous and Heterozygous

Each parent (diploid) has two alleles for a trait - that may be

1. Homozygous: homozygous genotype indicates that the organism possesses two identical alleles for a trait. (TT or tt)
2. Homozygous dominant genotypes possess two dominant alleles for a trait (TT)
3. Homozygous recessive genotypes possess two recessive alleles for a trait (tt)
4. Heterozygous: heterozygous genotype indicates that the organism has one of each allele for a particular trait/characters. (Tt)

Dominant and Recessive alleles

A dominant allele masks or hides expression of a recessive allele and it is represented by an uppercase letter. A recessive allele is an allele that exerts its effect only in the homozygous state and in heterozygous condition its expression is masked by a dominant allele. It is represented by a lowercase letter.

Homozygous

Wild type versus Mutant alleles

Prevalent alleles in a population are called wild-type alleles. These typically encode proteins that are made in the right amount and function normally. Alleles that are present at less than 1% in the population and have been altered by mutation are called mutant alleles. Such alleles usually result in a reduction in the amount or function of the wild-type protein and are most often inherited in a recessive fashion.

Homologous and non-homologous chromosomes

The term homologous refers to chromosomes that carry the same set of genes in the same sequence, although they may not necessarily carry identical alleles of each gene. Non-homologous chromosomes carry different sets of genes.

Genotype and Phenotype

To distinguish physical appearance from the genetic constitution, two different terms are used in genetics i.e. genotype and phenotype

1. Genotype is defined as the genetic constitution of an individual for any character or trait. It is represented by a symbol e.g. tt, Tt or TT, etc.
2. Phenotype is defined as the physical appearance of an organism for any particular trait. Phenotype of an individual is dependent on its genetic constitution.

Mendel chose the garden pea, *Pisum sativum*, for his experiments since it had the following advantages.

1. Well-defined discrete characters
2. Bisexual flowers
3. Predominant self fertilization
4. Easy hybridization
5. Easy to cultivate and relatively short life cycle

Characters studied by Mendel

The characteristics of an organism are described as characters or traits. Traits studied by Mendel were clear cut and discrete. Such clear-cut, discrete characteristics are known as Mendelian characters. Mendel studied seven characters/trait (all having two variants) and these are:

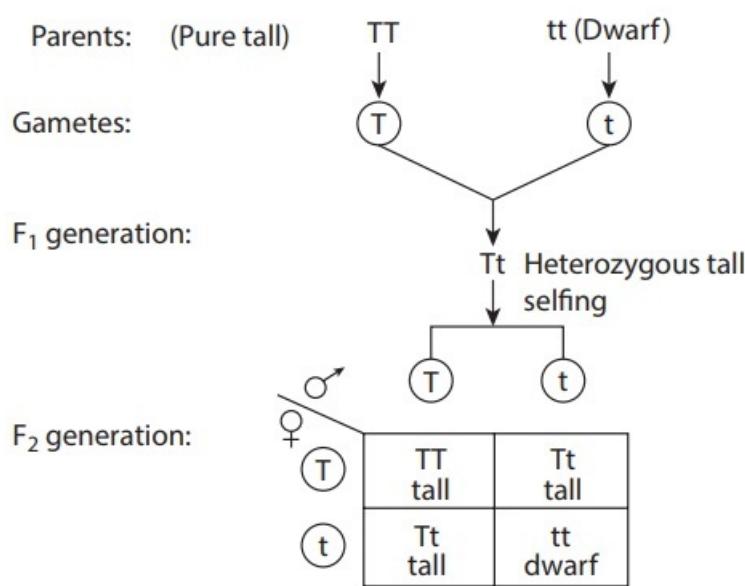
Mendel's Laws

	Flower color	Seed shape	Seed color	Pod color	Pod shape	Plant height	Flower position
DOMINANT							
RECESSIVE							

Gregor Johann Mendel gave three laws of inheritance:

1. Law of Dominance
2. Characters are controlled by discrete units called factors.
3. Factors occur in pairs.
4. In a dissimilar pair of factors one member of the pair dominates (dominant) the other (recessive).

The law of dominance explains the expression of only one of the parental characters in a monohybrid cross in the F1 and the expression of both in the F2. It also explains the proportion of 3: 1 obtained at the F2.



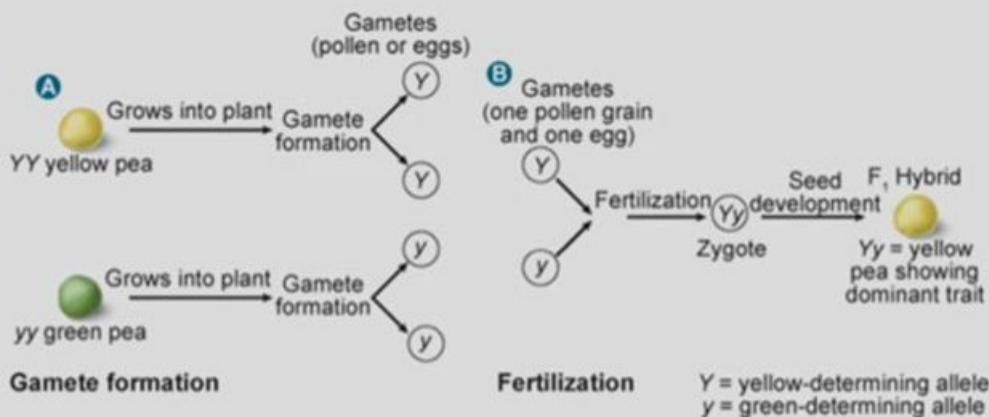
In F₁ generation : The phenotype and genotype ratio is 4:0. All plants were heterozygous. The trait which is expressed in the F₁ generation is dominant.

In F₂ generation: Both parental characters are expressed in F₂ generation.

1. Law of segregation

This law is based on the fact that the alleles do not show any blending and both the characters are recovered as such in the F₂ generation though one of these is not seen at the F₁ stage. The law states that during gamete formation the two alleles of a gene get segregated or separated. It is also known as the law of purity of gametes because even if the gametes are formed from the heterozygous individual the alleles are separated and each gamete possesses an allele which is pure for that gamete. Gamete receives only one allele of the gene.

LAW OF SEGREGATION



1. Law of Independent Assortment

It can be explained when dihybrid cross is considered.

A Dihybrid Cross

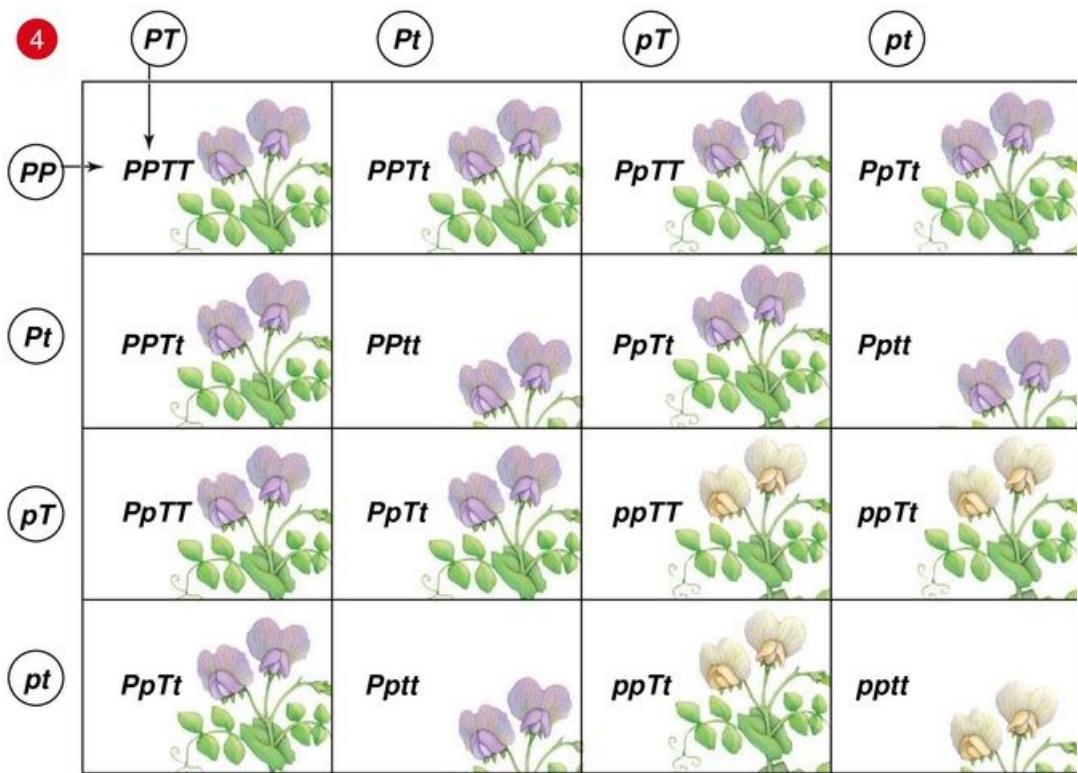
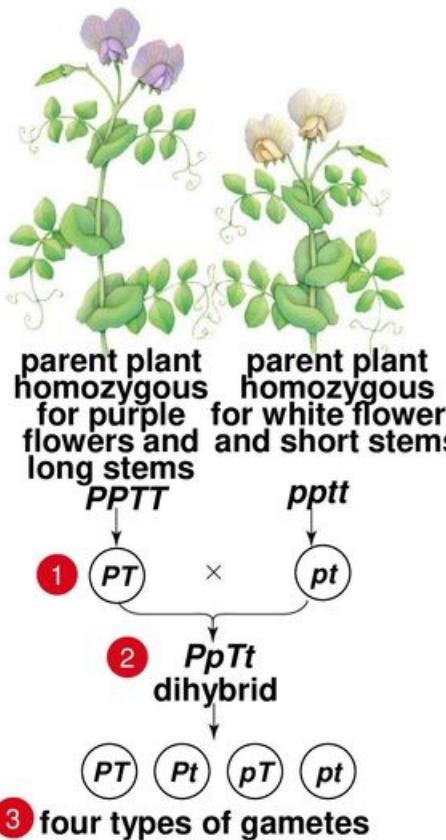


Fig. 13.8, p

Here, the gamete formation is independent of the number of traits taken into consideration or the number of genes. Alleles will be separated independently without affecting other alleles. This is the law of independent assortment.

Monohybrid cross

phenotypes of parents	tall	dwarf			
genotypes of parents	Tt	\times	Tt		
gametes	(T)	(t)	\times	(T)	(t)
punnett square					
		(T)	(t)		
	(T)	TT	Tt		
	(t)	Tt	tt		
F ₁ genotypes	1 TT, 2 Tt, 1 tt				
F ₁ phenotypes	tall	tall	dwarf		
ratio	3 tall : 1 dwarf				

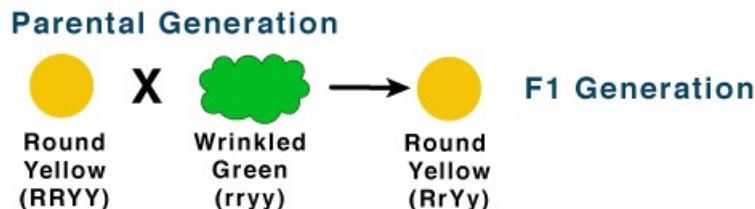
Dihybrid cross

Dihybrid Cross

Seed Shape	Seed Color
R = round ○ r = wrinkled ⚡	Y = yellow ■ y = green ■■

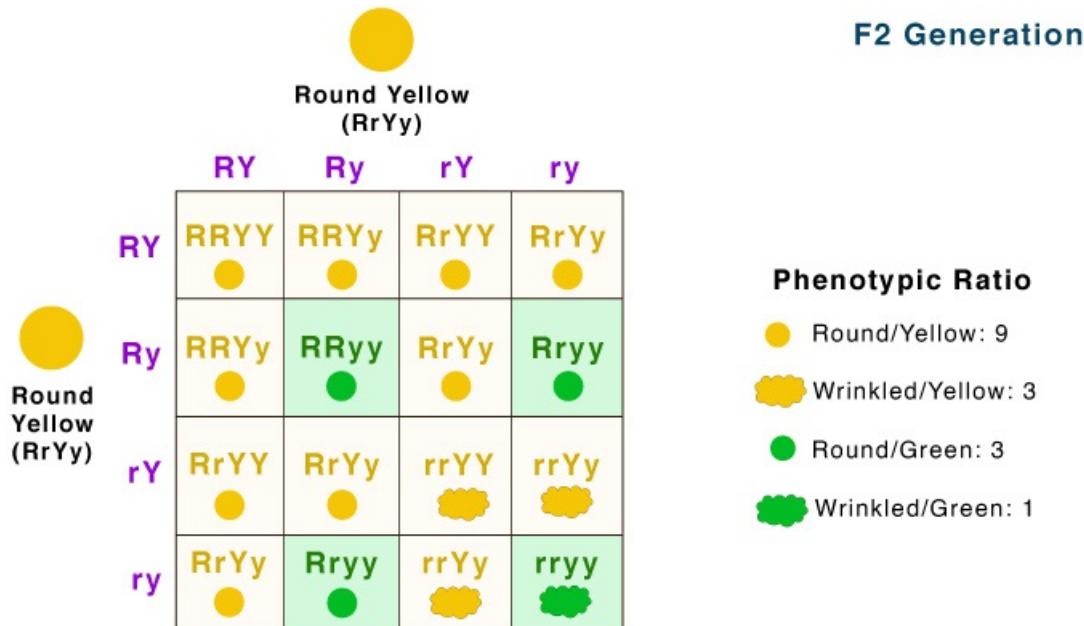
Cross Fertilization

Fusion of gametes from different individuals of the same species



Self Fertilization

Fusion of gametes from the same individual



Yellow round : Yellow wrinkled : Green round : Green wrinkled
 9 : 3 : 3 : 1

Genotypic ratio:

YYRR YYRr YyRR YyRr YYrr Yyrr yyRR yyRr yyrr
 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1

Non-Mendelian Inheritance/Variations from Mendel's laws

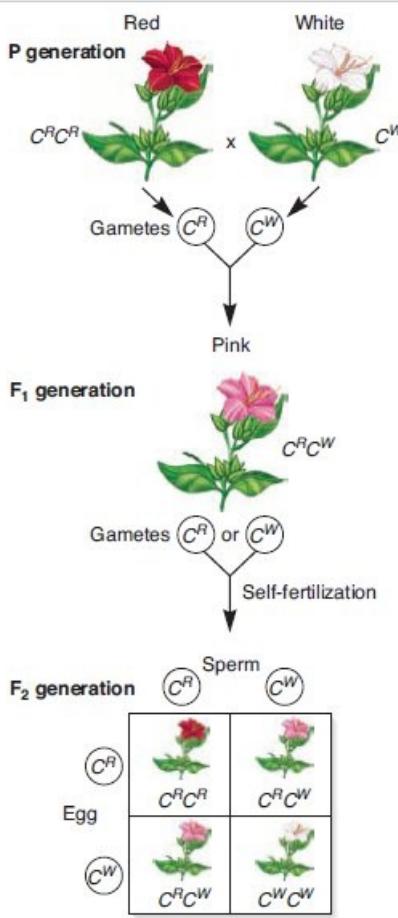
Non-Mendelian Inheritance does not follow the iconic Mendel's Laws and can be defined as any inheritance pattern that fails to follow one or more laws of Mendelian genetics.

1. Non-mendelian traits are not determined by dominant or recessive alleles. They can involve more than one gene leading to a complex pattern of inheritance.
2. Some traits exhibited blending where the organisms' offspring had two separate traits from the parent, meaning that certain alleles were not dominant.
3. Non-mendelian inheritance can also be a result of issues in reproduction.

Types of Non-Mendelian Inheritance

Incomplete dominance

As the name indicates, in this type of inheritance, a single trait isn't fully dominant, so you'll see resulting progeny with a mixed phenotype of a recessive and a dominant trait.



Codominance

The genetic traits expressed in the cases of co-dominance include both of the different alleles that are clearly visible in the phenotype. It's almost like the dominant traits compete with each other to be represented on the offspring's phenotype, and they then reach a compromise - both the traits show up on the offspring!

Inheritance of the ABO Blood System in Humans

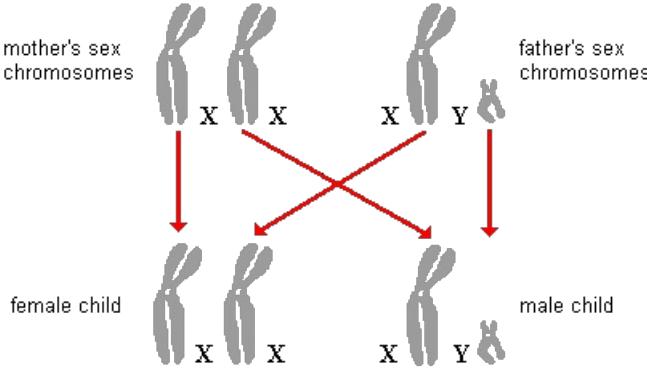
	I^A	I^B	i
I^A	$I^A I^A$ A	$I^A I^B$ AB	$I^A i$ A
I^B	$I^B I^A$ AB	$I^B I^B$ B	$I^B i$ B
i	$i I^A$ A	$i I^B$ B	$i i$ O

Multiple Alleles

In Mendelian Inheritance, there are only two alleles present for a specific trait. Multiple alleles, on the other hand, are named so because some traits could be encoded by more than just those two alleles. A very easy example of this would be our blood groups! Alleles that encode for our blood groups include A, B, and O.

Sex-linked Inheritance

Some genetic traits are located on the X chromosomes as well. Often, some recessive traits that may not show up in females are more likely to show up in males, because males only need to inherit ONE recessive allele on the X chromosome to represent that trait, while females inherit two X chromosomes.



Polygenic Traits

Certain traits are displayed as a result of interaction between various genes. As the name indicates, there is a need for a number of genes to work together for the offspring to show certain traits. Height, weight, skin color, etc are all examples of polygenic traits.

Conclusion

- Non-mendelian Inheritance does not follow Mendel's laws of inheritance.
- The different types of non-Mendelian inheritance are incomplete dominance, codominance, multiple alleles, sex-linked inheritance, and polygenic traits.

Molecular genetics/Molecular Basis of Inheritance: Structure of DNA & RNA

Characteristics of genetic material:

1. Genetic material should be able to replicate
2. It should show stable mutation and it should be inheritable
3. It should be able to translate the information by transcription.

Historical background

Nucleic acid was discovered by Friedrich Miescher in 1869. It was observed in pus cells. He called it "Nuclein". The term nucleic acid was given by Altman in 1889. Nucleic acids are of two types i.e. DNA (genetic materials) and RNA (act as genetic material in few viruses and in other organisms help in protein synthesis).

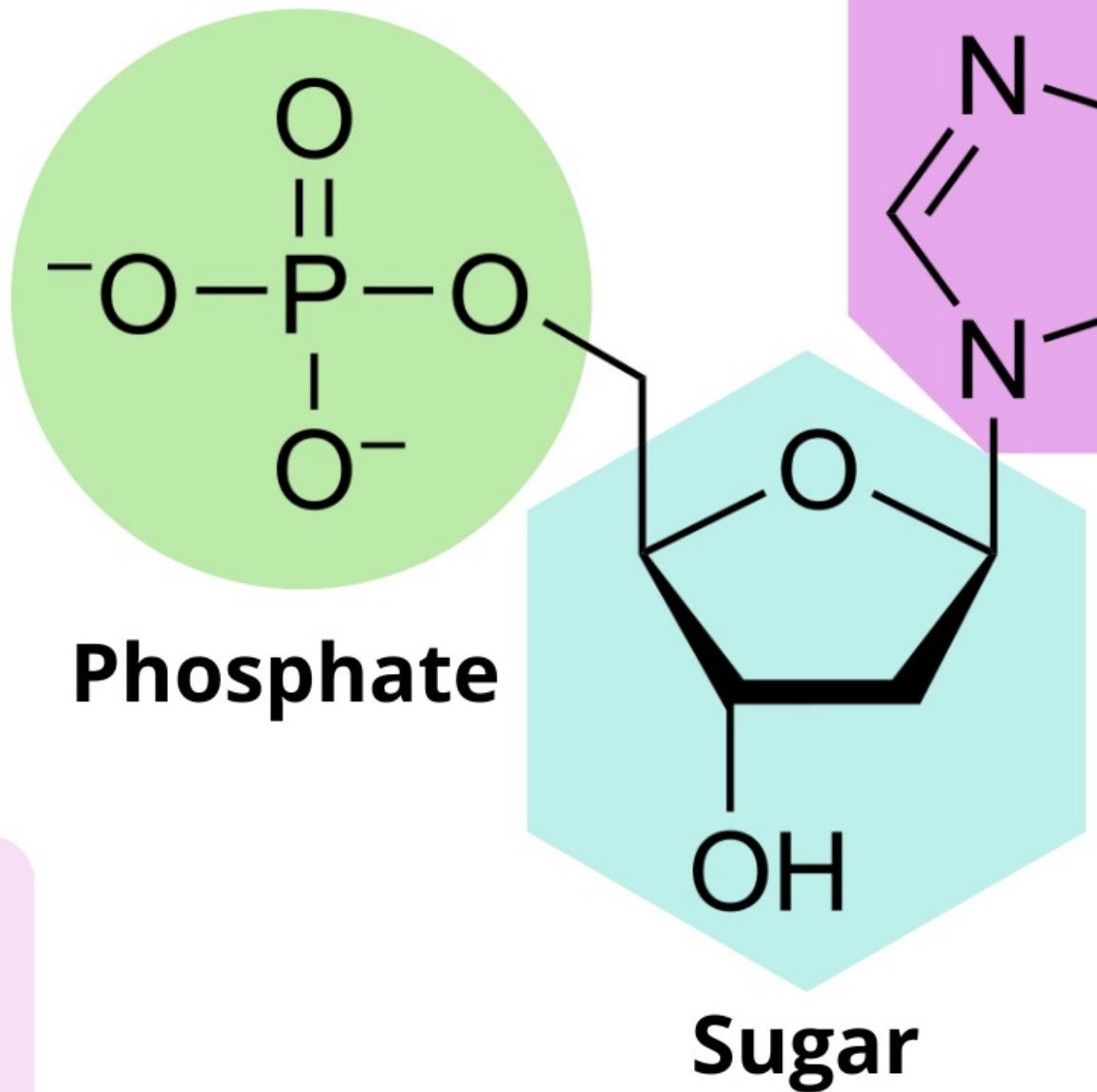
Structure of DNA and RNA

The term DNA was given by Zacharis.

- Structure of DNA was given by Watson and Crick
- Watson and Crick described DNA as 1. DNA is a double helix made up of two strands of polynucleotide chains
- 2. Two strands are arranged in antiparallel direction.
- Base Pair Rule: Purines pair with pyrimidines. A with T/U by double hydrogen bonds and G with C by triple hydrogen bonds.
- Chargaff's rule: number of Adenine will be equal to no. of Thymine and number of Guanine will be equal to Cytosine. Applicable in case of only double stranded DNA. Ratio of A/T and G/C will be equal to 1.

Structure of DNA and RNA:

3 Parts of a Nucle

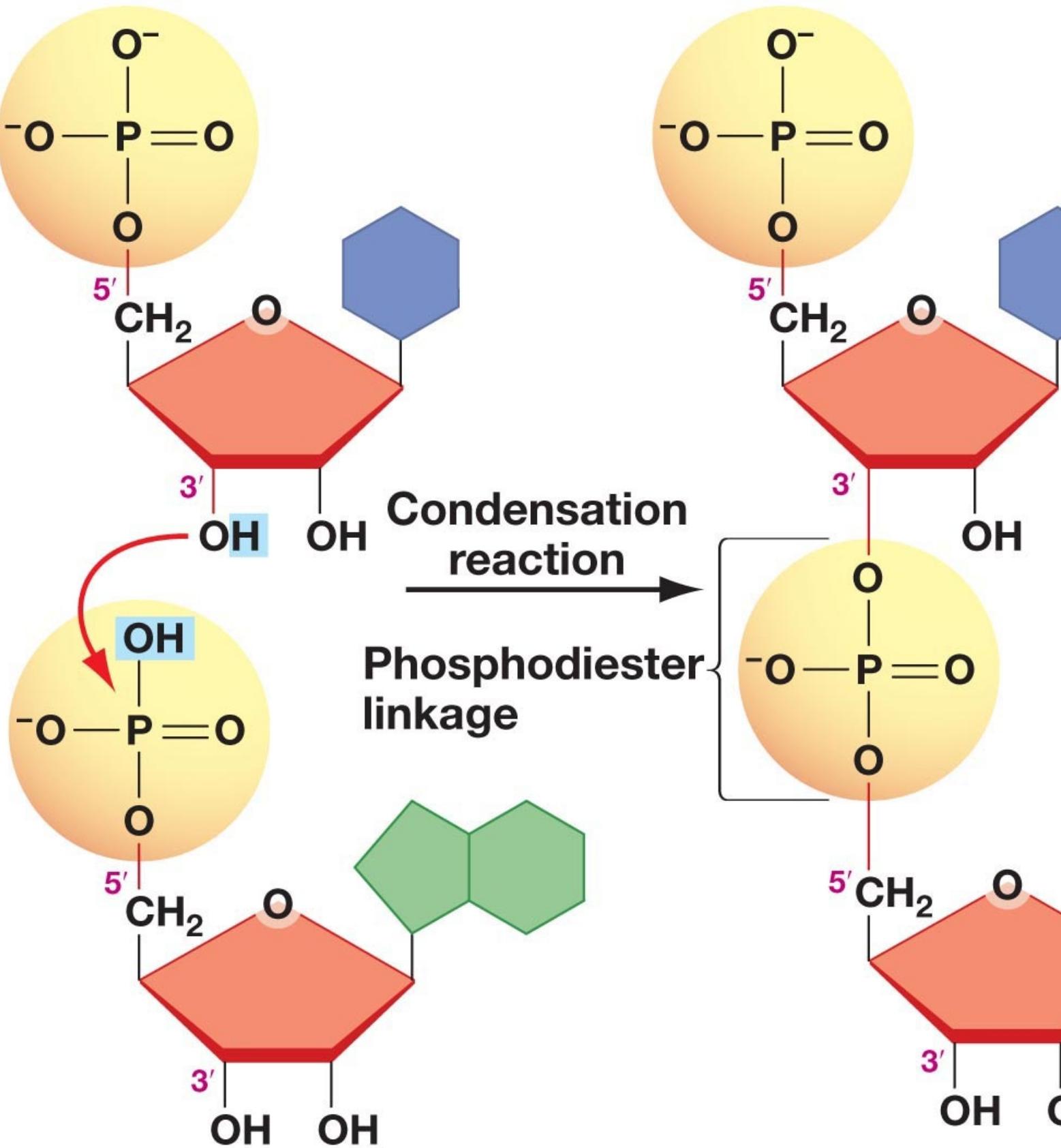


Nucleotides are the building blocks of DNA and RNA, which are the genetic material found in all living organisms. Each nucleotide is composed of three components:

A nitrogenous base: There are four different types of nitrogenous bases found in nucleotides: adenine (A), thymine (T), cytosine (C), and guanine (G) in DNA; adenine (A), uracil (U), cytosine (C), and guanine (G) in RNA. These nitrogenous bases are responsible for the sequence of genetic information encoded in DNA or RNA.

A five-carbon sugar: The sugar in DNA is called deoxyribose, while the sugar in RNA is called ribose. The sugar component provides the backbone of the nucleic acid polymer.

A phosphate group: The phosphate group is attached to the 5' carbon of the sugar molecule, and it links the nucleotides together in a linear chain via phosphodiester bonds, forming the backbone of the DNA or RNA molecule.



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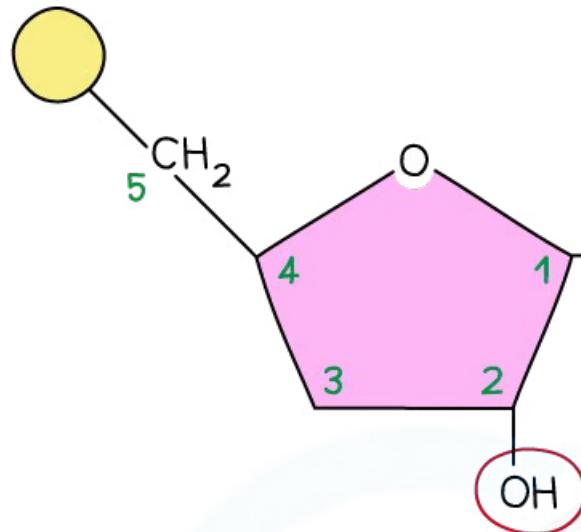
Phosphodiester bond formation is a chemical reaction that joins two nucleotides together in a DNA or RNA molecule. It involves the formation of a covalent bond between the 3'-OH group of one nucleotide and the 5'-phosphate group of another nucleotide. This reaction is catalyzed by enzymes called DNA or RNA polymerases.

During DNA replication or RNA transcription, a nucleoside triphosphate (NTP) molecule with a free 3'-OH group base-pairs with the complementary nucleotide on the template strand of DNA or RNA. The polymerase enzyme then catalyzes the formation of a phosphodiester bond between the 3'-OH group of the incoming nucleotide and the 5'-phosphate group of the growing chain, releasing two phosphate groups in the process.

The phosphodiester bond is a strong covalent bond that provides stability to the DNA or RNA molecule, as well as allowing for the transmission of genetic information through the generations.

RNA NUCLEOTIDE

PHOSPHATE GROUP

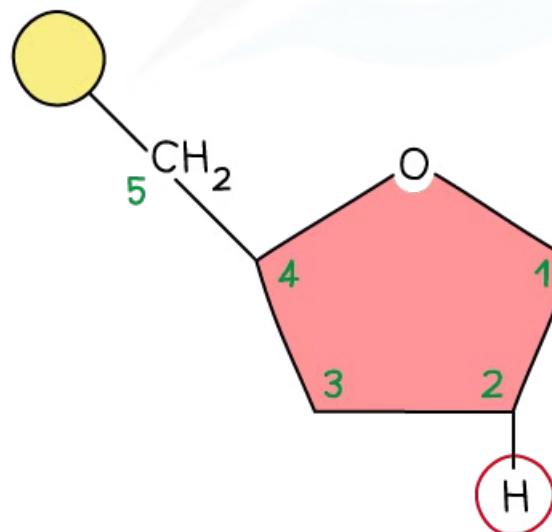


NITROGENOUS BASE
(A, C, G, U)

PENTOSE SUGAR
(RIBOSE)

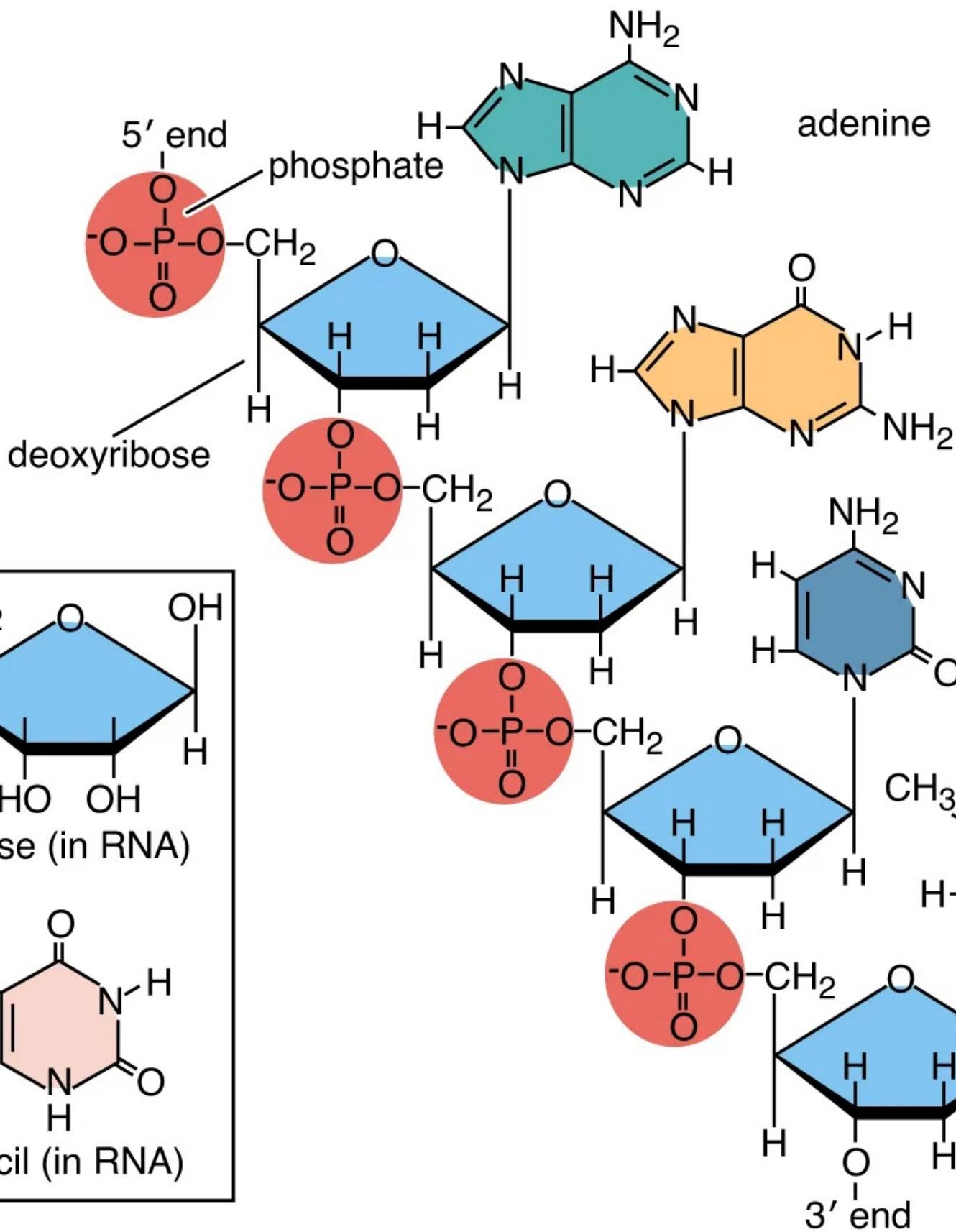
DNA NUCLEOTIDE

PHOSPHATE GROUP

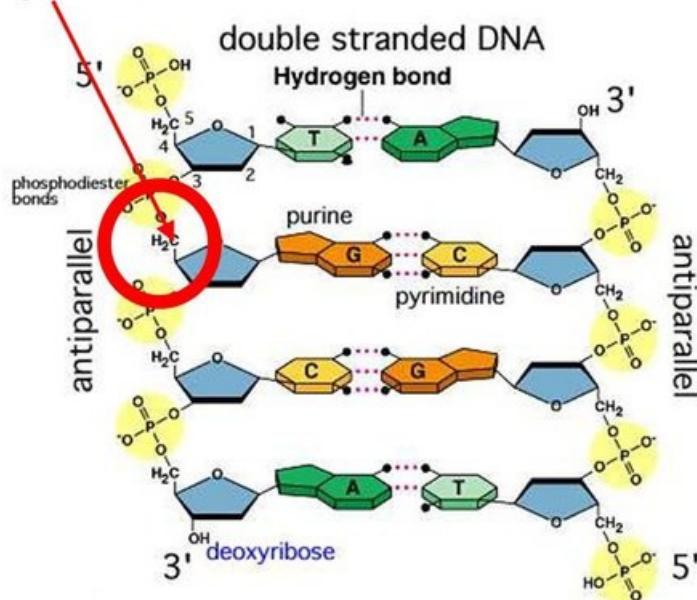


NITROGENOUS BASE
(A, C, G, T)

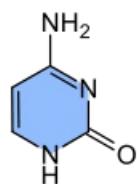
PENTOSE SUGAR
(DEOXYRIBOSE)



The nucleotides are joined together using phosphodiester bonds

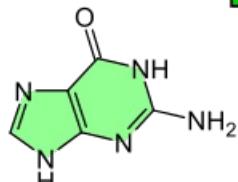


Cytosine



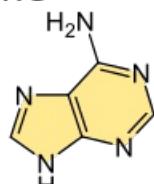
C

Guanine



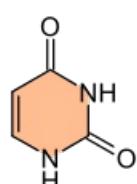
G

Adenine



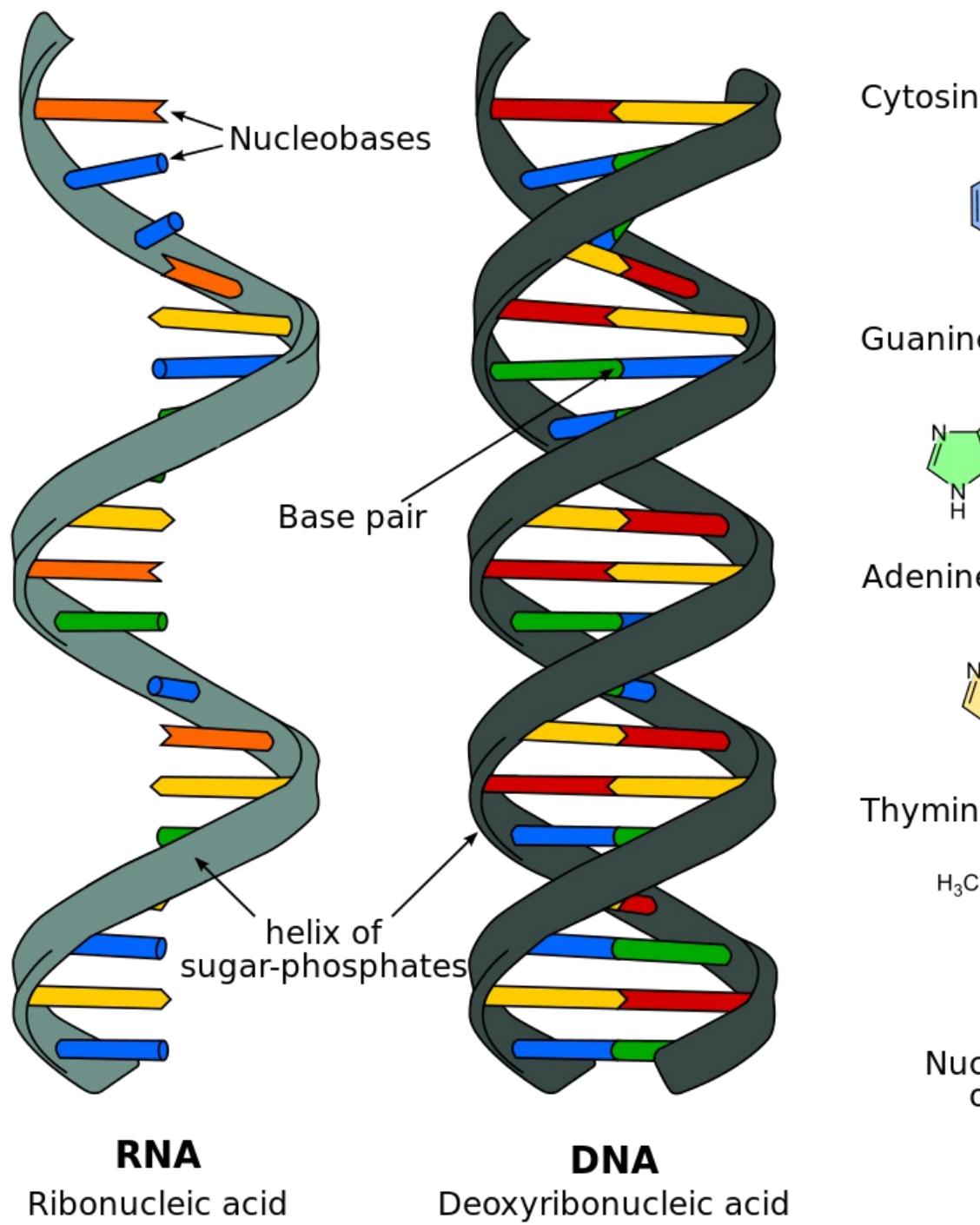
A

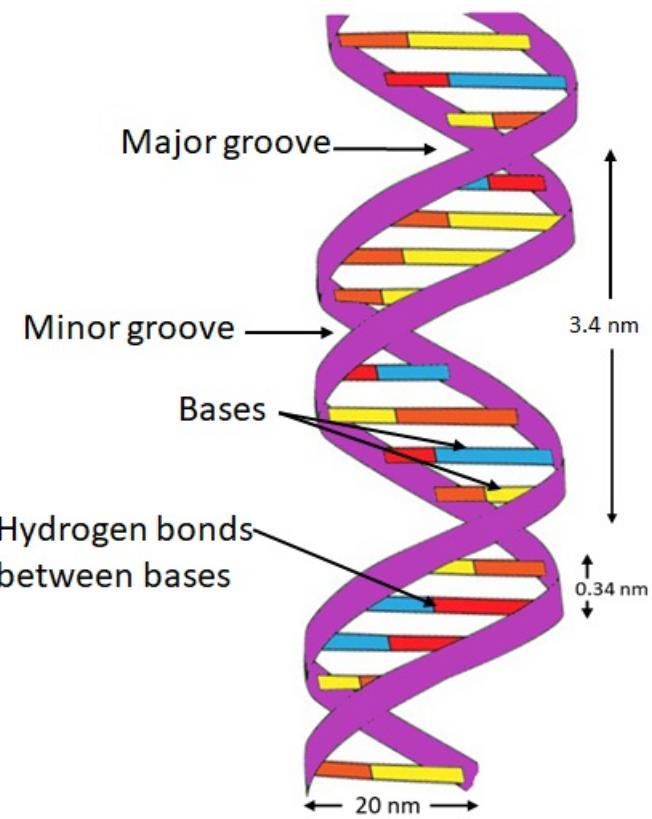
Uracil



U

Nucleobases of RNA





The DNA molecule has a double helix structure composed of nucleotides. A nucleotide is made up of three components: a sugar molecule (deoxyribose), a phosphate group, and a nitrogenous base. There are four types of nitrogenous bases in DNA: adenine (A), thymine (T), cytosine (C), and guanine (G).

The nitrogenous bases form hydrogen bonds with each other in a complementary fashion. Specifically, A always pairs with T, and C always pairs with G. This base pairing creates the rungs of the DNA ladder.

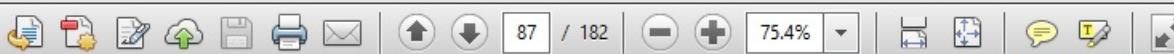
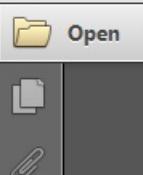
The sugar and phosphate molecules make up the vertical support structures of the DNA ladder, with the sugar of one nucleotide binding to the phosphate of the next nucleotide in the sequence. The result is a twisted, ladder-like structure that has a uniform width.

The double helix structure of DNA is vital for its function as a carrier of genetic information. The sequence of the nitrogenous bases along the DNA molecule serves as the genetic code, determining the sequence of amino acids that make up proteins, which are essential for the growth and development of all living organisms.

Function of DNA

- It is the genetic material, therefore responsible for carrying all the hereditary information.
- It has the property of replication essential for passing genetic information from one cell to its daughter cells or from one generation to the next.
- Crossing over produces recombination.
- Changes in sequence and number of nucleotides causes mutation which is responsible for all variations and formation of new species.
- It controls all the metabolic reactions of cells through RNAs and RNA directed synthesis of proteins.

Humans have a total of 46 chromosomes. Chromosomes are made up of DNA and histone proteins. Total length of the human genome is 3.2 billion bp or approximately 2m long.



87

/ 182

75.4%

▼

▲

+/-

X

Y

Z

A

B

C

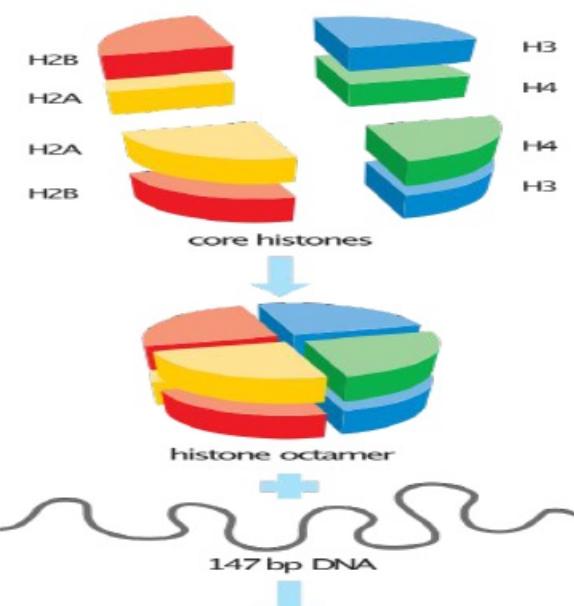
D

E

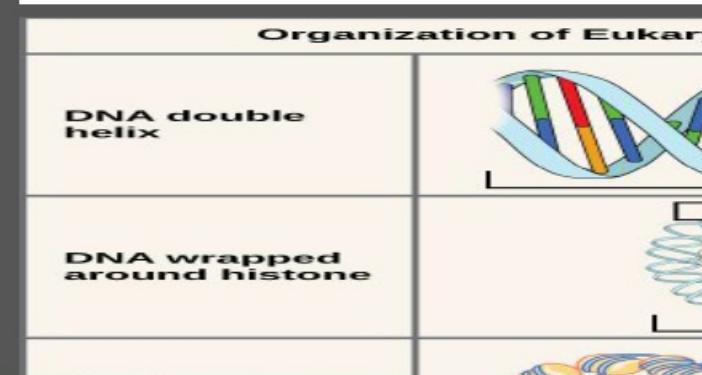
F

G

- Total length of DNA is about 3 billion bp or about 1 m per cell.
- Average size of cell nucleus is about 10⁻¹⁰ m³.



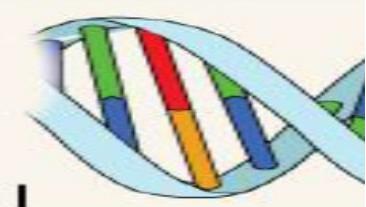
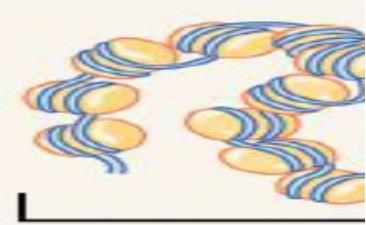
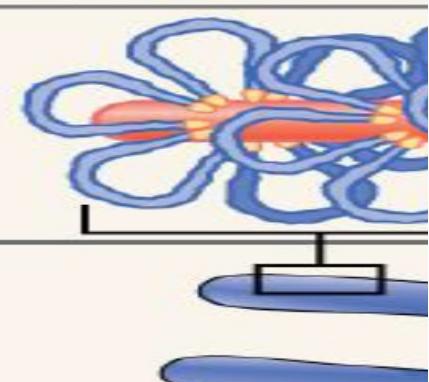
A.



Type here to search



A.

Organization of Eukaryotic Chromosomes**DNA double helix****DNA wrapped around histone****Nucleosomes coiled into a chromatin fiber****Further condensation of chromatin****Duplicated chromosome****Functions**

Type here to search



Types of RNA (Ribonucleic Acid)

There are 3 types of RNA

Ribosomal RNA (rRNA) : It is the non-coding RNA. rRNA varies in its length and form ribosomes along with proteins i.e. it is the component of the ribosome. It is also termed as ribozyme which carries out protein synthesis in ribosomes. It forces tRNA and mRNA to process and translate the mRNA into proteins.

Genetic disorders due to mutation

Disorders are caused in humans due to single gene mutation or change in the chromosome numbers.

Single gene mutation is also known as Mendelian disorders. These disorders are transmitted to the offspring on the same lines as the characters are passed onto the next generation according to Mendel's laws. The pattern of inheritance of these disorders can be traced in a family by the pedigree analysis. Few such common disorders are Haemophilia, Cystic fibrosis, Sickle-cell anaemia, colour blindness, phenylketonuria, Thalassemia, etc. Mendelian disorders can be either due to dominant or recessive alleles.

Mutation Definition

"Mutation is the change in our DNA base pair sequence due to various environmental factors such as UV light, or mistakes during DNA replication."

What Are Mutations?

The DNA sequence is specific to each organism. It can sometimes undergo changes in its base-pairs sequence. It is termed as a mutation. A mutation may lead to changes in proteins translated by the DNA. Usually, the cells can recognize any damage caused by mutation and repair it before it becomes permanent.

A mutation is a sudden, heritable modification in an organism's traits. The term "mutant" refers to a person who exhibits these heritable alterations. Mutations usually produce recessive genes.

Classification & Types of Mutations

Mutation Classifications	Types	Description	Examples of Human Disease(s)
Point mutation	Substitution	During replication, one base is inserted incorrectly, replacing the pair at the appropriate location on the complementary strand.	Sickle-cell anemia
	Insertion	In replicating DNA, one or more additional nucleotides are added, frequently causing a frameshift.	One form of beta-thalassemia
	Deletion	During replication, one or more nucleotides may be "skipped" or removed, which usually causes a frameshift.	Cystic fibrosis
Chromosomal mutation	Inversion	The flipping and reinserting of a single chromosomal region.	Opitz-Kaveggia syndrome
	Deletion	When a chromosome segment is lost, all the genes in that segment are also gone.	Cri du chat syndrome
Copy number variation	Duplication	A chromosomal segment is repeated, increasing the concentration of the genes in that area.	Some cancers
	Translocation	A section of one chromosome is inappropriately joined to another chromosome.	One form of leukemia
	Gene amplification	An increase is made in the tandem copies of a locus.	Some breast cancers
	Expanding trinucleotide repeat	There are more repeating trinucleotide sequences than usual.	Fragile X syndrome, Huntington's disease

Causes of Mutations

The mutation leads to genetic variations among species. Positive mutations are transferred to successive generations.

E.g. Mutation in the gene coding for haemoglobin causes sickle cell anaemia. The R.B.Cs become sickle in shape. However, in the African population, this mutation provides protection against malaria.

A mutation in the gene controlling the cell division leads to cancer.

Let us have an overview of the causes and impacts of mutation.

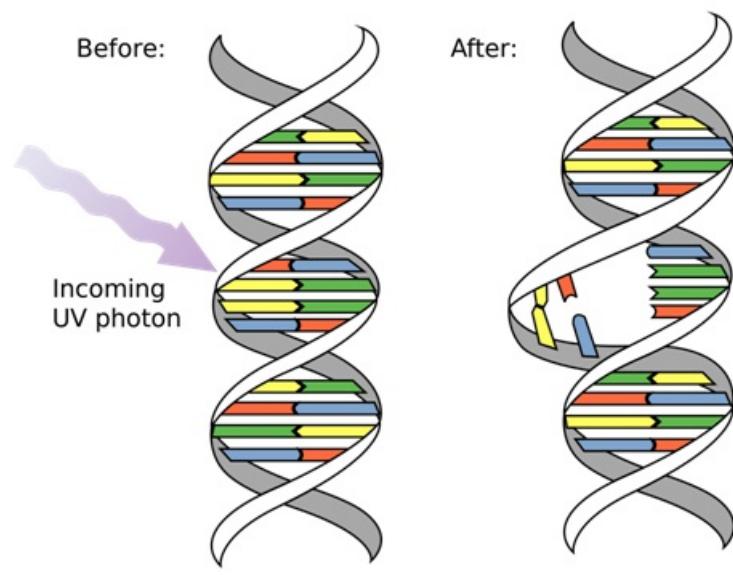
The mutation is caused due to the following reasons:

Internal Causes

Most of the mutations occur when the DNA fails to copy accurately. All these mutations lead to evolution. During cell division, the DNA makes a copy of its own. Sometimes, the copy of the DNA is not perfect and this slight difference from the original DNA is called a mutation.

External Causes

When the DNA is exposed to certain chemicals or radiations, it causes the DNA to break down. The ultraviolet radiations cause the thymine dimers to break resulting in a mutated DNA.



DNA Mutation

Effects of Mutation

There are several mutations that cannot be passed on to the offsprings. Such mutations occur in the somatic cells and are known as somatic mutations.

The germline mutations can be passed on to successive generations and occur in the reproductive cells.

Let us have a look at some of the effects of mutation:

Beneficial Effects of Mutation

- 1. Few mutations result in new versions of proteins and help the organisms to adapt to changes in the environment. Such mutations lead to evolution.
 2. Mutations in many bacteria result in antibiotic-resistant strains of bacteria that can survive in the presence of antibiotics.
 3. A unique mutation found in the population of Italy protects them from atherosclerosis, where fatty materials build up in the blood vessels.

Effects of Mutations

1. Genetic disorders can be caused by the mutation of one or more genes. Cystic fibrosis is one such genetic disorder caused by the mutation in one or more genes.
2. Cancer is another disease caused by the mutation in genes that regulate the cell cycle.

Origin of Life

Period of origin of earth is proposed to be about 4,500-5,000 million (i.e., 4.5-5 billion) years ago. In the beginning, it was a spinning ball of hot gases and vapours of elements. But due to gradual cooling, the gases condensed into molten core and different elements got stratified according to their density.

Theories of Origin of Life:

I. Theory of special creation:

It states:

1. Living organisms were formed on our planet by some supernatural power called God or Creator, so it believed in the divine creation of life.
2. The living organisms were formed all of a sudden and out of nothing. These are created as such.
3. There was no inter-relationship between these organisms.
4. These have not undergone any change since their formation (Life is immutable).

It was proposed by Hebrew et. al. and was very strongly supported by Father Suarez (1548-1671 A.D.). According to Christianity, the Bible states that the creator formed all the living organisms about 4004 B.C. within six-natural days— materia prima, heaven and earth on first day; sky was separated from water on second day; dry land and plants on third day; the sun, the moon and the stars on fourth day; fish and fowl on fifth day and animals including human beings on sixth day.

Man was created on the sixth day as Anima rationalis. The Bible says that Adam, the first man, was formed from clay about 6,000 years ago, while the first woman, Eve, was formed from one of his ribs. According to Hindu mythology, Brahma is the God of creation and created various forms of life in one stroke. Manu and Shraddha were the first man and woman on the earth.

This idea has no scientific support. It is further refuted by various evidence of evolution.

II. Abiogenesis or Theory of Spontaneous Creation or Autobiogenesis:

It was proposed by Von Helmont (1577-1644) and states that life originated abiogenetically from non-living decaying and rotting matter like straw, mud, etc., by spontaneous generation about 3.5 billion years ago. e.g.,

1. Anaximander (588-524 B.C.) proposed the air as sole cause of life.
2. Aristotle (384-322 B.C.) proposed that worms, insects, fish, frogs and even mice developed from soil and filth; tapeworms from excreta of animals; crabs and salamanders from earth and slime.
3. Hair of white horse tail forms living horse-hair worm, Gordius, when dropped into water.
4. The mud of Nile gave rise to living organisms when warmed in sun.
5. Von Helmont proposed that both sexes of mice will be developed when human sweat and wheat are kept together for 21 days.

This theory also proposed the formation of insects from dew, frogs and toads from the muddy bottom of ponds; butterflies from cheese and maggots (larvae of house flies) from decaying meat.

But abiogenesis was experimentally rejected by Francisco Redi (1668 A.D.).

III. Biogenesis (*omne vivum ex vivo*):

It states life arises from pre-existing life only. The idea of spontaneous generation came to an end with the experiment of Francisco Redi (1668). He founded the theory of biogenesis.

1. Redi's experiment:

Francisco Redi (Italian physician) took the flesh and cooked it so that no organisms were left alive. He placed the flesh in three jars (Fig. 7.2). One jar was covered with parchment, one was covered with muslin and third one was left open.

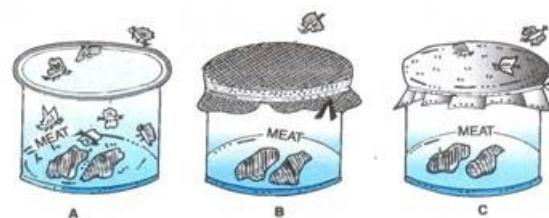


Fig. 7.2. Redi's experiment to disprove abiogenesis in large animals.
A. Uncovered jar, B. Jar covered with parchment, C. Jar covered with muslin.

The flesh/meat decayed in all the jars and flies were attracted towards all the three jars. He observed that maggots developed in uncovered jars though the flies visited other jars. The flies entered only the open jar and laid eggs which produced larvae. This confirms that maggots arise from eggs and not from decaying meat.

2. Spallanzani's experiment:

L. Spallanzani (1765 A.D.) poured hay infusion in eight bottles and boiled all of them. Four of them were loosely corked while the other four were made air tight. After a few days, he found that there was thick growth of microbes in all the loosely corked bottles but no organism in the air tight bottles. He concluded that air contains microbes and new microorganisms arise from existing micro-organisms.

3. Pasteur's experiment:

Louis Pasteur (1864) showed that minute organisms like protists and bacteria arise from pre-existing organisms of the same kind. He took a flask almost half filled with sugar and yeast (Fig. 7.3). By heating he gave S shaped structure to its neck. The contents of swan-necked flask were boiled and tube was sealed. No life appeared in the flask. But when neck of the flask was broken, micro-organisms appeared.

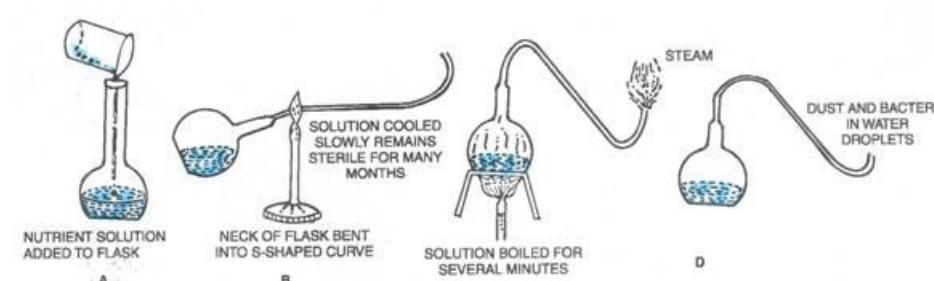


Fig. 7.3. Louis Pasteur's experiment showing the theory of biogenesis.

Spontaneous generation theory was rejected on the basis that it did not answer about the mode of formation of the first life form.

IV. Cosmozoic or Extraterrestrial or Interplanetary or Panspermia theory:

It was proposed by Richter (1865 A.D.) and was supported by Arrhenius (1908 A.D.). It states that life came on the earth from some other planet in the form of a seed or spore called panspermia, so is also called spore theory.

Spontaneous generation theory was rejected on the basis that it did not answer about the mode of formation of the first life form.

The Oparin-Haldane Hypothesis is a theory concerning how the Origin of Life on Earth came to be. In 1924 Russian scientist Aleksandr Oparin and in 1929 English scientist J. B. S. Haldane independently proposed new theories for the origins of life - what we now refer to as the Oparin-Haldane Hypothesis. They suggested life emerged from a series of step by step reactions between inorganic matter driven by a large energy input. These reactions initially produced the 'building blocks' of life (e.g., amino acids and nucleotides), then more and more complex molecules until primitive life forms arose.

The Oparin-Haldane Hypothesis proposes early life evolved through the process of abiogenesis.

Both Oparin and Haldane believed life could have arisen through the abiogenesis of non-living materials subjected to an external energy source, though their ideas on exactly how this occurred differ slightly. Their theories outlined the conditions in which this may have happened.

Both Oparin and Haldane theories described :

- The presence of a primitive reducing (oxygen-deprived) atmosphere containing ammonia, water vapour and other gases.
- Early life forms arising in the oceans.
- Early life forms were heterotrophic (they obtained nutrients already available during the primitive conditions).

The Oparin-Haldane Hypothesis described how life may have emerged from the primordial soup.

The origins of life according to Oparin

Oparin believed the earliest life forms developed from coacervates. Coacervates are small liquid droplets made up of 2+ different liquids that when mixed will not form a homogenous solution (a solution where the same share of components are found throughout). Coacervates are often produced and held together by the union of oppositely charged or hydrophobic molecules.

Oparin observed how coacervates formed, seemingly of their own accord, and suggested this mechanism is what formed the first pre-cells in the primordial soup. Oparin undertook experiments which proved that critical-for-life metabolic reactions were more efficient when contained in this way, as opposed to the reactants floating freely through aqueous solutions.

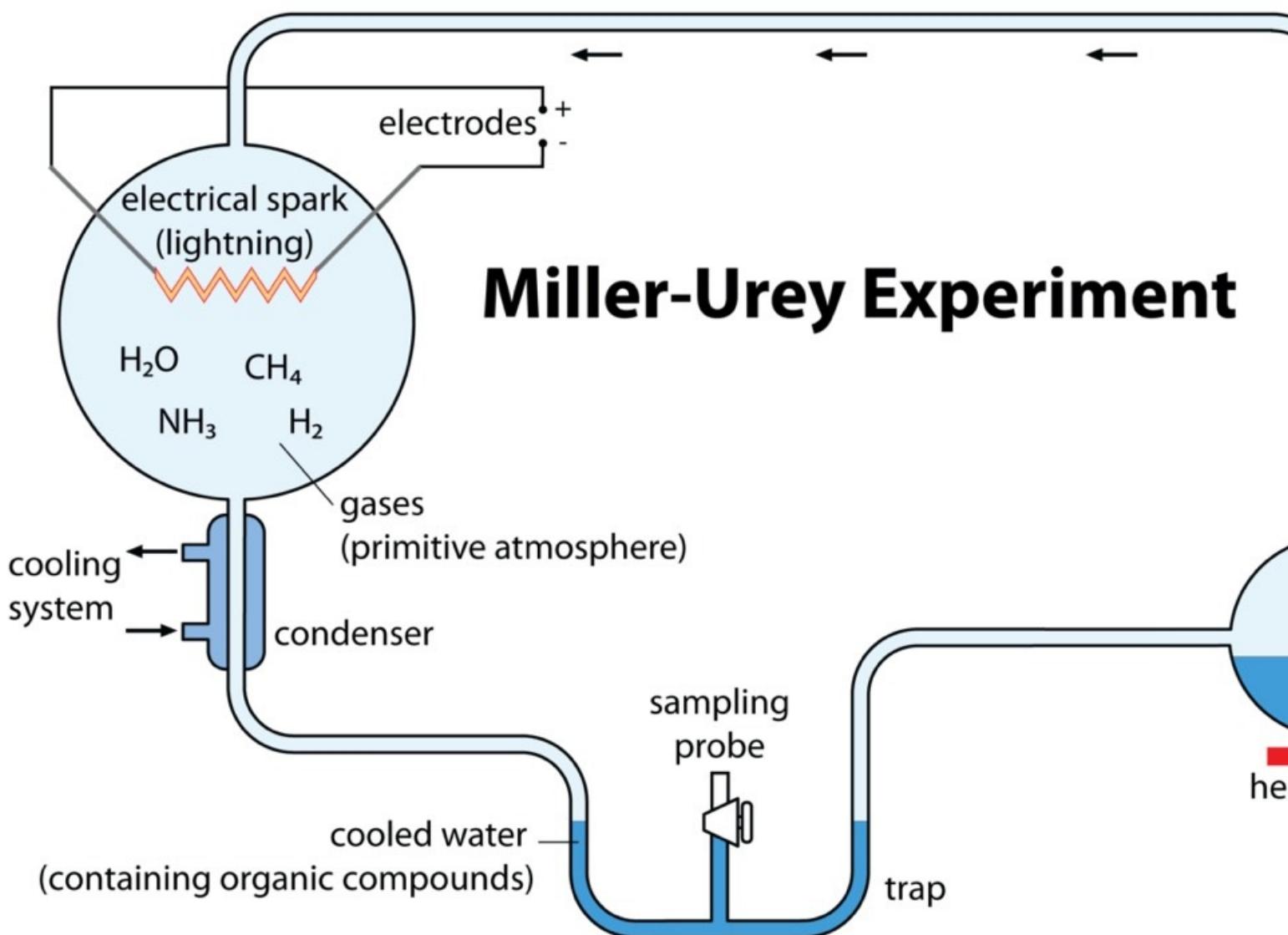
The origins of life according to Haldane

Haldane, at the time of his initial origin of life proposal, was unfamiliar with Oparin's work concerning coacervates. Haldane believed ultraviolet light provided energy for reactions to produce simple organic molecules. According to Haldane, these organic molecules continued to react until they eventually formed the first primitive cells.

Evidence supporting the Oparin-Haldane Hypothesis

In 1953 American chemists, Harold C. Urey and Stanley Miller, set out to test the Oparin-Haldane Hypothesis. Miller and Urey attempted to recreate the reducing primordial atmospheric conditions laid out by Oparin and Haldane (Figure 2) by combining four gases:

1. Water vapor
2. Methane
3. Ammonia
4. Molecular hydrogen



Miller-Urey Experiment

The pair of scientists then stimulated their faux atmosphere with electrical pulses to simulate energy provided by lightning, UV rays or hydrothermal vents. After a week, simple organic molecules, including amino acids, were produced by the experiment - proving organic molecules could form under the conditions laid out in the Oparin-Haldane Hypothesis. These findings became known as the Miller-Urey Experiment. The Miller-Urey Experiment was the first evidence that organic molecules could be spontaneously produced from only inorganic molecules.

Weaknesses of the Oparin-Haldane Hypothesis: unlikely coacervates

When Oparin first published his book, *Origins of Life*, little was known about genetic material. As knowledge about DNA and RNA emerged, coacervates as the first early life forms lost support. With the favor tipping towards theories which acknowledge the crucial role of genetic material for Darwinian evolution, such as the most widely supported RNA World Hypothesis.

Evolution: Modern concept of natural selection and speciation- Lamarckism, Darwinism/ Neo-Darwinism

Darwin's Theory of Evolution:

Darwin had the following ideas regarding the theory of natural selection:

- Species keep on evolving or changing with time. As the environment changes, the requirements of an organism also change and they adapt to the new environment. This phenomenon of changing over a period of time as per the natural requirements is called adaptation.
- As per Darwin's theory, only the superior changes are naturally selected and the inferior ones are eliminated. Thus, not all adaptations contribute to progressive evolution. For example, people living in tropical countries have more melanin in their bodies to protect them from the sunlight.
- Almost all organisms share common ancestry with some organisms. According to Darwin, all organisms had one common ancestor at some point in time and kept on diverging ever since. His evolutionary theories support the convergent theory and divergent theory of evolution with examples.
- He also studied that the birds of Galapagos Island (Darwin's finches) developed different beaks as per the availability of the food. This proved adaptive radiation. Similarly, he also observed the Australian Marsupials which showed a number of marsupials emerging from an ancestor.
- According to Charles Darwin, evolution is a very slow and gradual process. He concluded that evolution took place over a very long period of time. As we talk about the time period in evolution we usually refer to billions of years. The generation of a species from another takes a long period of time. It is a very steady process as the changes and adaptation take a long time to stabilize and give rise to a new species.

Natural selection takes place in four different ways as follows:

1. Variation – The changes accumulated over a period of time in an organism usually give rise to a new species.

2. Inheritance – It is the passing on of the variations over generations which ultimately leads to speciation.
3. A high rate of growth of population – This gives rise to more organisms being reproduced by a species than the environment can support.
4. Differential survival and reproduction – The superior variations lead to the survival of a particular organism and the inferior or negative variations lead to extinction. The superior variations are the ones inherited during reproduction.

Lamarckism was proposed by Jean-Baptiste de Monet Lamarck in the year 1744-1829. This theory was based on the principle that all the physical changes occurring in an individual during its lifetime are inherited by its offspring. For eg., the development of an organ when used many times. This theory has been explained here.

Lamarck's Theory

Lamarck's theory includes four main propositions:

Change Through Use And Disuse

The organs which are used frequently by the organism develop and the characteristics that are used seldom are lost in the succeeding generations. For eg., a giraffe stretches its neck to eat leaves, a “nervous fluid” would flow in its neck and it enlarges. The organs which the organisms have stopped using would shrink with time.

Organisms Driven To Greater Complexity

As the organisms adapted to their surroundings, they became increasingly complex from the simpler forms. Lamarck believed in the spontaneous generation of life.

Inheritance of Acquired Characters

An individual acquires certain characteristics during its lifetime. These characters are inherited by their offspring as well. He explained this with an example of a blacksmith. A blacksmith has strong arms due to the nature of their work. He proposed that any children a blacksmith conceives will inherit the development of strong muscles.

Effect of Environment and New Needs

The environment influences all the organisms. A slight change in the environment brings about changes in the organisms. This gives rise to new needs which in turn produces new structures and changes the habits of the organisms.

Examples of Lamarckism

Few of the examples of Lamarckism are mentioned below:

Evolution of giraffe

The ancestors of the giraffe looked like horses with small necks and forelimbs. They lived in areas where there was no surface vegetation. Therefore, they had to stretch their neck and forelimbs to eat leaves from tall plants. Consequently, these parts got elongated. This trait was transmitted in the successive generations.

Aquatic Birds with Webbed Toes

Aquatic birds such as ducks are believed to have evolved from terrestrial animals.

Extinction of Limbs in Snakes

The snakes are believed to have evolved from lizard-like ancestors that have two pairs of limbs.

Flightless Birds

It is believed that the ancestors of birds such as Ostrich were able to fly. Due to some environmental changes, they had a lot of food and were well protected. They stopped using their wings and as a result, the wings became vestigial.

Cave Dwellers

The ancestors of the animals living in caves are believed to have powerful eyesight. Due to living under continuous dark conditions, they lost their power to see.

Lamarckism v/s Darwinism

- Lamarck proposed theories like the inheritance of acquired characters, use and disuse, increase in complexity, etc. whereas Darwin proposed theories like inheritance, differential survival, species variation, and extinction.
- Darwin did not completely believe in his theory of acquired characters and proposed that the complexity in the organisms arise by the adaptation to the environment for several generations. Whereas Lamarck proposed that complexity arises due to usage or disuse of particular characters.

As the environment of an organism changes, so do his basic needs. The behaviour of the individual changes that eventually change organ usage and organism development. This gradual change in the species in response to the environment is known as “transmutation of species”.

Lamarckism was reformed by Giard and Cope by incorporating a few points of the opponents. This theory is known as Neo-Lamarckism.

Neo-Darwinism

- This is an altered explanation of Darwin's theory with regards to modern synthesis of natural selection and Mendelian genetics.
- The main force driving speciation is the gathering of genotypic variations in a gene pool
- Sometimes this theory is also referred to as the Modern synthetic theory of natural selection.
- Reproductive isolation has a major part in speciation allowing differential amplification of the fittest genes in a gene pool. Thus, natural selection of the most suitable genes is associated with the origination of new species.

Listed below are some important differences between Darwinism and neo-Darwinism:

Attributes	Darwinism	Neo-Darwinism
What it states	Evolution theory of species advanced by Darwin by natural selection	Modern interpretation of Darwin's theory by natural selection with discoveries of genetics
Adaptation	Original theory	Altered as per the information based on modern synthesis of natural selection and Mendelian genetics
Main driving force	Collection of phenotypic variations	Collection of genetic variations
Natural selection	Survival of fittest and removal of unfit entities with time	Differential amplification of fittest genotypes and genes
Purpose for variation	Not explained	Reason explained as genetic recombination, mutation, reproductive isolation and natural selection
Role of isolation in evolution	Does not have a role	Has a major role

Bioinformatics- brief idea

Bioinformatics is an emerging branch of biological science that emerged from the combination of both biology and information technology. It is an interdisciplinary field of study that uses Biology, Chemistry, Mathematics, Statistics, and Computer Science that have merged to form a single discipline. This sector is mainly involved in analyzing biological data, and developing new software using biological tools.

According to the NCBI- National Center for Biotechnology Information, the branch of NLM- National Library of Medicine and NIH- National Institutes of Health, **Bioinformatics is defined as the analysis, collection, classification, manipulation, recovery, storage and visualization of all biological information using computation technology.**

The term Bioinformatics was first coined in the year 1960 by the two Dutch biologists named Paulien Hogeweg and Ben Hesper. According to their research and discoveries, Bioinformatics was defined as the study of information processes in biotic systems.

Bioinformatic tools are software programs that are designed for extracting the meaningful information from the mass of molecular biology / biological databases & to carry out sequence or structural analysis

Factors that must be taken into consideration when designing bioinformatics tools, software and programmes are:

The end user (the biologist) may not be a frequent user of computer technology

These software tools must be made available over the internet given the global distribution of the scientific research community.

Major categories of Bioinformatics Tools :

There are both standard and customized products to meet the requirements of particular projects. There are data-mining software that retrieve data from genomic sequence databases and also visualization tools to analyze and retrieve information from proteomic databases. These can be classified as homology and similarity tools, protein functional analysis tools, sequence analysis tools and miscellaneous tools.

Here is a brief description of a few of these, everyday bioinformatics is done with sequence search programs like BLAST, sequence analysis programs, like the EMBOSS and Staden packages, structure prediction programs like THREADER or PHD or molecular imaging/modelling programs like RasMol and WHATIF.

Homology and Similarity Tools:

Homologous sequences are sequences that are related by divergence from a common ancestor. Thus the degree of similarity between two sequences can be measured while their homology is a case of being either true or false. This set of tools can be used to identify similarities between novel query sequences of unknown structure and function and database sequences whose structure and function have been elucidated.

Protein Function Analysis:

This group of programs allow you to compare your protein sequence to the secondary (or derived) protein databases that contain information on motifs, signatures and protein domains. Highly significant hits against these different pattern databases allow you to approximate the biochemical function of your query protein.

Structural Analysis:

This set of tools allow you to compare structures with the known structure databases. The function of a protein is more directly a consequence of its structure rather than its sequence with structural homologs tending to share functions. The determination of a protein's 2D/3D structure is crucial in the study of its function.

Sequence Analysis:

This set of tools allows you to carry out further, more detailed analysis on your query sequence including evolutionary analysis, identification of mutations, hydropathy regions, CpG islands and compositional biases. The identification of these and other biological properties are all clues that aid the search to elucidate the specific function of your sequence.

Some examples of Bioinformatics Tools:

BLAST:

BLAST (Basic Local Alignment Search Tool) comes under the category of homology and similarity tools. It is a set of search programs designed for the Windows platform and is used to

perform fast similarity searches regardless of whether the query is for protein or DNA. Comparison of nucleotide sequences in a database can be performed. Also a protein database can be searched to find a match against the queried protein sequence. NCBI has also introduced the new queuing system to BLAST (Q BLAST) that allows users to retrieve results at their convenience and format their results multiple times with different formatting options.

FASTA:

FAST homology search All sequences .An alignment program for protein sequences created by Pearsin and Lipman in 1988. The program is one of the many heuristic algorithms proposed to speed up sequence comparison. The basic idea is to add a fast prescreen step to locate the highly matching segments between two sequences, and then extend these matching segments to local alignments using more rigorous algorithms such as Smith-Waterman.

EMBOSS:

EMBOSS (European Molecular Biology Open Software Suite) is a software-analysis package. It can work with data in a range of formats and also retrieve sequence data transparently from the Web. Extensive libraries are also provided with this package, allowing other scientists to release their software as open source. It provides a set of sequence-analysis programs, and also supports all UNIX platforms.

Clustalw:

It is a fully automated sequence alignment tool for DNA and protein sequences. It returns the best match over a total length of input sequences, be it a protein or a nucleic acid.

RasMol:

It is a powerful research tool to display the structure of DNA, proteins, and smaller molecules. Protein Explorer, a derivative of RasMol, is an easier to use program.

PROSPECT:

PROSPECT (PROtein Structure Prediction and Evaluation Computer ToolKit) is a protein-structure prediction system that employs a computational technique called protein threading to construct a protein's 3-D model.

PatternHunter :

PatternHunter, based on Java, can identify all approximate repeats in a complete genome in a short time using little memory on a desktop computer. Its features are its advanced patented algorithm and data structures, and the java language used to create it. The Java language version of PatternHunter is just 40 KB, only 1% the size of Blast, while offering a large portion of its functionality.

COPIA (COnsensus Pattern Identification and Analysis) is a protein structure analysis tool for discovering motifs (conserved regions) in a family of protein sequences. Such motifs can be then used to determine membership to the family for new protein sequences, predict secondary and tertiary structure and function of proteins and study evolution history of the sequences.

Application of Programmes in Bioinformatics:

JAVA in Bioinformatics:

Since research centers are scattered all around the globe ranging from private to academic settings, and a range of hardware and OSs are being used, Java is emerging as a key player in bioinformatics. Physiome Sciences' computer-based biological simulation technologies and Bioinformatics Solutions' PatternHunter are two examples of the growing adoption of Java in bioinformatics.

Perl in Bioinformatics:

String manipulation, regular expression matching, file parsing, data format interconversion etc are the common text-processing tasks performed in bioinformatics. Perl excels in such tasks and is being used by many developers. Yet, there are no standard modules designed in Perl specifically for the field of bioinformatics. However, developers have designed several of their own individual modules for the purpose, which have become quite popular and are coordinated by the BioPerl project.

Application of Bioinformatics

Bioinformatics is mainly used to extract knowledge from biological data through the development of algorithms and software.

Bioinformatics is widely applied in the examination of Genomics, Proteomics, 3D structure modelling of [Proteins](#), Image analysis, Drug designing and a lot more. A

significant application of bioinformatics can be found in the fields of precision and preventive medicines, which are mainly focused on developing measures to prevent, control and cure dreadful infectious diseases.

The main aim of Bioinformatics is to increase the understanding of biological processes.

Listed below are a few applications of Bioinformatics.

- In Gene therapy.
- In Evolutionary studies.
- In Microbial applications.
- In Prediction of Protein Structure.
- For the Storage and Retrieval of Data.
- In the field of medicine, used in the discovery of new drugs.
- In Biometrical Analysis for identification and access control for improvising crop management, crop production and pest control.

Module 4

1. Concept of single-celled organisms, the concept of species and strains
2. Identification and classifications of microorganisms; Microscopy
3. Ecological aspects of single-celled organisms
4. Sterilization and media composition; Growth kinetics
5. Microbial diseases, epidemiology, and public health
6. Human immune mechanism- Types of immunities
7. Antigen-antibody reactions- Applications in human health
8. Immunological disorders: Auto-immune diseases

Concept of single-celled organisms, the concept of species and strains

Introduction of Microbiology

The field of microbiology has traditionally been concerned with information on how cells respond to their environment, interact with each other, and undergo complex processes such as cellular differentiation or gene expression. Here, we study invisible micro-organisms such as viruses, bacteria, many algae and fungi, and protozoa. Micro-organisms are necessary for the production of bread, cheese, beer, antibiotics, vaccines, vitamins, enzymes, etc. Modern biotechnology rests upon a microbiological foundation.

Concept of single-celled organisms

Most prokaryotes are unicellular and are classified into bacteria and archaea. Many eukaryotes are multicellular, but some are unicellular such as protozoa, unicellular algae, and unicellular fungi. Unicellular organisms are thought to be the oldest form of life, with early protocells possibly emerging 3.8–4.0 billion years ago. Although some prokaryotes live in colonies, they are not specialised cells with differing functions. These organisms live together, and each cell must carry out all life processes to survive. In contrast, even the simplest multicellular organisms have cells that depend on each other to survive. Most multicellular organisms have a unicellular life-cycle stage. Gametes, for example, are reproductive unicells for multicellular organisms. Some organisms are partially unicellular, like *Dictyostelium discoideum*. Additionally, unicellular organisms can be multinucleate, like *Caulerpa*, *Plasmodium*, and *Myxogastria*.

Most of the single-celled organisms are prokaryotic and show either vegetative or asexual mode of reproduction. A single cell carries out all life processes. Injury of the cell can cause death of the organism. Some examples of unicellular organisms are amoeba, slime mould, algae, bacteria, etc.

Single cell concept

Micro-organisms are capable of growing and reproducing. They also show characteristics such as:

1. Responsiveness to the environment
2. Growth
3. Ability to reproduce
4. Cellular organization
5. Ability to perform metabolism
6. Maintain homeostasis
7. Passing traits onto offspring

In the field of cellular biology, single cell analysis is the study of genomics, transcriptomics, proteomics and metabolomics at the single cell level. *In situ* sequencing and fluorescence *in situ* hybridization (FISH) do not require that cells be isolated and are increasingly being used for analysis of tissues.

Methods currently used for this purpose include:

- Enzymatic Digestion
- Serial Dilution
- Micromanipulation
- Laser capture microdissection
- Fluorescence-activated cell sorting(FACS)
- Microfluidics
- Manual picking
- Raman tweezers

Multicellular organisms studied in groups of cells, tissues, organs, and body give information which is not completely informative because individual cells of different parts of the body of multicellular organisms are not completely same.

To overcome this obstacle the concept of single cell isolation from multicellular organisms and studying their structure and metabolic pathways and genomic expression have been developed.

For example: Human brain is made of many types of cells like glial cells and nerve cells, etc. If a brain tumor develops the characteristics of that tumor will match the cell from which it arises. So, we isolate the different cells of the brain and assay them. Then the specific protein markers or gene markers are used to know the characteristics of the tumor which helps us to develop a particular type of treatment for it.

Benefits of Single Celled Concepts

1. In conventional methods of assays heterogeneity of individual cells is ignored. Cell to cell differences in RNA transcript and protein expression are ignored. To understand the variations from cell to cell, scientists need to use single cell analyses. This provides more detailed information for therapeutic decision making in precision medicine.
2. Single cell gene expression analysis can be used for tumor cell identification. Single cell DNA mutation analysis can be used for tumor cell monitoring and clinical decision making.
3. Intra-tumor heterogeneity has been widely reported in numerous human cancer types. Tumors in many cases are composed of individual, molecularly distinct clones that differ in proliferation rates, metastatic potential, and most importantly, they differ in their sensitivities and responses to drug treatment. Single cell analysis can show the better way for their treatment.
4. Stem cells are undifferentiated cells that are capable of self renewal and have the potential to differentiate into specialized types of cells. How stem cells balance their self renewal capacity and their ability to differentiate are still a central question in stem cell research. Single cell concept and analysis are helping the researchers to find out the causes.
5. Single cell analysis has influenced and impacted different domains of science such as neuroscience, immunology, etc.

The concept of species and strains:

The concept of species and strains is fundamental to understanding the diversity of living organisms. A species is a group of organisms that are capable of interbreeding and producing viable offspring. This definition is known as the biological species concept and is widely used in biology.

Strains, on the other hand, are subdivisions of a species that are distinguished by minor genetic or phenotypic differences. These differences can arise from mutations or selective pressures that lead to the development of distinct traits or characteristics.

Strains can also be used to describe subpopulations of microbes, such as bacteria or viruses, that have unique genetic or phenotypic features. In this context, strains are often used to identify different subtypes of a microbe, such as different strains of influenza virus.

These concepts are important for understanding the diversity of life and for identifying and categorizing different types of organisms.

Identification and classifications of microorganisms

Microorganisms are typically classified into five major groups, based on their cell structure, metabolism, and genetic makeup:

1. Bacteria: These are single-celled organisms that lack a nucleus and other membrane-bound organelles. They can be found in various environments, including soil, water, and living organisms. Bacteria can be further classified based on their shape (cocci, bacilli, spirilla) and staining characteristics (Gram-positive or Gram-negative).
2. Archaea: Archaea are similar to bacteria in size and shape but have a different cell wall composition and metabolic processes. They are often found in extreme environments such as hot springs and deep-sea vents.
3. Fungi: Fungi are eukaryotic organisms that are typically multicellular, but some are unicellular. They obtain their nutrients by absorbing organic matter from their environment. Fungi include molds, yeasts, and mushrooms.
4. Protists: Protists are eukaryotic microorganisms that are typically unicellular, but some are multicellular. They can be found in various aquatic and terrestrial environments and include algae, protozoa, and slime molds.
5. Viruses: Viruses are non-cellular particles that consist of genetic material (either DNA or RNA) enclosed in a protein coat. They cannot replicate on their own and require a host cell to reproduce.

Identification: It means to determine what species a microorganism is belonging to for the research or disease treatment.

Classification: It means to place an unknown microorganism in the most closely related species.

Further Identification and classification of the above five categories of microorganisms are done by observing morphological characteristics , certain metabolic activities, deciphering the genetic codes, DNA analysis, RNA analysis etc.

Goals of Classification

To identify different species of living organisms into a hierarchical pattern based on multiple criteria like morphology, metabolic activities, genome and genetic associations.

Reasons of Classification

Same species have different names in different languages in different regions of the earth.

To create a uniformity by creating a nomenclature on the basis of classification in scientific terms, so that the same species have the same name.

Different Techniques used for classifications are:

1. **Morphological Characteristics**: Based on morphological characteristics such as

- a. Size and shape
- b. Presence of endospores, flagella, capsules, pili, fimbriae
- c. Shape of the colonies developed by microorganisms.

2. **Differential Staining**

Gram positive or gram negative (Gram stain divides bacteria into two groups).

Acid- fast stain: usually used to identify Mycobacterium.

3. **Biochemical Tests**

- a. Fermentation of carbohydrates
- b. Fermentation end products
- c. Hydrolysis reactions
- d. Oxidation reactions
- e. Rapid identification methods: several tests performed simultaneously; each test result (+ or -) is given a coded value.

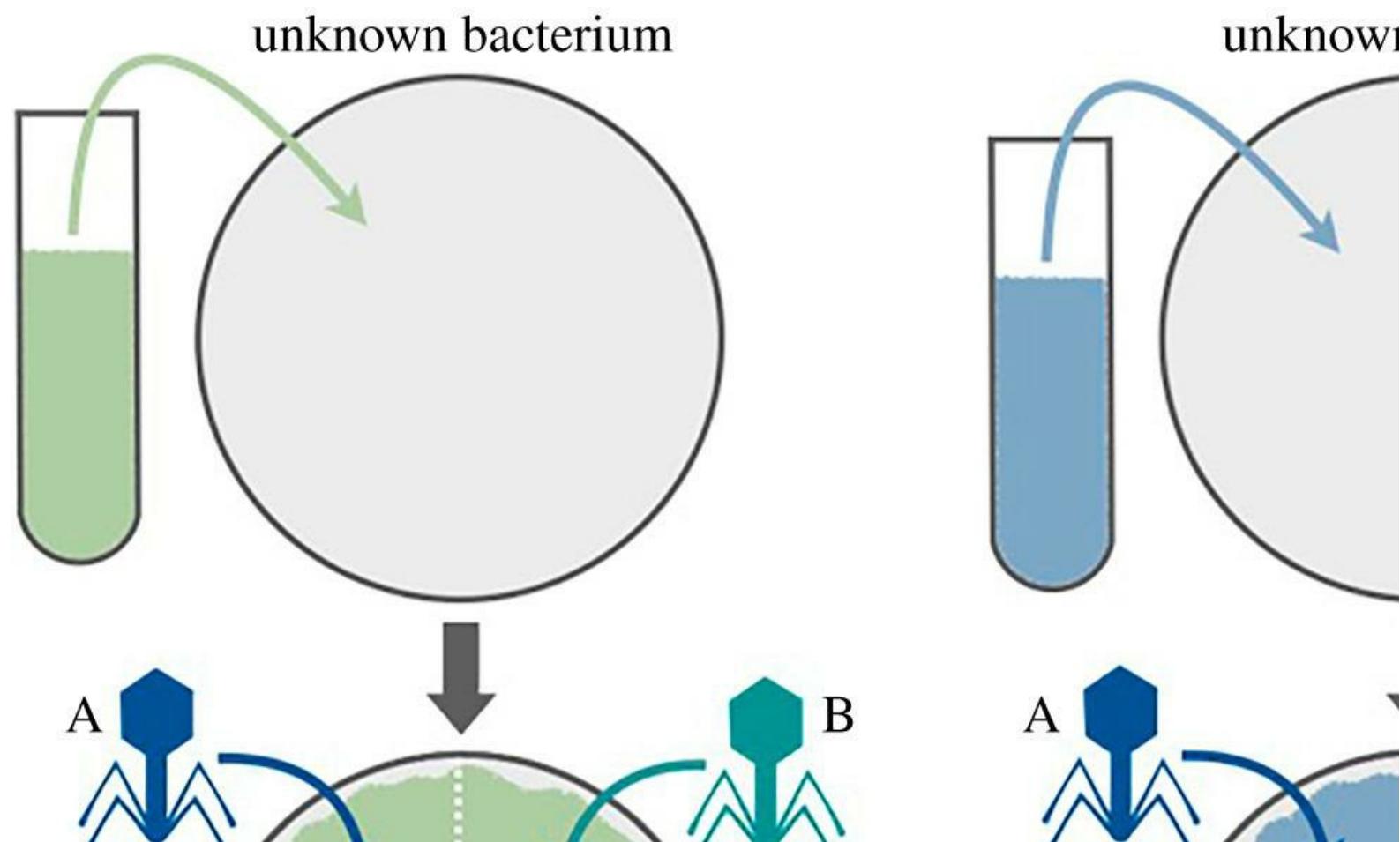
4. **Serological Testing**

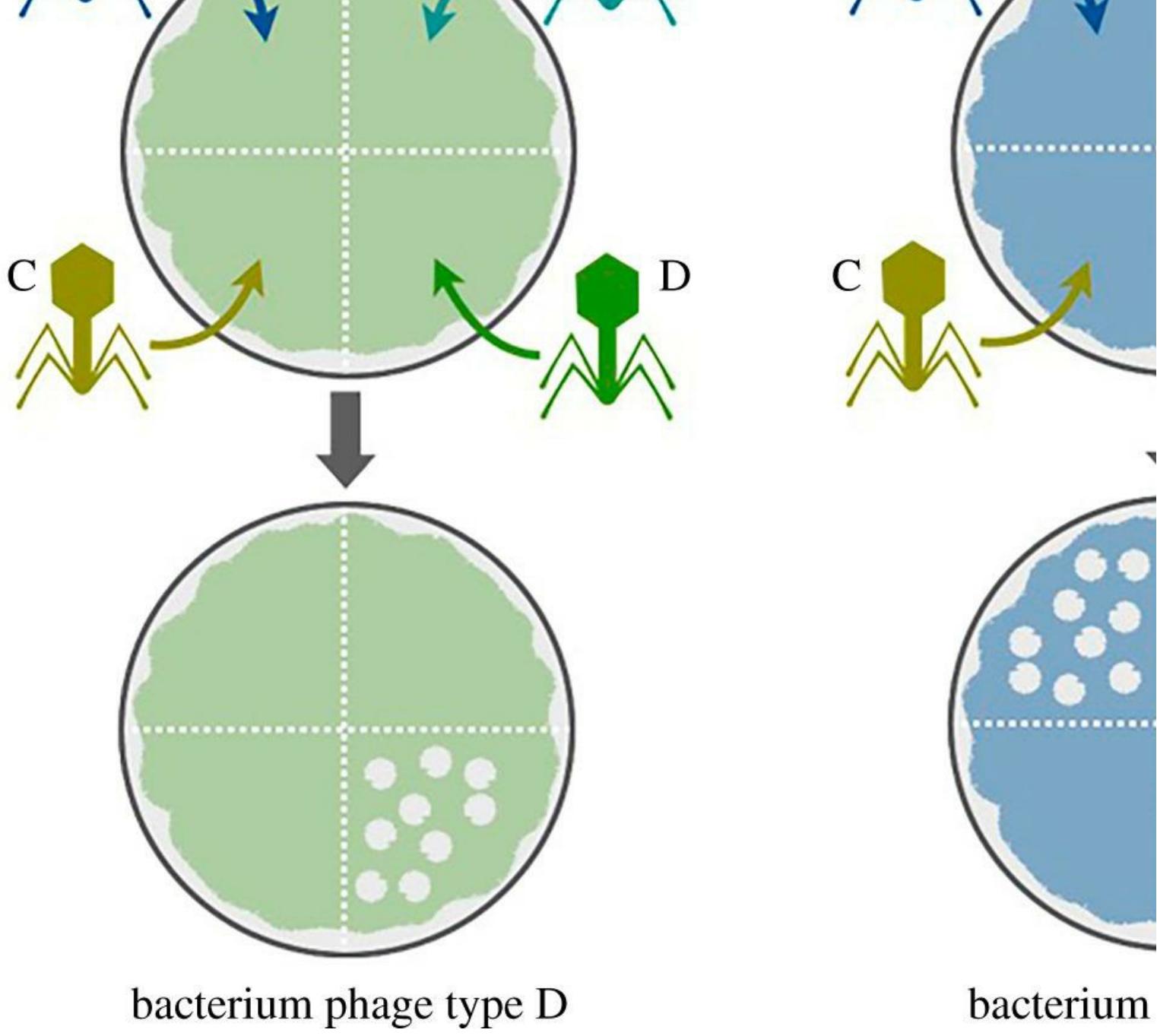
- a. Reaction of bacteria to antibodies that are specific for that organism's antigens.
- b. Agglutination tests: if an antibody is specific for a certain bacterium, it will cause agglutination (clumping) of the organisms.

(Serum: Blood plasma without white blood cells (leukocytes), red blood cells (erythrocytes), platelets, or clotting factors.

5. **Phage Typing**

Bacteriophages are specific to the bacterial species that they infect (similar idea to antigens/antibodies). If a phage that is specific for a bacterium is placed on agar with bacterial growth, it will destroy cells where it is placed creating a plaque.





6. Flow Cytometry

Bacteria flow through a tube and size/shape can be determined by how much light is scattered. It can also detect fluorescent cells (natural or using fluorescent dyes). And can be done without staining too.

7. DNA Base Composition

Determine percentage of GC pairs in DNA. If the difference is more than 10% between two species- they are not closely related. Very close percentage difference: might not be closely related since percentage could be similar but arrangements in genes could be quite different.

8. DNA Fingerprinting

Cut DNA with restriction enzymes; run electrophoresis to separate bands of DNA fragments. Compare bands of different bacteria: more bands similar- more closely related; exactly same bands- same species.

9. Ribosomal RNA Sequencing

Determine sequence of nucleotide bases on the ribosomal RNA in the small subunit of the ribosome. More similar sequences- more closely related. It is used to organize classification groups around this characteristic.

10. Nucleic Acid Hybridization

Separate the double stranded DNA of two organisms; mix the strands together and see how much DNA from one organism base pairs with the DNA from the other organism (hybridization). Higher percentage of DNAs hybridizing – more closely related. It can also hybridize DNA with mRNA.

Microscopy

Microscopy is the study of objects or specimens that are too small to be seen by the naked eye. It involves the use of a microscope, which magnifies and illuminates the object to allow for detailed observation and analysis.

There are several types of microscopes, including light microscopes, electron microscopes, and scanning probe microscopes. Light microscopes use visible light to illuminate the object and magnify it, while electron microscopes use beams of electrons to create an image. Scanning probe microscopes use a physical probe to interact with the surface of the specimen and create an image.

Microscopy has many applications in science and medicine. It is used in biology to study cells, tissues, and microorganisms, and in materials science to analyze the structure and properties of materials at the nanoscale. Medical professionals also use microscopy to diagnose and monitor diseases, such as cancer and infections.

Types of Microscopy

There are several types of microscopy, including:

1. Optical microscopy:

Optical microscopy is a scientific technique that uses visible light and a series of lenses to magnify and examine samples at a microscopic level. It allows scientists and researchers to observe the details of cells, tissues, and other small objects that are otherwise invisible to the naked eye.

There are several types of optical microscopy, including bright-field microscopy, dark-field microscopy, phase-contrast microscopy, fluorescence microscopy, etc. Each of these techniques has its own advantages and applications, depending on the sample being studied and the questions being asked.

Bright field microscopy is a type of light microscopy that uses visible light to observe samples. It is one of the most common forms of microscopy used in biology and is particularly useful for observing fixed and stained samples. In bright field microscopy, the sample is illuminated with a beam of light that passes through the specimen, which then absorbs or scatters the light. The resulting image is then viewed through the eyepiece of the microscope or captured using a digital camera.

Bright field microscopy is particularly useful for observing the morphology of cells and tissues, as well as for identifying various types of organisms and structures. However, it has limitations when it comes to observing unstained or transparent samples, as these may not be visible against a bright background. In addition, bright field microscopy does not provide any information about the chemical composition or internal structure of the sample. To overcome these limitations, other forms of microscopy, such as phase contrast microscopy, fluorescence microscopy, and electron microscopy, may be used.

Dark-field microscopy is a specialized form of microscopy that allows visualization of objects that are not visible under ordinary bright-field illumination. In dark-field microscopy, the specimen is illuminated with a cone of light that is positioned so that it does not enter the objective lens directly. This creates a dark background, and the only light that enters the lens is scattered by the specimen. As a result, objects in the specimen that scatter light will appear bright against a dark background.

Dark-field microscopy is particularly useful for the observation of live, unstained specimens, such as microorganisms, cells, and small particles. It is often used in microbiology to observe bacteria and other microorganisms that are difficult to see under normal bright-field illumination. It can also be used in the study of thin films and surfaces, such as in materials science and nanotechnology.

One of the advantages of dark-field microscopy is that it allows the visualization of objects that may be too small or too transparent to be seen using other forms of microscopy. Additionally, because the technique does not require staining or fixing of the specimen, it can provide more accurate information about the behavior and structure of live specimens.

Phase-contrast microscopy is a type of optical microscopy that allows visualization of transparent or translucent specimens that would otherwise be invisible under ordinary brightfield microscopy. It is a widely used technique in biological and medical research, as it allows visualization of live cells and tissues without the need for staining or other sample preparation methods.

The technique works by transforming the phase variations of light passing through a transparent specimen into variations in brightness or contrast, which can be observed and recorded using a specialized phase-contrast microscope. This is achieved by using a special optical system that converts the phase differences in the specimen into differences in light intensity, creating a high-contrast image.

Phase-contrast microscopy is particularly useful for visualizing the internal structures of cells, such as the nucleus, mitochondria, and other organelles, as well as for observing the movement of living cells and the dynamics of cellular processes in real time. It is a valuable tool for biological and medical research, as well as for clinical diagnostics and pathology.

Fluorescence microscopy is a type of microscopy that uses fluorescence to produce images of specimens. It involves the use of a fluorescent dye or a genetically encoded fluorescent protein that is specifically bound to the target molecule or structure in the specimen. When excited by a specific wavelength of light, the fluorescent molecule emits light of a longer wavelength, allowing it to be visualized and imaged.

Fluorescence microscopy is commonly used in biological research to study the distribution and behavior of molecules and structures within cells and tissues. It allows researchers to visualize and track specific proteins, organelles, and other molecular structures in living cells, and to study their dynamics and interactions over time.

There are several types of fluorescence microscopy techniques, including widefield microscopy, confocal microscopy, and super-resolution microscopy. Each technique has its own strengths and limitations, and the choice of technique depends on the specific research question being addressed.

In general, optical microscopy is a powerful tool for studying biological samples, as well as for examining materials, such as metals, minerals, and polymers. It is also widely used in medical diagnosis and treatment, for example, to analyze blood smears, to identify infectious agents, and to visualize tissues during surgery.

In recent years, advances in technology have led to the development of super-resolution microscopy techniques, which can overcome the diffraction limit of light and achieve resolutions of less than 100 nanometers. These techniques are revolutionizing our understanding of cellular processes and are enabling new discoveries in fields such as neuroscience, immunology, and cancer research.

2. Electron microscopy: This type of microscopy uses electrons to create an image. There are two main types of electron microscopy: transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Transmission electron microscopy (TEM) is a type of microscopy that uses a beam of electrons to examine the internal structure of thin samples. In a transmission electron microscope, a beam of electrons is focused onto a very thin sample, typically less than 100 nanometers thick, which is placed on a support structure called a grid. As the electrons pass through the sample, they interact with its atoms and are scattered in different directions.

The electrons that pass through the sample are collected by a detector on the other side, forming an image of the sample's internal structure. TEM allows scientists to examine the atomic structure of materials and biological samples, and it is widely used in fields such as materials science, nanotechnology, and biology.

In addition to imaging, TEM can also be used for diffraction experiments, which involve passing the electron beam through a crystal to produce a diffraction pattern. This pattern provides information about the crystal structure of the material being examined.

TEM requires specialized equipment and expertise to operate, and samples must be prepared carefully to ensure they are thin enough to allow the electrons to pass through. The technique has revolutionized our understanding of the microscopic world and has led to many important discoveries in science and engineering.

Scanning Electron Microscopy (SEM) is a type of microscopy that uses a beam of electrons to image the surface of a sample. In contrast to traditional light microscopy, which uses photons to produce images, SEM uses a focused beam of electrons to scan the surface of the sample. This beam of electrons interacts with the atoms on the surface of the sample, producing signals that can be used to generate an image.

SEM can be used to study a wide variety of samples, including biological samples, materials, and minerals. One of the key advantages of SEM is its ability to provide high-resolution images with a very large depth of field, which makes it useful for examining the surface features of samples in great detail. SEM is also capable of providing information about the composition of samples, as well as their structure and topography.

The basic components of an SEM include an electron source, a set of lenses to focus and direct the electron beam, a sample stage to hold the sample, and a detector to capture the signals produced by the interaction of the electrons with the sample. To create an image, the electron beam is scanned across the surface of the sample in a raster pattern, and the signals produced by the interaction of the electrons with the sample are detected and used to generate an image.

3. Scanning probe microscopy: Scanning probe microscopy (SPM) is a type of microscopy that uses a physical probe to interact with a sample and create an image. This technique is capable of producing high-resolution images of a wide range of materials, from biological molecules to electronic devices.

There are several types of scanning probe microscopy, including atomic force microscopy (AFM), scanning tunneling microscopy (STM), and magnetic force microscopy (MFM). In each of these techniques, a tiny probe is brought into contact with the sample and then scanned over its surface, recording data that can be used to create an image.

AFM is perhaps the most widely used type of scanning probe microscopy. It uses a sharp tip mounted on a cantilever to scan the surface of a sample. The tip is moved up and down as it is scanned over the surface, and the deflection of the cantilever is measured. This information is used to generate a topographic image of the sample.

STM, on the other hand, uses a conductive tip that is brought very close to the surface of a conductive sample. A voltage is applied between the tip and the sample, and the resulting tunneling current is measured. By scanning the tip over the surface and measuring the tunneling current, an image of the surface can be created.

MFM uses a magnetic probe to scan the surface of a magnetic sample. As the probe scans the surface, it detects the magnetic field emanating from the sample. This information is used to create an image of the sample's magnetic structure.

Scanning probe microscopy has become an important tool in materials science, physics, and biology, allowing researchers to study the structure and properties of materials at the nanoscale.

4. X-ray microscopy: This type of microscopy uses X-rays to create images of samples. X-rays have shorter wavelengths than visible light, which allows them to penetrate denser materials and create detailed images of the internal structure of objects. X-ray microscopy can be used to image a wide range of materials, including biological samples, minerals, and electronic devices.

There are several types of X-ray microscopy techniques, including scanning X-ray microscopy, transmission X-ray microscopy, and full-field X-ray microscopy. In scanning X-ray microscopy, a tightly focused beam of X-rays is scanned across the sample, and the scattered or absorbed X-rays are detected and used to create an image. In transmission X-ray microscopy, the sample is illuminated with a broad beam of X-rays, and the transmitted X-rays are detected and used to create an image. Full-field X-ray microscopy uses an X-ray microscope objective to magnify the image of the sample and project it onto a detector.

X-ray microscopy has several advantages over other imaging techniques, including its ability to image dense materials, its high resolution, and its ability to image samples in their natural state without the need for staining or fixing. However, X-ray microscopy also has some limitations, including the potential for radiation damage to the sample and the need for specialized equipment and facilities.

5. Confocal microscopy: Confocal microscopy is a type of microscopy that uses a laser beam to illuminate a single point on a sample, and then collects the emitted light using a pinhole aperture. The laser light is focused onto the sample at a very small point, and the emitted light is collected through the same point. This process is repeated for every point on the sample, resulting in a three-dimensional image of the specimen.

The main advantage of confocal microscopy over other types of microscopy is its ability to obtain high-resolution images of thick specimens. This is because the pinhole aperture allows for the collection of light only from the focal plane, while rejecting out-of-focus light from other regions of the sample. This produces images that are sharp and clear, with high contrast and minimal background noise.

Confocal microscopy is widely used in the field of biology, particularly in the study of cells and tissues. It has many applications, including in the investigation of the structure and function of cells, the visualization of subcellular structures, and the examination of cellular dynamics. It is also used in biomedical research, such as the study of cancer cells and the development of new drugs.

6. Super-resolution microscopy: Super-resolution microscopy is a set of imaging techniques that overcome the diffraction limit of conventional optical microscopy, allowing for higher resolution imaging of biological samples. In conventional optical microscopy, the resolution is limited by the wavelength of light,

which prevents the visualization of structures smaller than approximately 200 nanometers.

Super-resolution microscopy techniques use various approaches to improve resolution beyond this limit. One common approach is to use fluorescent molecules that can be switched on and off with specific wavelengths of light, allowing for the precise localization of individual molecules. This technique is called super-resolution single-molecule localization microscopy (SMLM) or photoactivated localization microscopy (PALM).

Another approach is to use structured illumination microscopy (SIM), which involves projecting a pattern of light onto the sample and then using sophisticated algorithms to reconstruct the high-resolution image from the resulting pattern of fluorescence.

A third approach is stimulated emission depletion (STED) microscopy, which uses a laser to selectively turn off fluorescence in certain regions of the sample, allowing for the visualization of structures as small as 20 nanometers.

Super-resolution microscopy has enabled researchers to study a wide range of biological structures at unprecedented levels of detail, including proteins, organelles, and even individual molecules. It has revolutionized our understanding of many cellular processes and has the potential to lead to new insights into disease mechanisms and drug development.

Ecological aspects of single-celled organisms

Single-celled organisms play an essential role in the ecology of many ecosystems. Here are some ecological aspects of single-celled organisms:

1. Nutrient cycling: Single-celled organisms such as bacteria and protists are important decomposers in ecosystems. They break down dead organic matter and release nutrients such as nitrogen and phosphorus, which are essential for the growth of plants and other organisms.
2. Primary production: Some single-celled organisms, such as algae, are capable of photosynthesis and are the primary producers in aquatic ecosystems. They convert sunlight into energy and produce organic matter, which forms the base of the food chain.
3. Bioremediation: Certain types of bacteria and fungi can break down pollutants and contaminants in the environment, a process known as bioremediation. They can detoxify contaminated soil, water, and air, and are thus essential for cleaning up polluted environments.
4. Symbiosis: Many single-celled organisms form symbiotic relationships with other organisms. For example, some bacteria live in the gut of animals and help with digestion, while others live in the roots of plants and help with nutrient absorption.
5. Disease: Some single-celled organisms can cause disease in humans and other animals. Examples include bacteria that cause pneumonia or food poisoning and protozoa that cause malaria or sleeping sickness.

The single-celled organisms are crucial to the functioning of ecosystems and play diverse roles in nutrient cycling, energy production, bioremediation, symbiosis, and disease.

Sterilization

Sterilization is the process of killing or removing all microorganisms from a surface, a material, or a biological medium. There are several sterilization techniques available, each with its own advantages and limitations. Here are some common techniques:

1. Autoclaving: This is a method of sterilization that uses high temperature and pressure to kill microorganisms. Autoclaves are commonly used in hospitals, laboratories, and other settings where sterilization is important.
2. Chemical sterilization: This technique involves the use of chemicals to kill microorganisms. Examples include using ethylene oxide gas or hydrogen peroxide vapor to sterilize medical equipment.
3. Radiation sterilization: This technique involves using high-energy radiation, such as gamma rays or electron beams, to kill microorganisms. This method is often used for sterilizing medical equipment and certain types of food products.
4. Filtration: This technique involves passing a fluid or gas through a filter that traps and removes microorganisms. This method is often used for sterilizing liquids or gases that cannot be autoclaved or chemically sterilized.
5. Dry heat sterilization: This technique involves heating materials to a high temperature, typically between 160-180°C, for a prolonged period of time to kill microorganisms. This method is often used for sterilizing glassware and other heat-resistant materials.

The choice of sterilization technique depends on the nature of the material or surface to be sterilized, the type of microorganisms present, and other factors such as cost and availability of equipment.

Media and its composition

Microbiology is the study of microorganisms, which includes bacteria, viruses, fungi, protozoa, and algae. Media or culture media is a crucial component in microbiology because it provides an environment for the growth and proliferation of microorganisms.

The composition of media used to grow microorganisms in the lab depends on the specific nutritional requirements of the microorganism being cultured. However, there are some general types of media that are commonly used:

1. Nutrient agar: This is a general-purpose medium that supports the growth of a wide range of microorganisms. It is made by dissolving beef extract, peptone, and agar in water.
2. Blood agar: This medium contains sheep blood and is used to grow bacteria that require extra nutrients, such as Streptococcus and Staphylococcus species.
3. MacConkey agar: This is a selective and differential medium that is used to isolate and differentiate Gram-negative bacteria. It contains bile salts and crystal violet, which inhibit the growth of Gram-positive bacteria, and lactose, which allows for the differentiation of lactose-fermenting and non-lactose-fermenting bacteria.
4. Sabouraud agar: This medium is used for the isolation of fungi and yeasts. It contains peptones, glucose, and agar, and has a low pH to inhibit the growth of bacteria.
5. Thioglycollate broth: This medium is used for the growth of anaerobic bacteria. It contains thioglycollate, which removes oxygen from the medium, and

resazurin, which acts as an indicator of oxygen levels.

6. Tryptic soy agar: This medium is a general-purpose medium that is commonly used for the growth of a wide range of bacteria and fungi. It contains tryptone, soy peptone, and agar.
7. Chocolate agar: This medium contains heated blood, which releases factors that promote bacterial growth. It is often used to culture fastidious bacteria, such as *Haemophilus influenzae*.

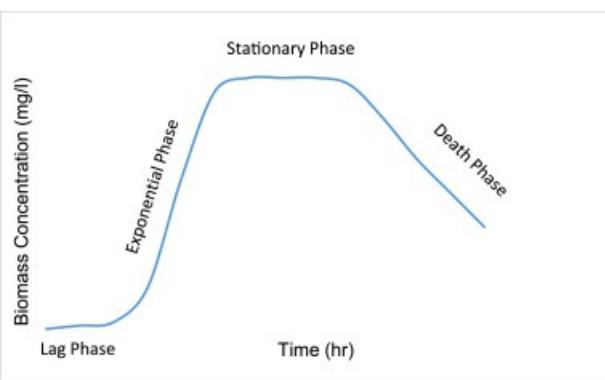
There are many other types of media available, and the choice of medium depends on the specific requirements of the microorganism being cultured.

Growth Kinetics of microorganisms

Growth kinetics of microorganisms refers to the study of how microorganisms grow and reproduce over time in a given environment. This can include the study of the population growth rate, the rate of reproduction, the length of the lag phase (the time it takes for the microorganisms to begin growing), the maximum population density that can be supported by the environment, and the factors that affect these growth parameters.

The growth of microorganisms typically follows a predictable pattern known as the bacterial growth curve, which is divided into several distinct phases:

1. Lag phase: This is a period of adaptation during which the microorganisms are adjusting to their new environment and preparing for growth. There is little or no increase in the population size during this phase.
2. Exponential phase/Log Phase: During this phase, the microorganisms are reproducing at their maximum rate and the population is growing exponentially. This is the phase of most interest to microbiologists, as it is the phase during which the cells are most active and can be most easily studied.
3. Stationary phase: As the environment becomes more crowded and resources become limited, the growth rate of the microorganisms slows down and eventually reaches a plateau. This is the stationary phase, during which the population size remains constant.
4. Death phase: Eventually, the microorganisms will run out of resources and begin to die off. This is the death phase, during which the population size declines.



The growth kinetics of microorganisms can be affected by a wide range of factors, including temperature, pH, nutrient availability, and oxygen levels. By studying the growth kinetics of microorganisms, microbiologists can gain a better understanding of how microorganisms behave in different environments, and can use this knowledge to develop new strategies for controlling their growth in industrial, medical, and environmental settings.

Microbial diseases, epidemiology, and public health

Microbial diseases are diseases that are caused by microorganisms such as bacteria, viruses, fungi, and parasites. These microorganisms can infect humans, animals, and plants, and can cause a range of illnesses from mild to severe.

Some examples of microbial diseases include:

- Bacterial diseases: tuberculosis, strep throat, Lyme disease, meningitis, cholera, pneumonia
- Viral diseases: influenza, HIV/AIDS, hepatitis, measles, chickenpox, Ebola, COVID-19
- Fungal diseases: athlete's foot, ringworm, candidiasis, aspergillosis
- Parasitic diseases: malaria, giardiasis, toxoplasmosis, trichomoniasis, leishmaniasis

Prevention and treatment of microbial diseases depend on the specific microorganism causing the illness. Some preventive measures include practicing good hygiene, getting vaccinated, and avoiding contact with infected individuals. Treatment may involve the use of antibiotics, antiviral drugs, antifungal medications, or antiparasitic drugs, depending on the type of microorganism causing the disease.

These diseases can be spread from person to person, through contact with contaminated surfaces or objects, through the air, or through ingestion of contaminated food or water.

Epidemiology is the study of the distribution and determinants of health-related states or events in populations, and the application of this study to control health problems. In other words, it is the study of how diseases or health conditions spread within populations and what factors contribute to their spread.

Epidemiologists use a variety of methods to investigate the patterns, causes, and effects of health and disease in populations. This includes collecting and analyzing data on the occurrence and distribution of diseases, identifying risk factors and protective factors for specific health conditions, and evaluating interventions to prevent or control diseases.

The field of epidemiology is important for understanding and managing public health issues, such as infectious disease outbreaks, chronic diseases, environmental health hazards, and occupational health concerns. Epidemiological research can inform public health policy, guide the development of effective interventions, and help to reduce the burden of disease on populations.

Public health is the science of protecting and improving the health of communities through education, promotion of healthy behaviors, and research for disease and injury prevention. Public health professionals work to prevent the spread of infectious diseases, promote healthy lifestyles, and ensure access to healthcare services.

The study of microbial diseases, epidemiology, and public health is important in preventing and controlling the spread of infectious diseases. Effective measures such as vaccination, good hygiene practices, and early detection and treatment of infectious diseases can help reduce the burden of these diseases on society.

Human immune mechanism- Types of immunities

Introduction

Immunology is the science that is concerned with immune response to foreign challenges. The term immunity is derived from the Latin term **immunis**, meaning exempt. This overall ability of the host to fight the disease-causing organisms, conferred by the immune system is called immunity. Immune system is a complex system that involves various organs, tissues, cells, and molecules that work together to protect the body from harmful agents such as viruses, bacteria, fungi, and parasites. The term immunity was coined by Burnet.

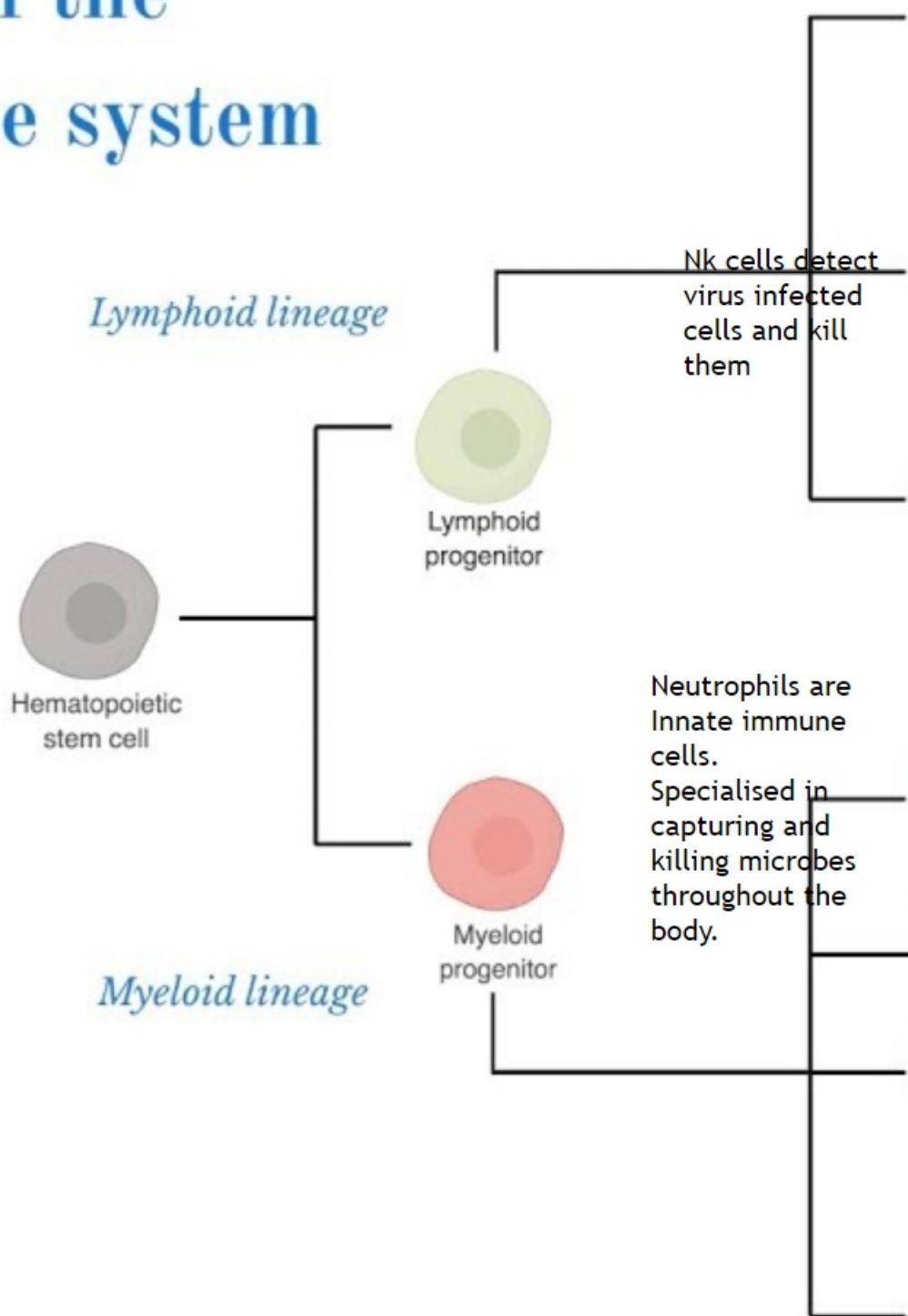
There are two main types of immunity: innate immunity and adaptive immunity. Innate immunity is the first line of defense against pathogens and is present at birth. It includes physical barriers such as the skin, mucous membranes, and secretions, as well as various cells and molecules that can recognize and eliminate pathogens. Adaptive immunity, on the other hand, develops after exposure to a specific pathogen and provides long-term protection against that pathogen. It involves the production of specific antibodies and immune cells that can recognize and eliminate the pathogen if it enters the body again in the future. There are many factors that can affect the immune system, including genetics, age, nutrition, stress, and lifestyle factors such as sleep and exercise. A strong and healthy immune system is essential for overall health and well-being.

Cells of immune system

The immune system is a complex network of cells, tissues, and organs that work together to defend the body against pathogens, foreign substances, and abnormal cells. The cells of the immune system are divided into two main categories: innate immune cells and adaptive immune cells.

Cells of the immune system

In the bone marrow



Innate immune cells are the first line of defense against infections and include:

Neutrophils: They are the most abundant white blood cells and play a key role in the body's defense against bacteria and fungi.

Macrophages: They are phagocytic cells that engulf and digest invading pathogens and debris.

Natural killer cells (NK cells): They are a type of lymphocyte that can identify and kill abnormal cells, including tumor cells and virus-infected cells.

Dendritic cells: They are specialized cells that present antigens to T cells and initiate an adaptive immune response.

Adaptive immune cells are activated by specific antigens and include:

T cells: They are a type of lymphocyte that can recognize and destroy cells that have been infected with viruses or other pathogens.

B cells: They are a type of lymphocyte that produces antibodies that can recognize and bind to specific antigens.

Memory cells: They are long-lived lymphocytes that can recognize and respond to previously encountered antigens, providing long-term immunity.

Plasma cells: They are short-lived cells that produce large quantities of antibodies in response to an antigen.

There are two types of immunity: innate immunity and adaptive immunity.

Innate Immunity: It is the first line of defense against pathogens and is present from birth. It is a non-specific response that provides immediate protection against a wide range of pathogens. Innate immunity includes physical barriers like the skin and mucous membranes, as well as chemical barriers such as stomach acid and enzymes in tears and saliva. Innate immunity also involves white blood cells called phagocytes that engulf and destroy foreign invaders.

Adaptive Immunity: It is a specific response that develops over time as a result of exposure to pathogens. Adaptive immunity involves the production of antibodies that specifically recognize and target the pathogen that triggered their production. It includes two types of responses: humoral immunity and cell-mediated immunity. Humoral immunity involves the production of antibodies by B cells, while cell-mediated immunity involves the activation of T cells that directly attack infected cells.

Overall, the immune system works to protect the body from pathogens and other harmful agents, and both innate and adaptive immunity play critical roles in this process.

Defense Mechanism

Defense mechanism is basically divided into two categories: a. non-specific Defense b. Specific Defense

1. Non-specific Defense Mechanism:

The non-specific defense mechanisms of the immune system, also known as innate immunity, are the body's first line and second line of defense against infections and other foreign substances. These mechanisms are present at birth and do not require previous exposure to the invading pathogen to be activated.

First line of defense mechanisms include:

Physical and chemical barriers: The skin and mucous membranes lining the respiratory, digestive, and urinary tracts form physical barriers that prevent pathogens from entering the body. In addition, these barriers contain antimicrobial substances that kill or inhibit the growth of microorganisms.

Second line of defense mechanism:

The second line of defense of the body is the non-specific or innate immune system, which includes various cells, proteins, and processes that work together to identify and eliminate potential threats to the body. This line of defense is activated when the first line of defense, such as physical barriers like skin or mucous membranes, is breached. The innate immune system includes phagocytes, such as neutrophils and macrophages, which engulf and destroy invading pathogens, as well as natural killer cells, which target and kill infected or abnormal cells. The complement system, a group of proteins that work together to promote inflammation and destroy pathogens, is also part of the innate immune system.

Phagocytosis: Phagocytic cells, such as macrophages and neutrophils, engulf and digest invading microorganisms.

Inflammatory response: When tissues are damaged or infected, the body responds with inflammation, characterized by redness, swelling, heat, and pain. The purpose of inflammation is to isolate and destroy the offending agent, and to promote tissue repair.

Natural killer cells: These cells can recognize and destroy virus-infected cells and cancer cells.

Complement system: This is a series of proteins that work together to destroy pathogens. They can either directly kill pathogens or promote phagocytosis.

Overall, the non-specific defense mechanisms of the immune system provide a rapid and broad-spectrum response to invading pathogens, allowing the body to contain and eliminate infections before they can cause serious harm.

1. Specific defense mechanism

The specific defense mechanism of immunity refers to the immune system's ability to recognize and mount a targeted response against specific foreign substances, known as antigens.

The key players in the specific defense mechanism are lymphocytes, which are specialized white blood cells that can recognize and respond to specific antigens. There are two types of lymphocytes: B cells and T cells.

B cells produce antibodies, which are proteins that can bind specifically to antigens and mark them for destruction by other cells in the immune system. T cells, on the other hand, directly attack cells that are infected or abnormal.

The specific defense mechanism is also characterized by the ability of the immune system to remember previous encounters with antigens. This is known as immunological memory and allows the immune system to respond more quickly and effectively to subsequent exposures to the same antigen.

Hence, the specific defense mechanism of immunity is a highly sophisticated system that plays a critical role in protecting the body from a wide range of pathogens and foreign substances.

Antigen:

It is defined as any foreign substance invading the body and capable of stimulating an immune response.

Or

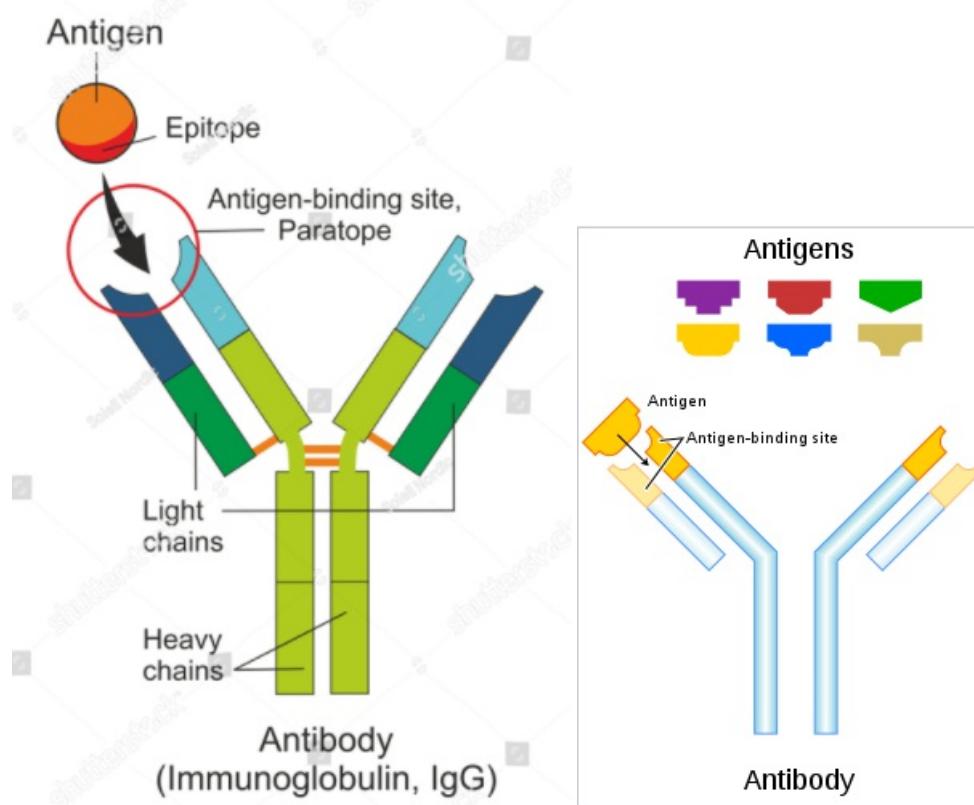
An antigen is any substance that causes your immune system to produce antibodies against it.

An antigen may be a substance from the environment, such as chemicals, bacteria, viruses, or pollen. Antigens can be large protein molecules.

It should have following properties:

- a. High molecular weight.
- b. Immunogenicity: capacity of antigen to induce an immune reaction
- c. Reactivity: capacity of antigen to react with an antibody.
- d. It should be unrelated to the body.

Antigens with both immunogenicity and reactivity are considered complete antigens. Antigens have a specific region on their surface which acts as an antigenic determinant. It is called epitope. Antibodies identify antigens by epitope.



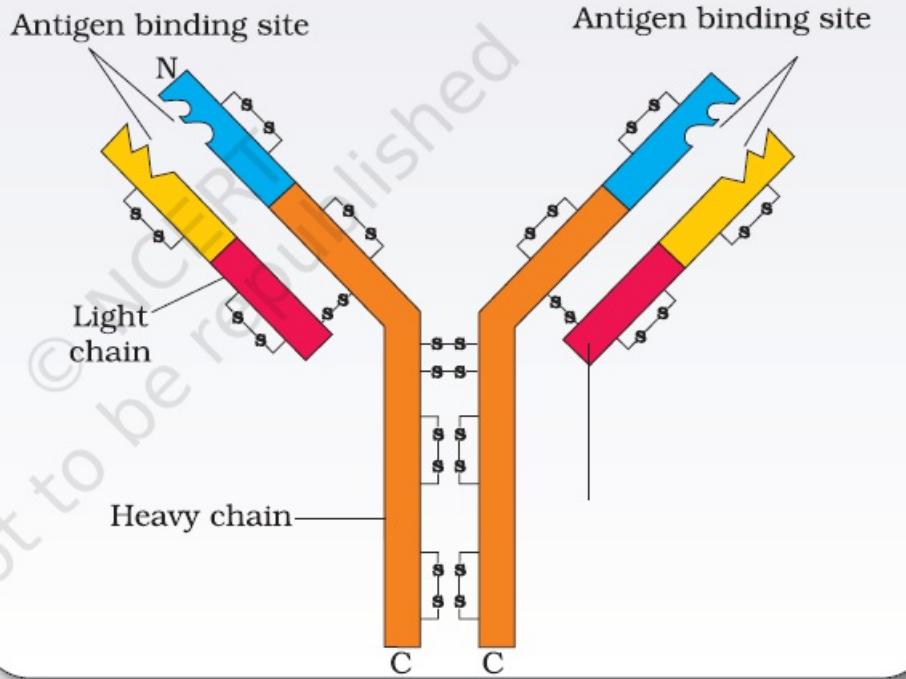
Antibody/Immunoglobulins(Ig)

It is defined as the protective chemical produced by immune cells in response to antigens.

Chemically antibodies are glycoproteins belonging to class of globulin proteins. Antibodies are highly specific to antigens. Antibodies are produced by plasma cells (B lymphocytes). These are also called antibody factories.

Structure of an antibody

- Antibody is a Y-shaped tetrapeptide molecule formed of
- 2 identical light chains (L chains)
- 2 identical heavy chains (H chains).
- The four polypeptide chains are held together by disulfide bonds (-S-S-).
 - Each light chain has a molecular weight of 25,000 Daltons.
 - Each light chain is made up of 214 amino acids.



Light chain & Heavy chain

The amino acid sequence of a light chain is formed of two parts:

- Outer variable region: It varies from one antibody to another.
- Inner constant region: It doesn't change.

The heavy chain is about twice as long as the light chains. The amino acid sequence of a heavy chain is also formed of two parts:

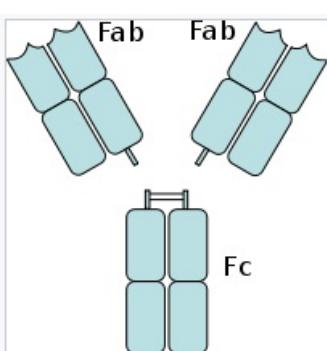
- Outer variable region: It varies from one antibody to another.
- Inner constant region: This region shows a hinge area to provide flexibility to the molecule.

The hinge area allows the antibody to simultaneously bind to two epitopes that are at some distance apart on the surface of antigen. Variable regions of light and heavy regions constitute the antigen-binding site called paratope. It recognizes and binds to the specific antigen forming an antigen-antibody complex.

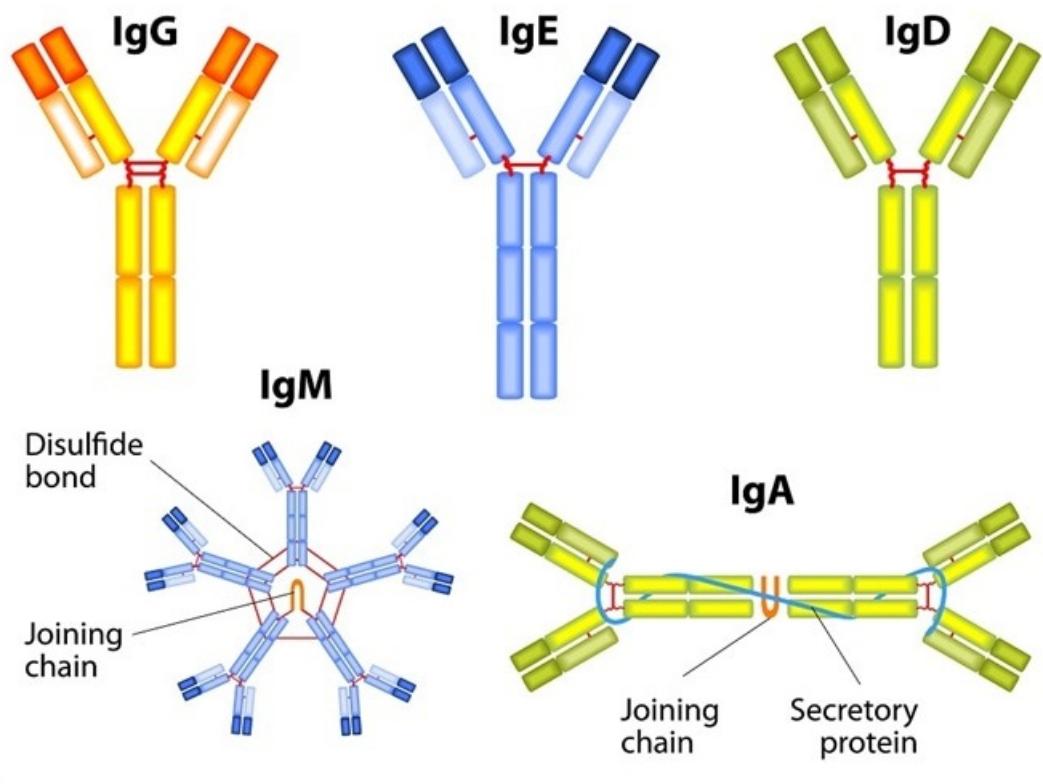
Fab & Fc region: An antibody digested by the enzyme papain (derived from papaya) yields three fragments, two Fab fragments and one Fc fragment.

Fab- Fragment antigen binding- It is made up of the entire light chain and a major part of heavy except the constant region beyond the hinge.

Fc- Fragment crystallisable- It is made up of the constant region of heavy chain.



Types of Antibodies:



There are five different class of antibodies on the basis of amino acid sequence in constant region of heavy chain:

1. Ig G(immunoglobulin γ):

- It is a monomer, the smallest antibody with minimum molecular weight.
- Most abundant antibody (most abundant in blood serum).
- Only antibodies that can pass through the placenta, from mother to fetus.

2. Ig A (immunoglobulin α):

- It is a dimer.
- It is also called secretory antibody.
- Present in colostrum (breast milk), sebum, sweat, semen and tears.
- Gives protection from inhaled and ingested pathogens.

3. Ig E (Immunoglobulin ϵ):

- It is a monomer.
- It is produced during allergic reactions.
- It causes release of histamine.

4. Ig D (Immunoglobulin δ):

- It is a monomer.
- Present on the surface of B cells. It functions as a B cell antigen receptor and may participate in B cell maturation, maintenance, activation and silencing. Exact function is still unclear, it may be involved in humoral immune responses by regulating B cell selection and homeostasis.

5. Ig M (Immunoglobulin μ):

- It is pentamer.
- Largest in size and is also called macroglobulin.
- It is the heaviest antibody which has maximum molecular weight.
- Formed during primary immune response.
- It is produced in response to ABO blood group incompatibility.

Types of immunity based on whether our body produces antibodies or get artificially:

1. Active immunity

a. Naturally acquired

b. Artificially acquired

2. Passive immunity

a. Naturally acquired

b. Artificially acquired

Naturally Acquired Active Immunity:

- It is the immunity produced when active microorganisms (pathogens) from nature enter the body i.e. Natural infection.
- Immune system is stimulated, hence the body produces antibodies.
- Memory-cells are formed, hence it is lifelong immunity.
- Example: a person recovered from measles or chickenpox becomes immune to it for a lifetime.

Artificially Acquired Active Immunity:

- It is produced when microorganisms inactivated by heat/ radiation enter the body (vaccination).
- This artificial preparation of inactive/dead microorganisms is called a vaccine.
- Vaccines are made up of:

a. Dead or alive but attenuated (artificially weakened) pathogens.

b. Toxoids consist of microbial components.

c. Toxins (inactive) secreted by the pathogens.

- Memory-cells are formed; hence immunity persists for a long time.
- Example: B.C.G. vaccine against tuberculosis, polio vaccine.

Naturally Acquired Passive Immunity:

- Newborn infants don't have a well developed immune system.
- Their immune system is not working; hence antibodies are not produced by their body.
- They are protected by maternal antibodies.
- Maternal antibodies reach child in following ways:

1. Before delivery: maternal antibodies (IgG) reach the placenta and enter the child's circulation through the umbilical cord.
2. After delivery: maternal antibodies (IgA) reach the child through colostrum/ first breast milk.

- Memory cells are not produced; hence this immunity is short lived.

Artificially Acquired Passive Immunity:

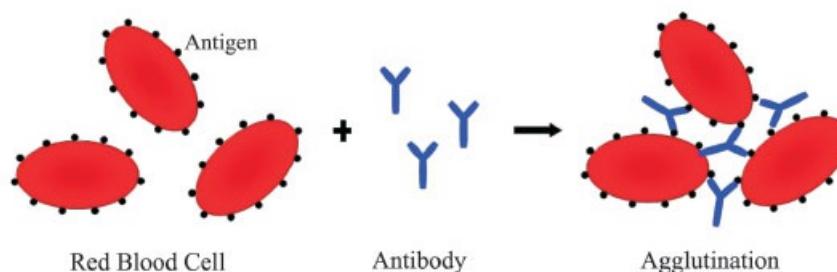
A person suffers from severe disease when his immune system isn't producing antibodies for that particular disease. Hence external antibodies are given in the form of anti-serum. Anti-serum contains antibodies against a particular disease. They may be obtained from humans or other animals.

Following are the examples of antisera:

- Anti rabies serum
- Anti tetanus serum
- Anti diphtheria serum
- Antivenom against snake bite

Antigen-antibody interactions

The antigen-antibody reaction is similar to an enzyme-substrate reaction. It is highly specific in nature. The specificity of antigen-antibody interaction has led to development of a variety of immunogenic assays, which can be used to detect presence of any particular antigen or antibody. A particular antigen binds to a particular antibody at its epitope region. The interaction between antibody and a specific antigen results in visible clumping or agglutination.



Applications of Agglutination reaction:

1. Determining blood typing:

Agglutination reaction is commonly performed to know the blood group.

Blood typing is a medical test that determines a person's blood type. This is important information to know because in certain medical situations, such as blood transfusions or organ transplants, it is necessary to match the blood type of the donor with the blood type of the recipient to avoid serious complications.

There are four main blood types: A, B, AB, and O. Blood type is determined by the presence or absence of certain antigens on the surface of red blood cells, as well as the presence or absence of certain antibodies in the blood plasma.

To determine a person's blood type, a blood sample is collected from the person and tested in a laboratory. The blood is mixed with antibodies that target specific antigens on the surface of red blood cells. If the blood cells clump together (agglutinate), it means that the antigen is present on the cells and the person has that blood type.

For example, if a person's blood sample agglutinates with anti-A antibodies, it means that the person has the A antigen on their red blood cells and their blood type is either A or AB. If the blood sample agglutinates with anti-B antibodies, it means that the person has the B antigen on their red blood cells and their blood type is either B or AB. If the blood sample agglutinates with both anti-A and anti-B antibodies, it means that the person has both A and B antigens on their red blood cells and their blood type is AB. If the blood sample does not agglutinate with either anti-A or anti-B antibodies, it means that the person does not have either A or B antigens on their red blood cells and their blood type is O.

In addition to testing for the A and B antigens, blood typing also involves testing for the presence or absence of the Rh factor, which is another antigen that is either present (+) or absent (-) on the surface of red blood cells. This determines whether a person's blood type is positive or negative, such as A+ or B-.

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	A and B antigens	None

2. Radioimmunoassay (RIA):

It is one of the most sensitive techniques for detecting antigen or antibody.

This technique uses antigens labelled with radioisotopes.

It is useful in measuring hormones, serum proteins, drugs and vitamin concentration in the body.

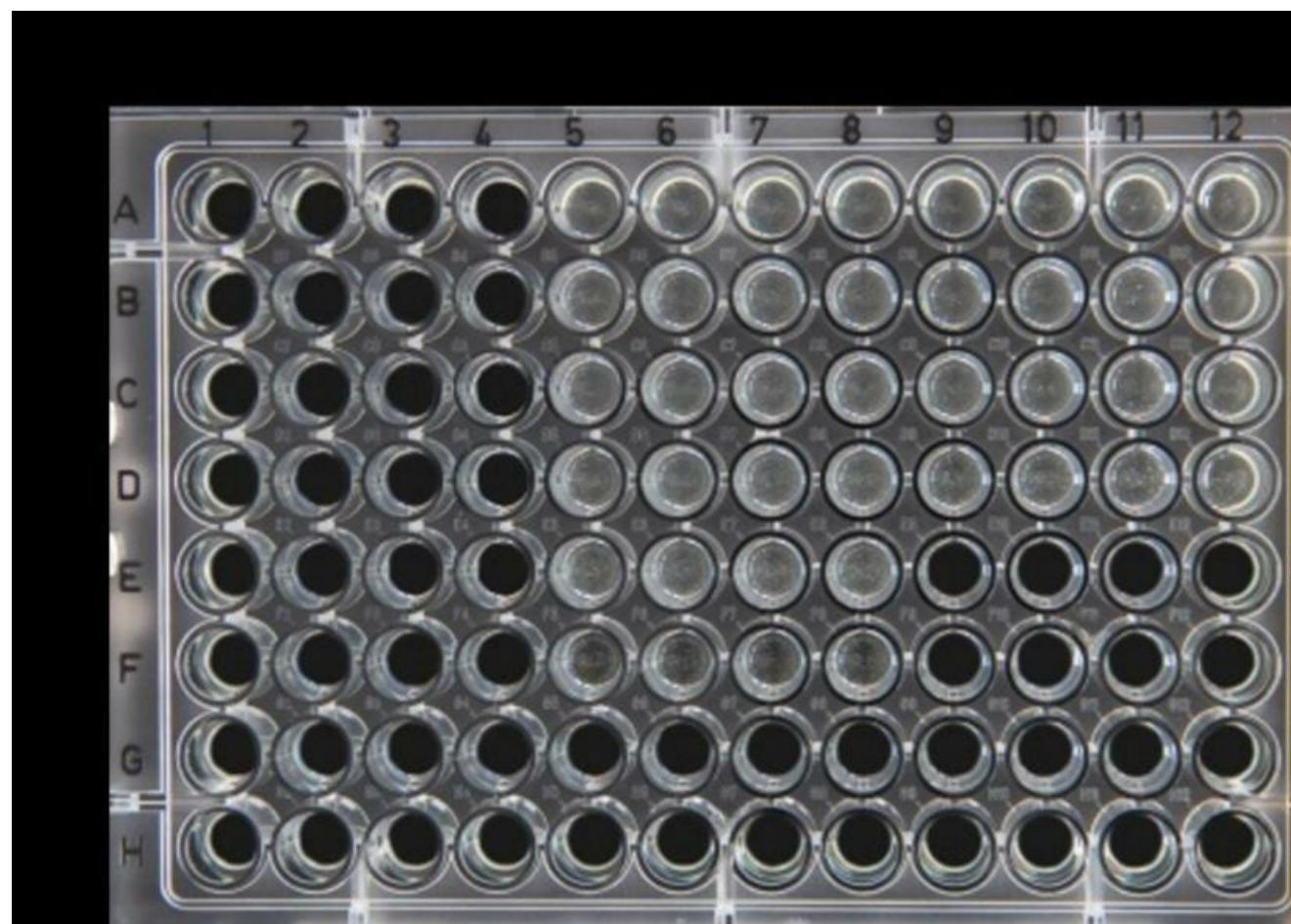
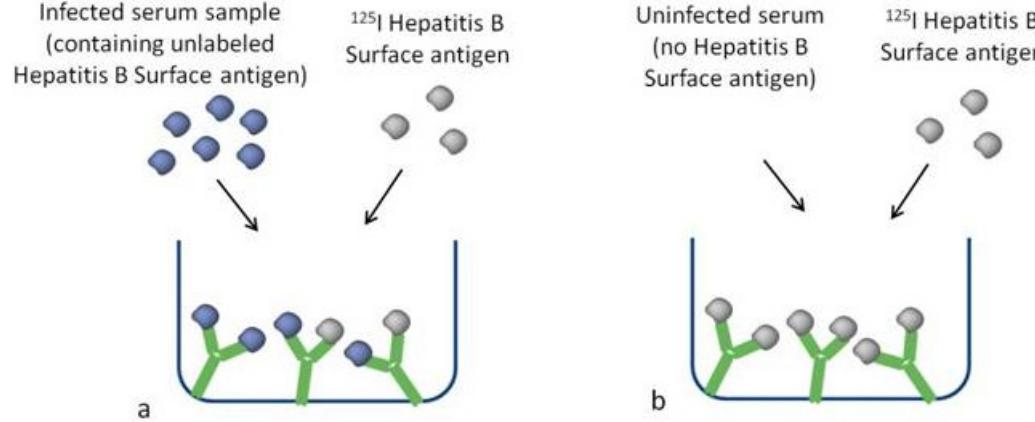


Image: Microtitre plate containing wells. Wells are coated with either an antigen or antibody

A microtitre plate, also known as a microplate or a multiwell plate, is a flat plate with multiple wells or small depressions arranged in a grid pattern. These plates are commonly made of plastic or glass and are used in various laboratory applications, including biomedical research, drug discovery, and diagnostics.

Microtitre plates come in different formats and well sizes, ranging from 6-well to 1536-well plates. The most commonly used formats are 96-well and 384-well plates. Each well can hold a small volume of liquid, usually ranging from a few microliters to a few hundred microliters, depending on the well size.

Microtitre plates are used for high-throughput screening and analysis of large numbers of samples. They can be used for a variety of applications, including enzyme assays, protein-protein interactions, DNA sequencing, and drug discovery. The wells in microtitre plates are typically coated with various reagents, antibodies, or biomolecules to facilitate specific assays or analyses.



Radioimmunoassay (RIA) is a laboratory technique used to measure the concentration of a specific substance in a sample, typically a biological sample such as blood or urine. RIA involves the use of a radioactive tracer molecule and an antibody that binds specifically to the substance of interest.

In a typical RIA, a known amount of the radioactive tracer is mixed with the sample, along with a limited amount of the antibody. The antibody will bind to the substance of interest in the sample and the unbound tracer is separated from the bound tracer. The amount of bound tracer is then measured using a radiation detector.

The concentration of the substance in the sample can then be determined by comparing the amount of bound tracer to a standard curve, which relates the amount of bound tracer to known concentrations of the substance.

RIA has been widely used in medical research and clinical laboratories to measure the concentrations of hormones, drugs, and other small molecules in biological fluids. However, due to the use of radioactive materials, RIA has been largely replaced by non-radioactive methods such as enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA).

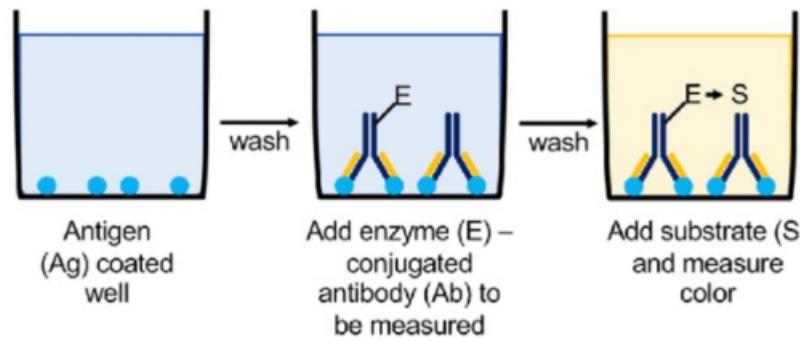
3. Enzyme-linked immunosorbent assay (ELISA):

Enzyme-linked immunosorbent assay, commonly known as ELISA, is a biochemical assay technique used to detect and quantify the presence of specific proteins, antibodies, or antigens in biological samples such as blood, saliva, or urine. It is widely used in research, clinical diagnosis, and quality control applications.

ELISA works by binding the antigen of interest to a solid surface, such as a microplate, and then detecting it using a specific antibody labeled with an enzyme. When the enzyme-labeled antibody binds to the antigen, a reaction is triggered, which produces a detectable signal, such as a change in color or fluorescence.

ELISA is a highly sensitive and specific technique, capable of detecting proteins or antibodies at very low concentrations. It is widely used in the diagnosis of infectious diseases, autoimmune disorders, and cancer, as well as in drug discovery and development.

(a) Direct ELISA



Direct ELISA (Enzyme-Linked Immunosorbent Assay) is a commonly used technique to detect the presence or absence of a specific antigen in a sample. The method is based on the principle of an antigen-antibody interaction, where a specific antibody binds to a target antigen.

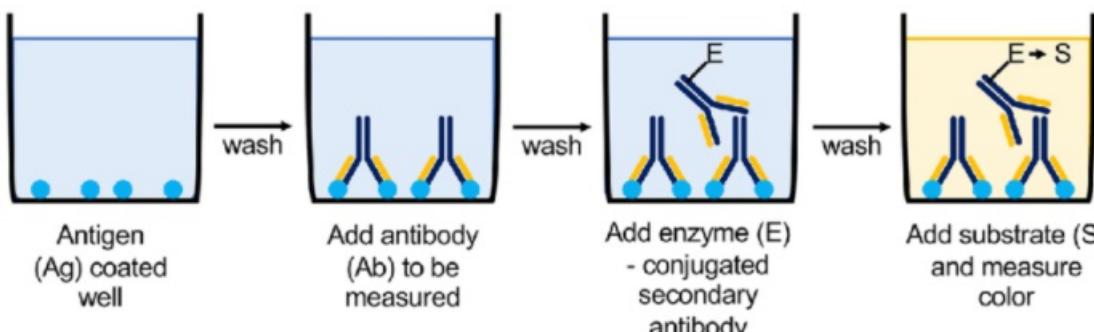
The steps involved in the Direct ELISA technique are as follows:

1. **Plate preparation:** First, a 96-well plate is coated with the antigen of interest. The plate is incubated for some time to allow the antigen to adhere to the wells of the plate.
2. **Blocking:** The plate is then blocked with a blocking solution, such as BSA or milk, to prevent non-specific binding of antibodies.
3. **Addition of primary antibody:** A primary antibody, conjugated to an enzyme such as horseradish peroxidase (HRP), is added to the wells. The secondary antibody recognizes and binds to the primary antibody.

4. Addition of substrate: A substrate solution is added to the wells, and the enzyme-linked to the secondary antibody catalyzes a reaction that produces a detectable signal, such as a color change.
5. Reading the results: The color intensity is measured using a spectrophotometer, and the amount of antigen in the sample is determined by comparing the results to a standard curve.

Direct ELISA is a simple and sensitive technique for the detection of antigens in biological samples, and it is widely used in research and clinical diagnostics.

(b) Indirect ELISA



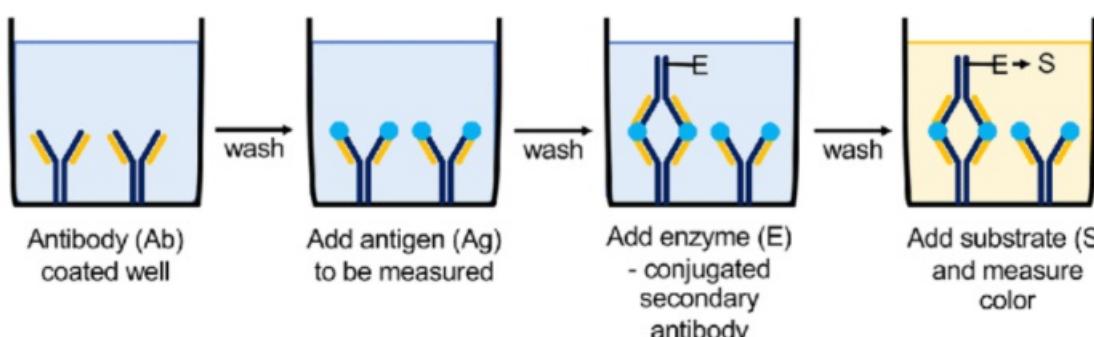
Indirect Enzyme-Linked Immunosorbent Assay (ELISA) is a technique used to detect the presence of antibodies in a sample. It involves immobilizing the antigen of interest onto a solid surface (such as a microplate), incubating the sample (containing antibodies) on the antigen-coated surface, and then detecting the bound antibodies using a secondary antibody that is conjugated with an enzyme.

Here are the general steps involved in an indirect ELISA:

1. Coat a microplate with the antigen of interest (e.g., a protein or peptide).
2. Block any remaining surface area on the microplate with a blocking agent (e.g., BSA or milk) to prevent non-specific binding.
3. Incubate the microplate with the sample (e.g., serum or plasma) containing antibodies that may bind to the antigen.
4. Wash the microplate to remove any unbound sample components.
5. Add a secondary antibody (labeled with an enzyme) that specifically binds to the primary antibody (i.e., the antibody in the sample).
6. Incubate the microplate with a substrate that the enzyme can convert to a detectable signal (e.g., a chromogenic substrate).
7. Measure the signal (e.g., color change or fluorescence) using a spectrophotometer or plate reader.

The signal generated in an indirect ELISA is proportional to the amount of primary antibody bound to the antigen, and therefore indirectly reflects the concentration of the primary antibody in the sample.

(c) Sandwich ELISA



Sandwich ELISA (Enzyme-linked immunosorbent assay) is a type of immunoassay that is used to detect and quantify specific proteins or antigens in a sample. It is called a "sandwich" assay because the protein of interest is captured between two layers of antibodies, one of which is immobilized on a solid surface (such as a microtiter plate) and the other is linked to an enzyme.

The steps involved in a sandwich ELISA include:

1. Coat the microtiter plate wells with a capture antibody that is specific for the protein of interest.
2. Block the remaining surface area of the wells with a blocking solution to prevent non-specific binding.
3. Add the sample containing the protein of interest to the wells, allowing the protein to bind to the capture antibody.
4. Wash the wells to remove any unbound sample components.
5. Add a detection antibody that is specific for a different epitope of the protein of interest. This detection antibody is conjugated to an enzyme, such as horseradish peroxidase.
6. Wash the wells to remove any unbound detection antibody.
7. Add a substrate solution to the wells. The enzyme conjugated to the detection antibody catalyzes a reaction with the substrate to produce a detectable signal, such as a color change.
8. Measure the signal using a spectrophotometer. The amount of signal is proportional to the amount of protein of interest in the sample.

Sandwich ELISA is a widely used technique in many fields of research, including immunology, diagnostics, and drug discovery.

4. Chemiluminescent Immunoassay

Chemiluminescent immunoassay (CLIA) is a type of immunoassay that uses chemiluminescence to detect the presence of an analyte, typically a protein or antibody, in a biological sample. The technique is based on the specific binding of an antibody to its antigen, and involves the use of a chemiluminescent substrate that emits light when it reacts with an enzyme that is conjugated to the antibody. The emitted light is then measured and quantified to determine the concentration of the analyte in the sample.

CLIA has several advantages over other immunoassay techniques, including high sensitivity and specificity, a wide dynamic range, and the ability to detect multiple analytes simultaneously. CLIA is commonly used in clinical laboratories for the diagnosis of infectious diseases, autoimmune disorders, and cancer, as well as for monitoring therapeutic drug levels in patients.

The basic steps involved in a CLIA include sample preparation, incubation with specific antibodies, washing to remove unbound antibodies, and addition of the chemiluminescent substrate. The emitted light is then measured using a luminometer or other detection device, and the concentration of the analyte in the sample is calculated based on a standard curve.

Autoimmune Diseases

Autoimmune diseases are a group of disorders that occur when the immune system mistakenly attacks healthy cells and tissues in the body. The immune system normally defends the body against harmful invaders like bacteria and viruses, but in autoimmune diseases, it can't distinguish between foreign substances and healthy cells. As a result, it produces antibodies that attack and destroy healthy tissues and organs.

There are over 80 different types of autoimmune diseases, each affecting different organs or tissues in the body. Some of the most common autoimmune diseases include:

1. Rheumatoid arthritis:
2. Systemic lupus erythematosus (lupus)
3. Multiple sclerosis
4. Type 1 diabetes
5. Inflammatory bowel disease (IBD)
6. Psoriasis
7. Hashimoto's thyroiditis
8. Graves' disease
9. Sjogren's syndrome
10. Myasthenia gravis

The symptoms of autoimmune diseases can vary depending on the type of disease and the organs or tissues that are affected. Some common symptoms include fatigue, joint pain, skin rashes, fever, and difficulty concentrating. Treatment for autoimmune diseases typically involves medications that suppress the immune system, as well as lifestyle changes such as a healthy diet and regular exercise.

Rheumatoid Arthritis: Rheumatoid arthritis (RA) is a chronic autoimmune disorder that primarily affects the joints. It occurs when the body's immune system mistakenly attacks the lining of the joints, causing inflammation and damage to the joint tissue.

The symptoms of RA typically include joint pain, swelling, and stiffness, particularly in the hands and feet. The symptoms often develop gradually over time and can be accompanied by fatigue, fever, and weight loss. RA can also cause damage to other parts of the body, such as the eyes, lungs, and heart.

There is currently no cure for RA, but there are a variety of treatments available that can help manage the symptoms and slow the progression of the disease. These treatments may include medication, physical therapy, and lifestyle modifications such as exercise and a healthy diet.

It's important for individuals with RA to work closely with their healthcare team to develop an individualized treatment plan that addresses their specific needs and concerns.

Systemic lupus erythematosus (lupus): Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease that can affect many organs and tissues throughout the body. In lupus, the immune system attacks healthy cells and tissues, leading to inflammation and damage.

The symptoms of lupus can vary widely and can be different for each person, but common symptoms include fatigue, joint pain and stiffness, skin rashes, fever, and swollen glands. Lupus can also affect other organs such as the kidneys, heart, lungs, and brain.

The exact cause of lupus is not fully understood, but it is thought to be a combination of genetic and environmental factors. Women are also more likely to develop lupus than men.

There is currently no cure for lupus, but treatments are available to help manage symptoms and prevent damage to organs. Treatment may include medications such as corticosteroids, immunosuppressants, and antimalarials, as well as lifestyle changes such as getting enough rest, exercise, and avoiding triggers that may exacerbate symptoms.

Multiple sclerosis: Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS), which includes the brain, spinal cord, and optic nerves. MS occurs when the immune system attacks the myelin, a protective covering that surrounds nerve fibers, causing inflammation and damage to the nerve fibers themselves.

Symptoms of MS vary widely and can include fatigue, numbness or tingling in the limbs, muscle weakness, blurred or double vision, difficulty with coordination and balance, problems with speech and swallowing, and cognitive impairment.

The cause of MS is not well understood, but it is believed to involve a combination of genetic and environmental factors. MS is not contagious, and it is not directly inherited.

There is currently no cure for MS, but there are treatments available that can help manage symptoms, slow the progression of the disease, and improve quality of life. These treatments can include medications, physical therapy, and lifestyle changes such as regular exercise and a healthy diet.

Type 1 diabetes: Type 1 diabetes, also known as juvenile diabetes or insulin-dependent diabetes, is a chronic autoimmune disease in which the body's immune system attacks and destroys the beta cells in the pancreas that produce insulin. Insulin is a hormone that regulates blood sugar levels and allows the body to use glucose for energy.

Without insulin, the body cannot regulate blood sugar levels properly, and glucose builds up in the bloodstream instead of being used for energy. This can lead to a variety of health problems, including damage to the eyes, kidneys, nerves, and blood vessels.

Type 1 diabetes is typically diagnosed in children and young adults, although it can occur at any age. The exact cause of type 1 diabetes is unknown, but it is thought to involve both genetic and environmental factors.

Treatment for type 1 diabetes involves regular insulin injections or the use of an insulin pump, along with careful monitoring of blood sugar levels and adherence to a healthy diet and exercise regimen. With proper management, people with type 1 diabetes can live long, healthy lives.

Inflammatory bowel disease: Inflammatory bowel disease (IBD) is a group of chronic conditions that cause inflammation in the digestive tract. The two main types of IBD are Crohn's disease and ulcerative colitis.

Crohn's disease can affect any part of the digestive tract, from the mouth to the anus, and causes inflammation that can penetrate the entire thickness of the bowel wall. This can lead to symptoms such as abdominal pain, diarrhea, weight loss, and fatigue.

Ulcerative colitis affects only the colon and rectum, causing inflammation and ulcers in the lining of the colon. Symptoms of ulcerative colitis include abdominal pain, diarrhea, rectal bleeding, and urgency to have a bowel movement.

The exact cause of IBD is not known, but it is believed to involve an abnormal immune response to the bacteria in the digestive tract in genetically susceptible individuals. There is no cure for IBD, but treatments such as medications, dietary changes, and surgery can help manage symptoms and improve quality of life for people with the condition.

Psoriasis: Psoriasis is a chronic autoimmune condition that affects the skin. It causes the skin to become thick, red, and scaly, and is often accompanied by itching and pain. Psoriasis can occur anywhere on the body, but is most commonly found on the scalp, elbows, knees, and lower back.

The exact cause of psoriasis is not known, but it is thought to be related to an immune system malfunction that leads to the rapid growth of skin cells. This causes the buildup of thick, scaly patches on the skin.

There is currently no cure for psoriasis, but there are many treatments available to help manage symptoms. These include topical creams, light therapy, and oral medications. In addition, lifestyle changes such as reducing stress, avoiding triggers, and maintaining a healthy diet and exercise regimen can also help manage psoriasis.

Hashimoto's thyroiditis: Hashimoto's thyroiditis is an autoimmune disorder that affects the thyroid gland, a butterfly-shaped gland located in the neck that produces hormones that regulate the body's metabolism. In Hashimoto's thyroiditis, the immune system attacks the thyroid gland, causing inflammation and damage to the gland over time. This can lead to an underactive thyroid, or hypothyroidism, in which the gland is not producing enough thyroid hormone.

Symptoms of Hashimoto's thyroiditis may include fatigue, weight gain, sensitivity to cold, dry skin, constipation, depression, and a slowed heart rate. Diagnosis typically involves a blood test to measure levels of thyroid hormones and antibodies.

Treatment for Hashimoto's thyroiditis usually involves hormone replacement therapy with synthetic thyroid hormone to bring hormone levels back to normal. In some cases, anti-inflammatory medications may also be prescribed to reduce inflammation in the thyroid gland. It's important for individuals with Hashimoto's thyroiditis to have regular check-ups with their healthcare provider to monitor thyroid hormone levels and adjust treatment as necessary.

Graves' disease: Graves' disease is an autoimmune disorder that causes hyperthyroidism, a condition in which the thyroid gland produces too much thyroid hormone. It is named after the doctor who first described it, Sir Robert Graves.

In Graves' disease, the immune system produces antibodies that stimulate the thyroid gland to produce more thyroid hormone than the body needs. This leads to a variety of symptoms, including weight loss, rapid heartbeat, anxiety, tremors, heat intolerance, and sweating. Graves' disease is also associated with eye problems, such as bulging eyes and double vision.

Treatment options for Graves' disease include medications to reduce the production of thyroid hormone, radioactive iodine to destroy some of the thyroid tissue, and surgery to remove part or all of the thyroid gland. Your doctor will work with you to determine the best treatment approach based on your individual circumstances.

Sjogren's syndrome: Sjogren's syndrome is a chronic autoimmune disorder that affects the moisture-producing glands of the body, such as the salivary and lacrimal glands. This condition can result in dryness of the mouth, eyes, and other parts of the body, as well as fatigue and joint pain.

The exact cause of Sjogren's syndrome is not known, but it is believed to involve a combination of genetic and environmental factors. Women are more likely to develop this condition than men.

The symptoms of Sjogren's syndrome can vary from person to person, but typically include dryness of the mouth and eyes, as well as difficulty swallowing, speaking, or chewing. Other symptoms can include joint pain, swelling, and stiffness, as well as fatigue and skin rashes.

There is currently no cure for Sjogren's syndrome, but treatment options are available to help manage the symptoms. These can include over-the-counter and prescription medications to increase saliva and tear production, as well as lifestyle changes to manage dryness and maintain good oral hygiene. In severe cases, surgery may be necessary to address complications of the condition.

Myasthenia gravis: Myasthenia gravis (MG) is a chronic autoimmune disorder that affects the muscles responsible for movement and breathing. The disorder is caused by an abnormal immune response in which the immune system mistakenly attacks and damages the receptors responsible for transmitting nerve signals to the muscles, leading to muscle weakness and fatigue.

The symptoms of MG typically include muscle weakness and fatigue, particularly in the muscles that control eye movement, facial expression, chewing, swallowing, and speaking. In some cases, the weakness may also affect the limbs and respiratory muscles, leading to difficulty breathing and other serious complications.

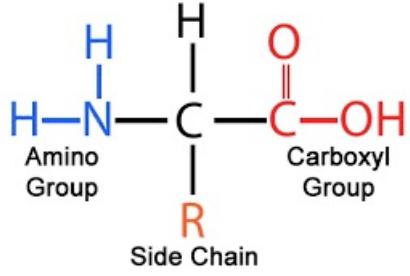
The diagnosis of MG typically involves a thorough medical history and physical exam, as well as blood tests and other diagnostic tests, such as nerve conduction studies and electromyography (EMG). Treatment for MG typically involves medications that help improve muscle strength and reduce symptoms, such as acetylcholinesterase inhibitors, immunosuppressants, and corticosteroids. In severe cases, surgical interventions may also be necessary to remove the thymus gland or to implant a device to help with breathing.

Module 5

- Amino acids & proteins- Classification based on function and structure
- Protein synthesis- components and regulatory mechanism
- Enzymes- An overview
- Carbohydrates and Lipids
- Nucleic Acids
- Basic concepts on Totipotency and cell manipulation
- Biotechnology- Basic concepts of recombinant DNA technology
- Plant & Animal tissue culture- Methods and uses in agriculture, medicine and health
- Biological indicators, Biofuel
- Bio-sensors, Biochips, Nano biomolecules

Amino Acids

Amino acids are organic compounds that are the building blocks of proteins. They contain an amino group (-NH₂) and a carboxyl group (-COOH) attached to a carbon atom, which is also bonded to a side chain (R-group). The side chain gives each amino acid its unique chemical and physical properties.



There are 20 different amino acids that are commonly found in proteins, and they can be classified based on the functional groups present in their side chains. These functional groups include aliphatic, aromatic, acidic, basic, sulfur-containing, and hydroxyl-containing groups.

1. Aliphatic amino acids:

These amino acids have side chains that are composed of only carbon and hydrogen atoms. The aliphatic amino acids can be further divided into four categories: glycine, alanine, valine, and leucine. Glycine is the only amino acid that does not have a chiral carbon atom and has a hydrogen atom as its side chain.

1. Aromatic amino acids:

These amino acids have an aromatic ring in their side chain. There are three aromatic amino acids: phenylalanine, tyrosine, and tryptophan. Phenylalanine and tyrosine have a phenyl ring in their side chain, while tryptophan has an indole ring.

1. Acidic amino acids:

These amino acids have a carboxyl group (-COOH) in their side chain, which makes them acidic. There are two acidic amino acids: aspartic acid and glutamic acid. The carboxyl group in these amino acids can donate a proton, making them negatively charged at physiological pH.

1. Basic amino acids:

These amino acids have a nitrogen-containing group in their side chain, which makes them basic. There are three basic amino acids: lysine, arginine, and histidine. The side chain of lysine contains an amino group (-NH₂), while the side chains of arginine and histidine contain guanidine (-NH-C(=NH)-NH₂) and imidazole (-C₃H₄N₂) groups, respectively.

1. Sulfur-containing amino acids:

These amino acids have a sulfur atom in their side chain. There are two sulfur-containing amino acids: cysteine and methionine. Cysteine contains a thiol (-SH) group in its side chain, which can form disulfide bonds with other cysteine residues in proteins. Methionine contains a sulfur-containing methyl group (-CH₂-S-CH₃) in its side chain and is the only amino acid that contains a non-polar sulfur atom.

1. Hydroxyl-containing amino acids:

These amino acids have a hydroxyl (-OH) group in their side chain. There are two hydroxyl-containing amino acids: serine and threonine. Serine has a hydroxyl

group attached to a carbon atom in its side chain, while threonine has a hydroxyl group attached to a carbon atom that is also bonded to a methyl group (-CH₃).

The classification of amino acids based on their functional groups provides insight into their chemical and physical properties, which can have important implications for protein structure and function. Understanding these properties is crucial for the design and synthesis of novel proteins with specific functionalities.

Amino acids are the building blocks of proteins and are essential for many functions in the body, such as building muscle, repairing tissue, and synthesizing enzymes and hormones. There are 20 different amino acids that the body needs to function properly. Of these 20, 9 are considered essential amino acids, and 11 are considered non-essential amino acids. Essential amino acids are those that the body cannot produce on its own, and therefore must be obtained through the diet. These include:

1. Histidine
2. Isoleucine
3. Leucine
4. Lysine
5. Methionine
6. Phenylalanine
7. Threonine
8. Tryptophan
9. Valine

Non-essential amino acids are those that the body can produce on its own, and therefore do not need to be obtained through the diet. These include:

1. Alanine
2. Arginine
3. Asparagine
4. Aspartic acid
5. Cysteine
6. Glutamic acid
7. Glutamine
8. Glycine
9. Proline
10. Serine
11. Tyrosine

While non-essential amino acids can be produced by the body, they are still important for maintaining good health and supporting various bodily functions.

Proteins are large, complex molecules that are essential to the structure and function of all living organisms. They are made up of long chains of smaller units called amino acids, which are linked together by peptide bonds.

Proteins serve a variety of functions in the body, including:

- Enzymes: Proteins act as enzymes, which are specialized molecules that catalyze biochemical reactions in the body.
- Structural support: Some proteins provide structural support for cells and tissues, such as collagen in skin and bone.
- Transport: Proteins can act as transporters to move molecules across cell membranes or within the bloodstream.
- Defense: Antibodies, which are proteins, help to defend the body against foreign invaders such as viruses and bacteria.
- Hormones: Some proteins act as hormones, which are signaling molecules that regulate various physiological processes.
- Energy: In times of starvation or prolonged exercise, the body can break down proteins to provide energy.

Proteins are synthesized in cells through a process called protein synthesis, which involves the transcription of DNA to RNA and the translation of RNA to protein. The sequence of amino acids in a protein is determined by the genetic code, and changes in this sequence can lead to protein dysfunction and disease.

Chemical properties of proteins

The chemical properties of proteins are determined by the chemical nature of the amino acid residues that make up the protein chain, as well as the three-dimensional structure of the protein. Some of the key chemical properties of proteins include:

1. Acidity and Basicity: Proteins can act as both acids and bases, due to the presence of acidic and basic amino acid residues in the protein chain. The pH at which a protein is most stable is called its isoelectric point (pI), which is determined by the balance between the acidic and basic residues in the protein.
2. Hydrophobicity and Hydrophilicity: The amino acid residues in a protein can be classified as either hydrophobic (water-repelling) or hydrophilic (water-attracting), depending on their chemical properties. The distribution of hydrophobic and hydrophilic residues in a protein is important for determining its solubility and interaction with other molecules.
3. Reactivity: Proteins can undergo a variety of chemical reactions, such as oxidation, reduction, and hydrolysis, which can affect their structure and function. For example, the oxidation of certain amino acid residues in a protein can lead to the formation of disulfide bonds, which can stabilize the protein structure.
4. Binding Specificity: Proteins can interact with other molecules, such as enzymes with their substrates, antibodies with antigens, and receptors with ligands. The binding specificity of a protein is determined by its three-dimensional structure and the chemical properties of its amino acid residues.
5. Conformational Stability: The three-dimensional structure of a protein is critical for its function, and is determined by the interactions between its amino acid residues. Changes in the environment, such as changes in temperature or pH, can disrupt these interactions and lead to changes in the protein structure, which can affect its function.

Physical properties of proteins

Proteins have a variety of physical properties that determine their function and behavior. Here are some of the most important physical properties of proteins:

1. Size: Proteins vary in size from small peptides containing just a few amino acids to large, multi-subunit complexes composed of thousands of amino acids.
2. Shape: Proteins have a specific 3D structure that is essential for their function. The shape of a protein is determined by the sequence of amino acids that make up the protein and the interactions between those amino acids.
3. Solubility: Proteins can be either soluble or insoluble in water, depending on their amino acid composition and overall 3D structure. Solubility is an important property of proteins because it affects their ability to interact with other molecules.
4. Stability: Proteins can be stable or unstable, depending on their structure and the environment in which they are found. Factors that can affect protein stability include pH, temperature, and the presence of other molecules.
5. Flexibility: Proteins are not rigid structures and can exhibit different levels of flexibility. Some proteins are highly flexible, allowing them to adapt to different environments and interact with a variety of other molecules.
6. Optical properties: Some proteins can absorb and emit light, making them useful as fluorescent probes for biological studies. Proteins that can absorb light are often colored, such as the green fluorescent protein (GFP).
7. Electrical properties: Proteins have electrical charges due to the presence of charged amino acids in their structure. These charges can affect the protein's solubility, stability, and ability to interact with other molecules.

Overall, the physical properties of proteins are critical for their function in biological systems, and understanding these properties is essential for advancing our knowledge of the molecular basis of life.

Protein synthesis- components and regulatory mechanism

Protein synthesis is the process by which cells make proteins, which are essential molecules for the structure, function, and regulation of the body. The process involves the translation of the genetic information stored in DNA into a functional protein.

The key components involved in protein synthesis are:

1. DNA - the genetic material that contains the instructions for making proteins
2. Messenger RNA (mRNA) - a single-stranded RNA molecule that carries the genetic information from DNA to the ribosome for protein synthesis.
3. Ribosomes - the cellular organelles that serve as the site of protein synthesis.
4. Transfer RNA (tRNA) - small RNA molecules that transport amino acids to the ribosome, where they are incorporated into a growing protein chain.

The regulatory mechanism of protein synthesis involves several steps, including transcription, RNA processing, translation initiation, elongation, and termination. The regulation of protein synthesis can occur at each step, and the process is tightly controlled to ensure that the right proteins are made at the right time and in the right amounts.

Some of the regulatory mechanisms include:

1. Transcription factors - proteins that bind to specific DNA sequences and regulate the rate of transcription.
2. RNA processing - the modification of mRNA after transcription, which can affect the stability, localization, and translation of the mRNA.
3. Ribosome biogenesis - the regulation of the number and activity of ribosomes.
4. Translation initiation factors - proteins that regulate the binding of mRNA to the ribosome and the recruitment of the initiator tRNA.
5. mRNA stability - the regulation of the lifespan of mRNA molecules.
6. Post-translational modifications - the modification of proteins after synthesis, which can affect their activity, localization, and stability.

Protein synthesis is a highly regulated process that ensures that the correct proteins are produced in the right amounts and at the right time to support the proper functioning of the cell and the organism as a whole.

The two main events of protein synthesis

1. Transcription:

Transcription is the first stage of protein synthesis, and it takes place in the nucleus of eukaryotic cells. The process of transcription begins when an enzyme called RNA polymerase binds to a specific region of the DNA known as the promoter region. The promoter region is the place where transcription starts, and it helps to recruit RNA polymerase to the correct location on the DNA.

Once RNA polymerase is bound to the promoter region, it begins to move along the DNA strand, unwinding the double helix and using one of the DNA strands as a template to make an RNA copy. The RNA polymerase adds nucleotides one at a time to the growing RNA chain, using complementary base pairing to ensure that the correct sequence is formed.

As the RNA polymerase moves along the DNA strand, the newly formed RNA strand begins to detach from the DNA, and the DNA strand is rewound. The RNA polymerase continues to add nucleotides to the RNA chain until it reaches a specific sequence of DNA bases known as the termination sequence. When the RNA polymerase reaches this sequence, it stops transcription, and the newly formed RNA molecule is released.

1. Translation:

Translation is the second stage of protein synthesis, and it takes place in the cytoplasm of the cell. The process of translation begins when the mRNA molecule formed during transcription binds to a ribosome. A ribosome is a large complex made up of proteins and RNA molecules that helps to translate the genetic information in mRNA into a protein.

The mRNA molecule is read in groups of three nucleotides called codons. Each codon codes for a specific amino acid, which is the building block of proteins. The ribosome reads the codons in the mRNA molecule and matches them to the appropriate amino acid.

Once the ribosome has matched a codon to an amino acid, it adds the amino acid to a growing chain of amino acids, called a polypeptide chain. The ribosome continues to move along the mRNA molecule, adding amino acids one at a time to the polypeptide chain until it reaches a stop codon. The stop codon signals the end of the protein synthesis process, and the newly formed polypeptide chain is released.

Post-Translational Modifications:

After the protein is synthesized, it undergoes several modifications to become fully functional. These modifications may include folding, cleaving, and adding chemical groups to the protein. The modifications are essential for the protein to function correctly and carry out its specific role in the cell.

Conclusion:

In summary, protein synthesis is a complex process that involves two main stages, transcription and translation. During transcription, the genetic information in DNA is used to make an RNA copy of the gene, while in translation, the RNA is used to synthesize a protein. The process of protein synthesis is regulated and finely tuned to ensure that proteins are produced at the right time and in the right amount to carry out their specific functions in the cell.

Enzymes- An overview

Enzymes are biological molecules that catalyze, or speed up, chemical reactions in living organisms. They are essential for life and play a critical role in nearly all cellular processes, including metabolism, DNA replication, and protein synthesis.

Enzymes are typically proteins, although some RNA molecules, known as ribozymes, also exhibit enzymatic activity. They are highly specific in their function, meaning that each enzyme catalyzes a particular chemical reaction or group of related reactions.

Enzymes work by lowering the activation energy required for a chemical reaction to occur. This allows reactions to take place at a faster rate and under milder conditions than they would without the enzyme. Enzymes are highly efficient catalysts and can accelerate chemical reactions by factors of up to a million or more.

Enzymes are named based on the type of reaction they catalyze. For example, hydrolases catalyze the breakdown of molecules through the addition of water, while dehydrogenases catalyze the removal of hydrogen atoms.

Enzymes are typically regulated through a variety of mechanisms, including feedback inhibition, allosteric regulation, and covalent modification. Feedback inhibition occurs when the end product of a metabolic pathway binds to and inhibits the activity of an enzyme earlier in the pathway, thereby regulating the rate of the pathway as a whole. Allosteric regulation occurs when a molecule binds to a site on the enzyme that is distinct from the active site, causing a conformational change that either activates or inhibits the enzyme. Covalent modification involves the addition or removal of a chemical group, such as a phosphate group, to or from the enzyme, which can either activate or inhibit its activity.

Enzymes are involved in a wide range of biological processes. Some of the most well-known enzymes include:

Proteases: These enzymes catalyze the breakdown of proteins into smaller peptides and amino acids. Proteases are essential for digestion, as well as for the turnover of proteins within cells.

Lipases: These enzymes catalyze the breakdown of fats and oils into fatty acids and glycerol. Lipases are important for the digestion of dietary fats and for the release of stored fats from adipose tissue.

Amylases: These enzymes catalyze the breakdown of starch into glucose. Amylases are found in saliva and pancreatic juice and are essential for the digestion of carbohydrates.

DNA polymerases: These enzymes catalyze the replication of DNA during cell division. DNA polymerases are highly accurate and are able to replicate DNA with a fidelity of one error in every billion base pairs.

RNA polymerases: These enzymes catalyze the synthesis of RNA from DNA. RNA polymerases are essential for gene expression and are regulated in a highly specific manner to ensure that the correct genes are expressed at the appropriate times.

Enzymes are also used extensively in industry and biotechnology. For example, enzymes are used in the production of a wide range of products, including food, pharmaceuticals, and biofuels. Enzymes are also used in diagnostic tests to detect diseases, and in the development of therapeutic drugs that target specific enzymes or enzyme pathways.

Despite their importance, enzymes are not without their limitations. Enzymes are highly specific in their function, which can make them difficult to engineer for new applications. Enzymes can also be inhibited by a variety of compounds, including drugs and toxins, which can have negative effects on health and the environment.

In summary, enzymes are essential biological molecules that catalyze chemical reactions in living organisms. They are highly specific and efficient catalysts that play critical roles in nearly all cellular processes. Enzymes are regulated through a variety of mechanisms and are involved in a wide range of biological and industrial applications.

Carbohydrates

Carbohydrates are one of the three macronutrients, along with protein and fat, that provide energy for the body. Carbohydrates are made up of carbon, hydrogen, and oxygen, and can be classified as either simple or complex.

Simple carbohydrates are made up of one or two sugar molecules, and are often referred to as "sugar" or "sweets". Examples of simple carbohydrates include table sugar, honey, and fruit.

Complex carbohydrates are made up of long chains of sugar molecules, and are often referred to as "starches". Examples of complex carbohydrates include whole grains, vegetables, and legumes.

Carbohydrates are an important source of energy for the body, and can be used for immediate energy or stored in the muscles and liver for later use. However, not all carbohydrates are created equal, and it's important to choose carbohydrates that are high in fiber and nutrients, rather than those that are high in sugar and low in nutrients.

Carbohydrates are a class of organic compounds that contain carbon, hydrogen, and oxygen in the ratio of 1:2:1. They are the most abundant biomolecules on Earth and serve as a primary source of energy for living organisms. The physical and chemical properties of carbohydrates include:

1. Solubility: Most carbohydrates are soluble in water due to their polar nature. However, some complex carbohydrates like cellulose are insoluble in water.
2. Sweetness: Simple carbohydrates like glucose, fructose, and sucrose are sweet in taste. The sweetness of carbohydrates increases with an increase in the number of monosaccharide units.
3. Melting and Boiling Point: Carbohydrates have high melting and boiling points due to their extensive hydrogen bonding.
4. Optical Activity: Carbohydrates are optically active due to the presence of chiral carbon atoms in their structure. They rotate the plane of polarized light either to the left (levorotatory) or to the right (dextrorotatory).
5. Reducing properties: Carbohydrates with free carbonyl groups, such as glucose and maltose, can act as reducing agents. They can reduce other compounds like Benedict's reagent and Fehling's solution.
6. Polymerization: Carbohydrates can undergo condensation reactions to form larger molecules. This process is called polymerization. Examples of carbohydrate polymers include starch, glycogen, and cellulose.
7. Reactivity: Carbohydrates can undergo various chemical reactions, such as oxidation, hydrolysis, and esterification.

Carbohydrates are a diverse group of biomolecules with many physical and chemical properties that allow them to serve a variety of functions in living organisms.

Carbohydrates are classified into several categories based on their chemical structure and the number of sugar molecules they contain. Here are some of the common classifications of carbohydrates:

1. Monosaccharides: These are the simplest carbohydrates and consist of a single sugar molecule.

Monomers of carbohydrates, monosaccharides, are the simplest form of 3 types of carbohydrates.

They (mono- = “one”; sacchar- = “sweet”) are simple sugars, the most common of which is glucose.

Most monosaccharide names end with the suffix -ose.

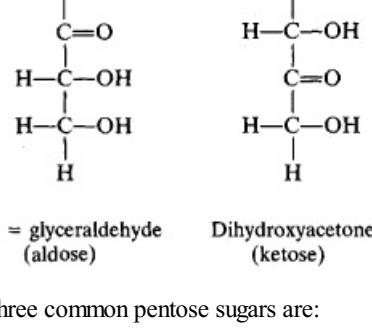
If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose, if it has a ketone group (the functional group with the structure R-C(=O)-R'), it is known as a ketose.

In monosaccharides, the number of carbons usually ranges from three to seven.

Depending on the number of carbons in the sugar, they also may be known as trioses (three carbons), pentoses (five carbons), and hexoses (six carbons).

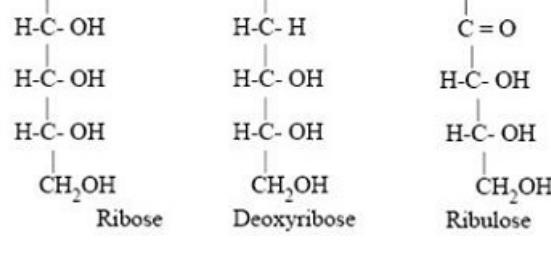
The two simplest monomers of carbohydrates are:

1. dihydroxyacetone (a triose with a ketone group),
2. glyceraldehyde (a triose with an aldehyde group).



Three common pentose sugars are:

1. ribose (a component of RNA)
2. deoxyribose (a sugar in DNA)
3. ribulose (used in photosynthesis)



Three important hexoses include glucose, fructose, and galactose.

The structure of glucose:

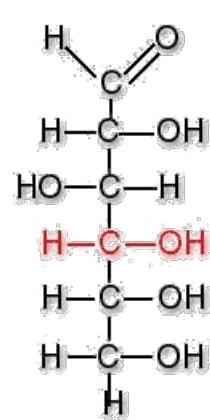
Glucose is a six-carbon sugar with the chemical formula C₆H₁₂O₆. Its structure can be represented as a linear chain or a cyclic structure. In its cyclic form, glucose exists in two isomeric forms: alpha-glucose and beta-glucose. The cyclic structure of glucose is formed by the reaction of the hydroxyl group on the fifth carbon atom with the aldehyde group on the first carbon atom.

The structure of fructose:

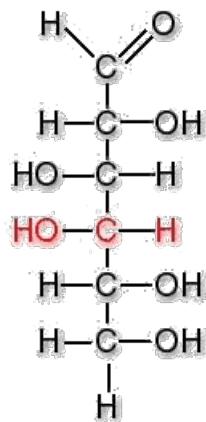
Fructose is also a six-carbon sugar with the same chemical formula C₆H₁₂O₆. It has a cyclic structure that is formed by the reaction of the hydroxyl group on the fifth carbon atom with the carbonyl group on the second carbon atom. The cyclic structure of fructose exists in two isomeric forms: alpha-fructose and beta-fructose.

The structure of galactose:

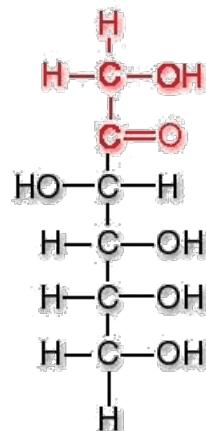
Galactose is a six-carbon sugar with the same chemical formula C₆H₁₂O₆ as glucose and fructose. It has a cyclic structure that is similar to glucose, but the hydroxyl group on the fourth carbon atom is oriented in the opposite direction. The cyclic structure of galactose also exists in two isomeric forms: alpha-galactose and beta-galactose.



Glucose



Galactose



Fructose

1. Disaccharides: These are carbohydrates made up of two sugar molecules linked together. Examples include sucrose (glucose + fructose), lactose (glucose + galactose), and maltose (glucose + glucose).

1) Maltose

Chemical structure of maltose is composed of two α - ring structures of glucose molecules held together by a 1-4 glycosidic linkage.

Maltose can be found in grains which are used in the production of beer.

2) Sucrose

Sucrose molecular structure consists of α - ring structure of glucose and α - ring structure of fructose with a 1-2 glycosidic linkage between them.

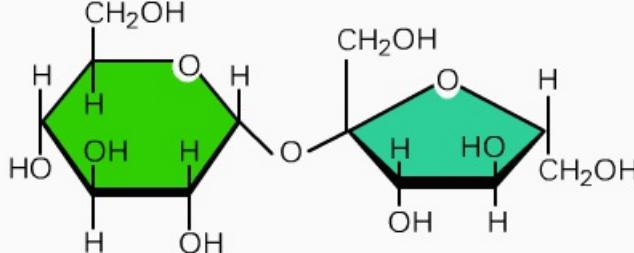
Sucrose is the most common table sugar.

3) Lactose

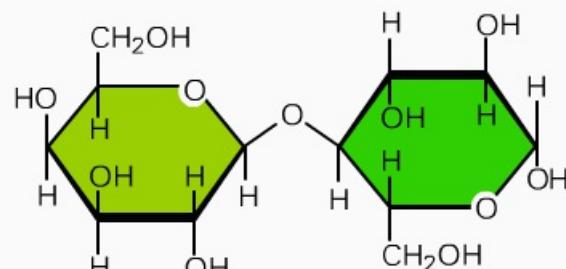
Molecular structure of lactose is composed of such monomers of carbohydrates as α - ring structure of glucose and α - ring structure of galactose.

Lactose is normally found in milk.

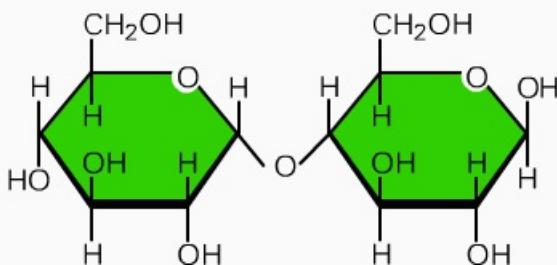
Sucrose
(glucose and fructose)



Lactose
(galactose and glucose)



Maltose
(glucose and glucose)



1. Oligosaccharides: These are carbohydrates made up of 3 to 10 sugar molecules linked together. Examples include raffinose and stachyose.
2. Polysaccharides: These are carbohydrates made up of many sugar molecules linked together. They are usually classified as either structural or storage carbohydrates.
3. Structural polysaccharides: These provide support and structure to cells and tissues. Examples include cellulose, chitin, and peptidoglycan.
4. Storage polysaccharides: These are used to store energy in plants and animals. Examples include starch (found in plants) and glycogen (found in animals).

Polysaccharides are the type of carbohydrate polymers that are made up of several hundred to several thousand monomers of carbohydrates - monosaccharides held together by glycosidic linkages.

Some complex carbohydrate polymers are straight chains, and some are branched.

Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Starch

Starch is the stored form of carbohydrate polymers in plants and is made up of a mixture of amylose and amylopectin (both polymers of glucose).

Starch is made up of monomer of carbohydrates - glucose that are joined by α 1-4 or α 1-6 glycosidic bonds.

The numbers 1-4 and 1-6 refer to the carbon number of the two residues that have joined to form the bond.

Amylose is starch formed by unbranched chains of glucose monomers (only α 1-4 linkages), whereas amylopectin is a branched polysaccharide (α 1-6 linkages at the branch points).

Glycogen

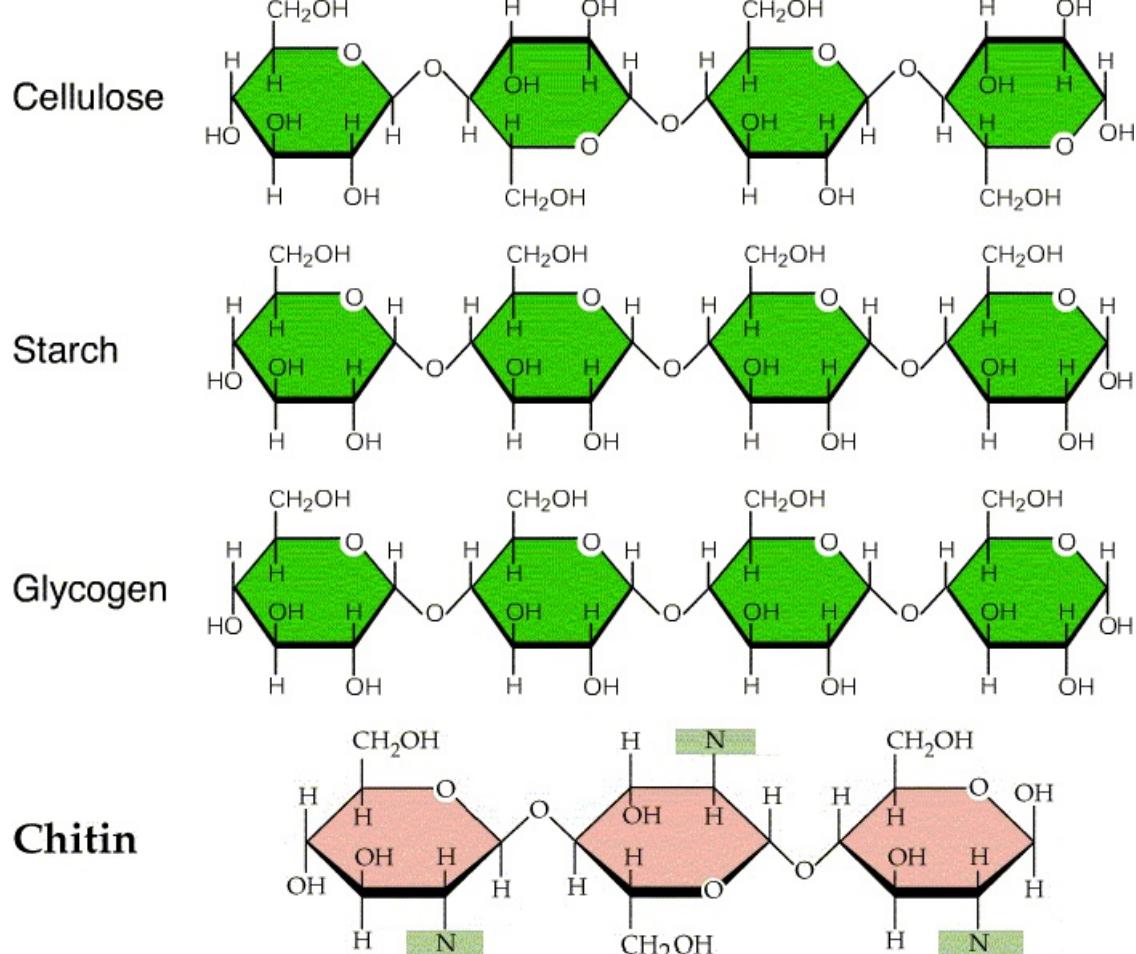
Glycogen is the storage form of glucose in humans and other vertebrates and is made up of monomers of glucose.

Cellulose

Cellulose is the primary structural polysaccharide in all plants, and is a major component in cell walls. It is a straight chain polymer of β - ring structure of glucose that is held together by 1-4 glycosidic linkages.

Every other glucose monomer in cellulose is flipped over, and the monomers are packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength - which is so important to plant cells.

While the β 1-4 linkage cannot be broken down by human digestive enzymes, herbivores such as cows, koalas, buffalos, and horses are able, with the help of the specialized flora in their stomach, to digest plant material that is rich in cellulose and use it as a food source.



Chitin

A cellulose-like polymer exists in the hard exoskeleton of insects, crustaceans. This polymer is known as chitin, which is a polysaccharide-containing nitrogen.

It is made of repeating units of N-acetyl- β -D-glucosamine, a modified monomer of carbohydrates - glucose.

Chitin is also a major component of fungal cell walls.

1. **Glycoconjugates:** These are carbohydrates that are covalently attached to lipids or proteins. Examples include glycoproteins, glycolipids, and proteoglycans.

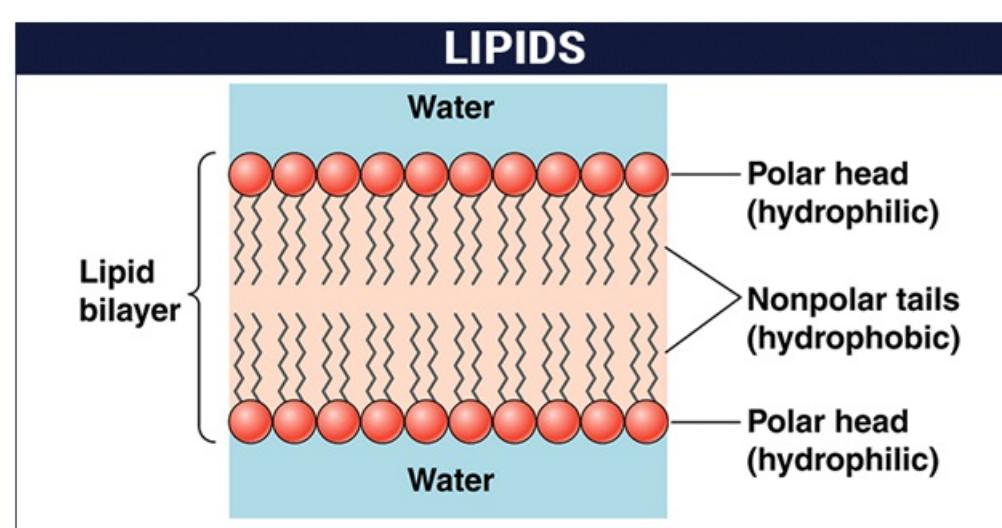
Simple and Complex Carbohydrates: Another way of classifying carbohydrates is based on their glycemic index and the rate at which they are absorbed by the body. Simple carbohydrates are rapidly absorbed and have a high glycemic index, while complex carbohydrates are slowly absorbed and have a low glycemic index. Examples of simple carbohydrates include sugar, honey, and fruit juice, while complex carbohydrates include whole grains, fruits, and vegetables.

Lipids

Lipids are organic compounds that contain hydrogen, carbon, and oxygen atoms, which form the framework for the structure and function of living cells.

These organic compounds are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water because water is a polar molecule. In the human body, these molecules can be synthesized in the liver and are found in oil, butter, whole milk, cheese, fried foods and also in some red meats.

Let us have a detailed look at the lipid structure, properties, types and classification of lipids.



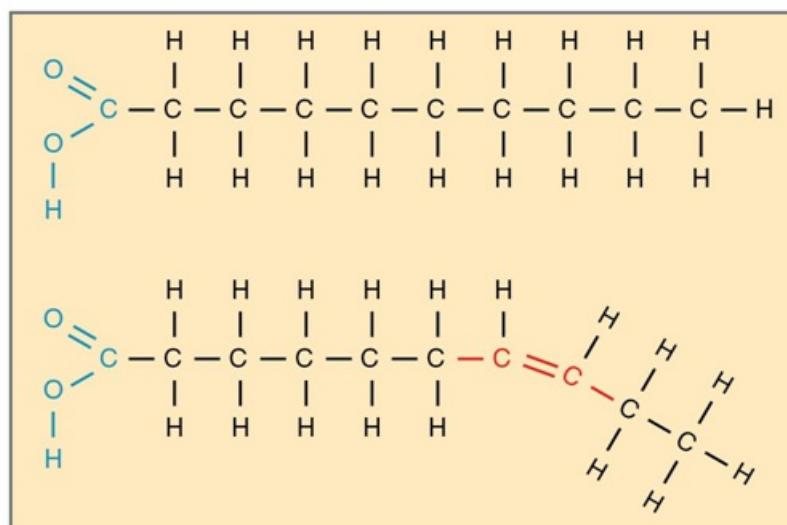
Properties of Lipids

Lipids are a family of organic compounds, composed of fats and oils. These molecules yield high energy and are responsible for different functions within the human body. Listed below are some important characteristics of Lipids.

1. Lipids are oily or greasy nonpolar molecules, stored in the adipose tissue of the body.
2. Lipids are a heterogeneous group of compounds, mainly composed of hydrocarbon chains.
3. Lipids are energy-rich organic molecules, which provide energy for different life processes.
4. Lipids are a class of compounds characterised by their solubility in nonpolar solvents and insolubility in water.
5. Lipids are significant in biological systems as they form a mechanical barrier dividing a cell from the external environment known as the cell membrane.

Lipid Structure

Lipids are the polymers of fatty acids that contain a long, non-polar hydrocarbon chain with a small polar region containing oxygen. The lipid structure is explained in the diagram below:



Classification of Lipids

Lipids can be classified into two main classes:

- Nonsaponifiable lipids
- Saponifiable lipids

Nonsaponifiable Lipids

A non saponifiable lipid cannot be disintegrated into smaller molecules through hydrolysis. Nonsaponifiable lipids include cholesterol, prostaglandins, etc.

Saponifiable Lipids

A saponifiable lipid comprises one or more ester groups, enabling it to undergo hydrolysis in the presence of a base, acid, or [enzymes](#), including waxes, triglycerides, sphingolipids and phospholipids.

Further, these categories can be divided into non-polar and polar lipids.

Nonpolar lipids, namely triglycerides, are utilized as fuel and to store energy.

Polar lipids, that could form a barrier with an external water environment, are utilized in membranes. Polar lipids comprise sphingolipids and glycerophospholipids.

Fatty acids are pivotal components of all these lipids.

Types of Lipids

Within these two major classes of lipids, there are numerous specific types of lipids, which are important to life, including fatty acids, triglycerides, glycerophospholipids, sphingolipids and steroids. These are broadly classified as simple lipids and complex lipids.

Simple Lipids

Esters of fatty acids with various alcohols.

1. Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state
2. Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols

Complex Lipids

Esters of fatty acids containing groups in addition to alcohol and fatty acids.

1. Phospholipids: These are lipids containing, in addition to fatty acids and alcohol, phosphate groups. They frequently have nitrogen-containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
2. Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine and carbohydrate.
3. Other complex lipids: Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category.

Precursor and Derived Lipids

These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesterol esters are termed neutral lipids. These compounds are produced by the hydrolysis of simple and complex lipids.

Some of the different types of lipids are described below in detail.

Fatty Acids

Fatty acids are carboxylic acids (or organic acid), usually with long aliphatic tails (long chains), either unsaturated or saturated.

- Saturated fatty acids

Lack of carbon-carbon double bonds indicate that the fatty acid is saturated. The saturated fatty acids have higher melting points compared to unsaturated acids of the corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape.

- Unsaturated fatty acids

Unsaturated fatty acid is indicated when a fatty acid has more than one double bond.

“Often, naturally occurring fatty acids possess an even number of carbon atoms and are unbranched.”

On the other hand, unsaturated fatty acids contain a cis-double bond(s) which create a structural kink that disables them to group their molecules in straight rod-like shape.

Role of Fats

Fats play several major roles in our body. Some of the important roles of fats are mentioned below:

- Fats in the correct amounts are necessary for the proper functioning of our body.
- Many fat-soluble vitamins need to be associated with fats in order to be effectively absorbed by the body.
- They also provide insulation to the body.
- They are an efficient way to store energy for longer periods.

Nucleic Acids

Nucleic acids are biomolecules that store and transmit genetic information. There are two main types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

DNA is a double-stranded nucleic acid that carries genetic information in the form of a sequence of nucleotide bases. These bases are adenine (A), thymine (T), cytosine (C), and guanine (G). The sequence of these bases determines the genetic code, which contains instructions for the development, function, and reproduction of all living organisms.

DNA is found in the nucleus of cells and is organized into structures called chromosomes. The human genome contains approximately 3 billion base pairs of DNA, which are organized into 23 pairs of chromosomes.

DNA replication is the process by which cells copy their DNA before cell division. During replication, the two strands of DNA separate, and each strand serves as a template for the synthesis of a new complementary strand. This process ensures that each new cell receives a complete copy of the genetic information.

DNA also serves as a template for the synthesis of RNA, which is involved in protein synthesis. The genetic code is read by the ribosome, which translates the sequence of RNA nucleotides into a sequence of amino acids, the building blocks of proteins.

DNA is a vital molecule that plays a crucial role in the development, function, and reproduction of all living organisms.

Types of RNA

There are several types of RNA (ribonucleic acid), each with different functions in the cell. Here are some of the most important types:

Messenger RNA (mRNA): mRNA carries genetic information from DNA in the nucleus to ribosomes in the cytoplasm, where it is translated into proteins.

Transfer RNA (tRNA): tRNA is responsible for carrying amino acids to the ribosome during protein synthesis, where they are assembled into polypeptide chains according to the instructions encoded in the mRNA.

Ribosomal RNA (rRNA): rRNA is a structural component of ribosomes, which are the cellular structures that carry out protein synthesis. It helps to catalyze the

formation of peptide bonds between amino acids.

Small nuclear RNA (snRNA): snRNA is involved in RNA splicing, the process by which introns (non-coding regions) are removed from pre-mRNA before it is translated into protein.

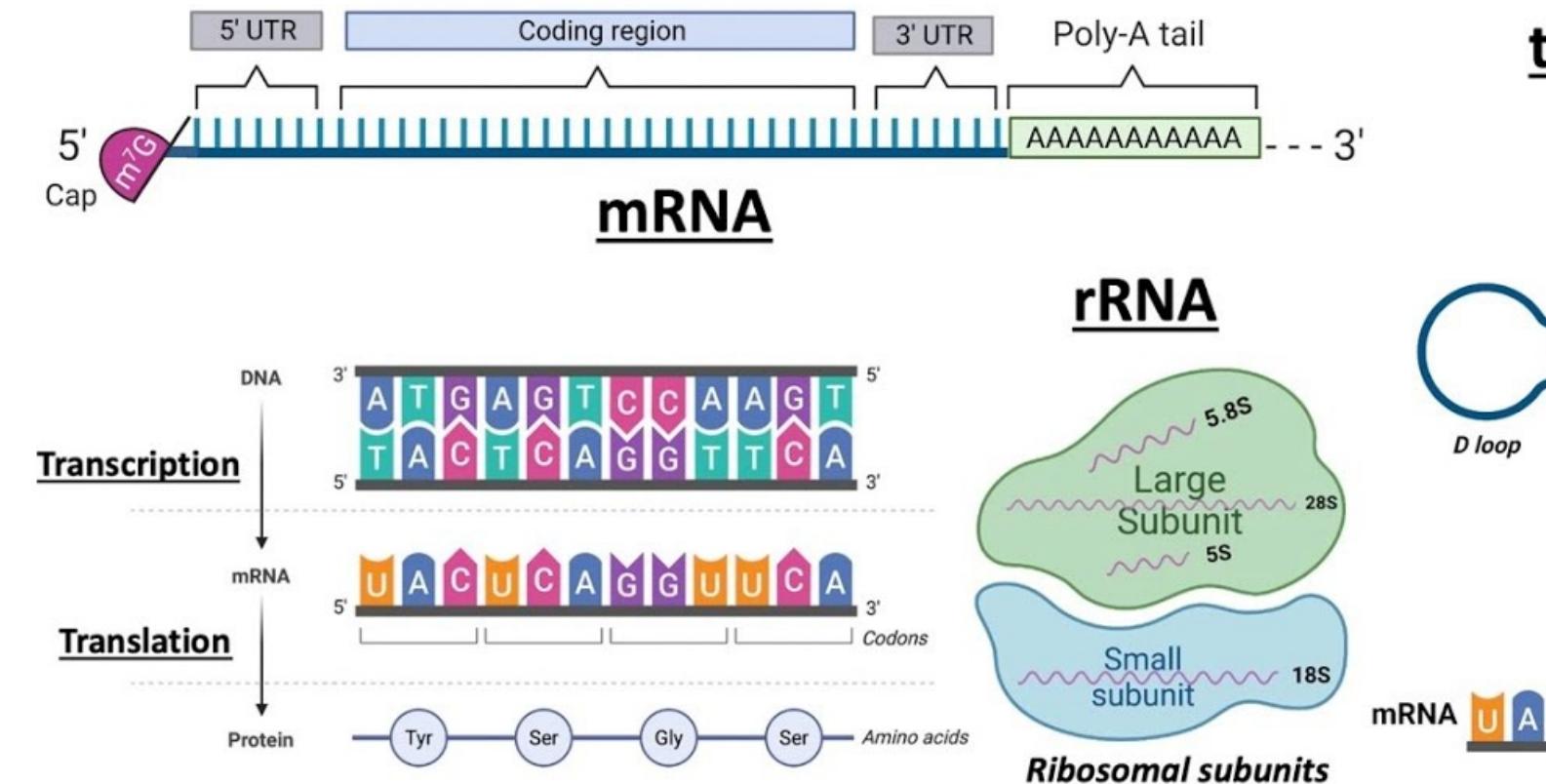
Small nucleolar RNA (snoRNA): snoRNA plays a role in modifying rRNA, which is important for proper ribosome function.

MicroRNA (miRNA): miRNA regulates gene expression by binding to complementary sequences in mRNA, causing it to be degraded or preventing it from being translated into protein.

Long non-coding RNA (lncRNA): lncRNA is a large and diverse group of RNA molecules that do not encode proteins, but instead play important roles in regulating gene expression and other cellular processes.

What are different types of RNA?

mRNA, tRNA and rRNA – Structure and Function



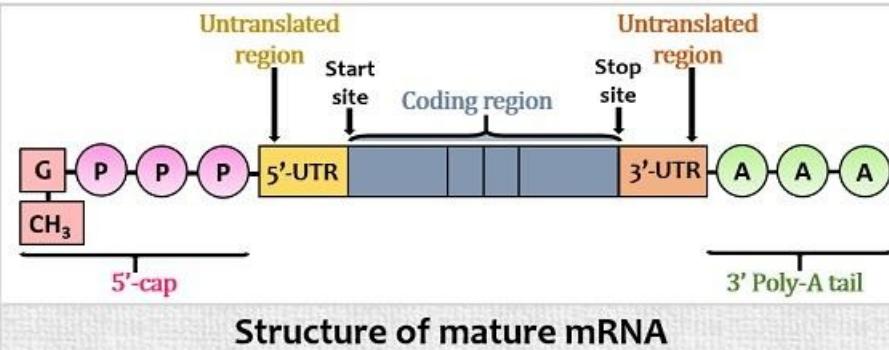
mRNA

mRNA stands for messenger RNA. It is a type of RNA molecule that carries genetic information from the DNA in the cell nucleus to the ribosomes in the cytoplasm, where it is translated into proteins.

During transcription, RNA polymerase enzyme binds to a specific gene on the DNA molecule and copies the DNA sequence into a pre-mRNA molecule. This pre-mRNA molecule then undergoes a process called splicing, in which non-coding regions called introns are removed and the remaining coding regions called exons are joined together to form a mature mRNA molecule.

The mature mRNA molecule is then transported out of the nucleus and into the cytoplasm, where it binds to ribosomes and the process of translation begins. The ribosomes read the sequence of nucleotides in the mRNA molecule and use this information to synthesize a specific protein.

mRNA plays a critical role in the central dogma of molecular biology, which describes how genetic information flows from DNA to RNA to proteins. It is also a key component of many modern vaccines, including those developed for COVID-19, as it can be used to instruct cells to produce specific proteins that can stimulate an immune response.



BIOLOGY READER

rRNA

rRNA stands for ribosomal RNA. It is a type of RNA (ribonucleic acid) molecule that is a major component of ribosomes, the cellular structures responsible for protein synthesis.

There are three main types of rRNA in prokaryotes (bacteria) and eukaryotes (organisms with cells containing a nucleus and other membrane-bound organelles): 16S rRNA, 23S rRNA, and 5S rRNA. In eukaryotes, there is an additional rRNA called 28S rRNA.

rRNA plays a crucial role in the process of translation, which is the conversion of the genetic code carried by mRNA (messenger RNA) into a specific sequence of amino acids to form a protein. During translation, ribosomes use rRNA to help match tRNA (transfer RNA) molecules carrying specific amino acids to the codons (three-nucleotide sequences) on the mRNA. The amino acids are then joined together to form a protein chain.

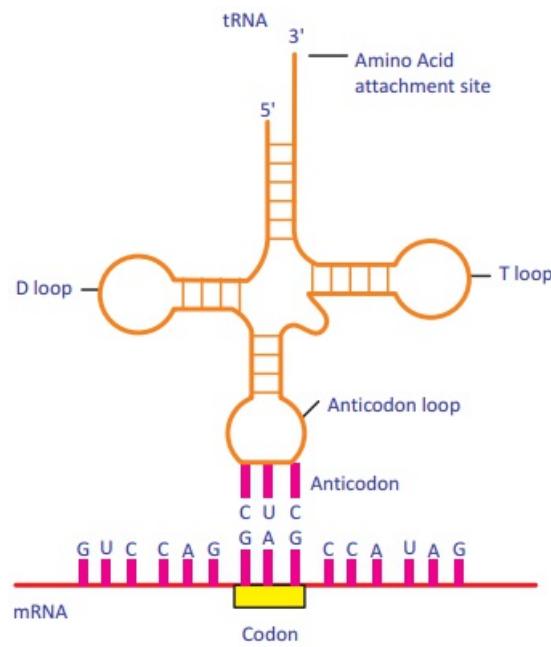
Because rRNA is highly conserved and evolves slowly, it is often used in molecular biology research to study the evolutionary relationships among organisms. This is because rRNA sequences can provide a useful measure of the degree of relatedness between different species or groups of organisms.

tRNA

tRNA stands for transfer RNA. It is a type of RNA molecule that plays a key role in the process of translation, which is the synthesis of proteins in the cell. tRNA is responsible for carrying amino acids to the ribosome, the molecular machine that assembles proteins.

Each tRNA molecule has a specific sequence of three nucleotides called the anticodon, which matches up with a complementary sequence of three nucleotides, called the codon, on the mRNA molecule that is being translated. Each amino acid is attached to a specific tRNA molecule through a process called aminoacylation, in which an enzyme known as an aminoacyl-tRNA synthetase attaches the appropriate amino acid to the tRNA molecule with a high degree of specificity.

During translation, tRNA molecules with their attached amino acids move to the ribosome and bind to the mRNA molecule in a specific order, dictated by the sequence of codons on the mRNA. As each tRNA molecule binds to the ribosome, the amino acid that it carries is added to the growing protein chain. This process continues until the ribosome reaches a stop codon on the mRNA molecule, at which point the protein is released and folds into its functional form.



Basic concepts on Totipotency and cell manipulation

Totipotency refers to the ability of a single cell to give rise to all the cell types in an organism, including both the embryonic and extra-embryonic tissues. This means that a totipotent cell has the ability to differentiate into any cell type, as well as to self-renew, or make copies of itself.

Totipotent cells are typically found in early embryos, before the formation of the blastocyst, which is the stage at which embryonic stem cells (ESCs) are derived. The totipotent cells in the embryo are capable of giving rise to all the different cell types that make up the embryo and the placenta, which is why they are sometimes referred to as "pluripotent".

Cell manipulation refers to the ability to modify or manipulate cells in order to achieve a desired outcome. This can be done in a number of ways, including genetic engineering, cell culture, and tissue engineering. Cell manipulation has many applications in medicine, biotechnology, and basic research.

Genetic engineering involves the alteration of an organism's DNA in order to change its characteristics. This can be done using a variety of techniques, including gene editing using CRISPR/Cas9, RNA interference, and gene therapy.

Cell culture involves growing cells outside of the body in a controlled environment. This allows researchers to study cell behavior, test drugs and treatments, and develop cell-based therapies.

Tissue engineering involves growing tissues or organs *in vitro* using a combination of cells, scaffolds, and growth factors. This has the potential to revolutionize medicine by allowing for the replacement of damaged or diseased tissues with functional, lab-grown organs.

Biotechnology- Basic concepts of recombinant DNA technology

Biotechnology is the controlled use of living organisms, proteins, enzymes, or any other parts for human benefits.

Or

Biotechnology is "the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogs for products and services.

Károly Ereky (German: Karl Ereky; 20 October 1878 – 17 June 1952) was a Hungarian agricultural engineer. The term 'biotechnology' was coined by him in 1919. He is known as the father of Biotechnology.

Paul Berg is considered as the father of Genetic Engineering.

Recombinant DNA technology, also known as genetic engineering, is a set of techniques used to manipulate and modify DNA molecules in order to produce new proteins or to modify the characteristics of an organism.

The basic steps of recombinant DNA technology include:

1. Isolation of DNA: DNA is extracted from the cells of an organism, purified and then amplified using polymerase chain reaction (PCR) if necessary.
2. Cutting DNA: The DNA is then cut into smaller pieces using restriction enzymes, which are enzymes that recognize specific DNA sequences and cut the DNA at those points.
3. Insertion of DNA: A DNA fragment containing a gene of interest is then inserted into a vector, which is typically a plasmid or a virus. The vector is used to transport the DNA fragment into the host cell.
4. Transformation: The host cell is then transformed with the vector containing the DNA fragment, allowing the DNA to integrate into the host cell's genome.
5. Selection and Screening: The transformed cells are then selected and screened for the presence of the desired gene or protein.
6. Expression: Finally, the gene is expressed, and the protein product is produced.

Recombinant DNA technology has numerous applications in fields such as medicine, agriculture, and biotechnology. For example, it can be used to produce human insulin for the treatment of diabetes, develop new drugs, improve crop yields, and create genetically modified organisms with desirable traits. However, it also raises ethical concerns, and its use is subject to regulatory oversight in many countries.

Tools of rDNA Technology

- Desired gene or fragment
- Restriction enzymes
- Polymerase enzymes
- Ligases
- Vectors
- Host organisms

Restriction enzymes

Restriction enzymes are the enzymes produced by certain bacteria that have the property of cleaving DNA molecules at or near specific base sequences.

Restriction enzymes are of two types: a. Restriction exonuclease b. Restriction exonuclease

Restriction endonucleases are commonly used in rDNA Technology.

These enzymes are also known as molecular scissors. These enzymes always cut double stranded DNA molecules at a particular point by recognizing a specific palindromic sequence of 6-7 base pairs. This specific base sequence is known as the recognition sequence. This specific base sequence is known as the recognition sequence. More than 800 different restriction enzymes that recognize and cut DNA at more than 100 different sequences have been isolated from bacteria. Some examples of restriction endonuclease are: EcoRI, BamHI, HindII.

A palindrome is a word or sequence that reads the same backward as forward. E.g. MADAM

5' — GAATTC — 3'
3' — CTTAAG — 5'

In a palindromic DNA sequence the following sequences reads

the same on the two strands in the $5' \rightarrow 3'$ direction. This is also true if read in the $3' \rightarrow 5'$ direction.

Nomenclature:

EcoRI: A restriction enzyme

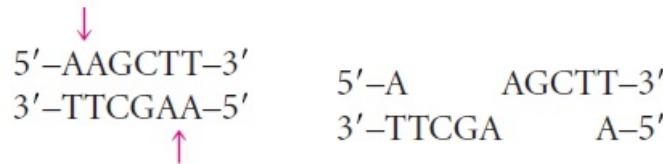
Genus name-Escherichia

Species name-col*i*

Strain name- R

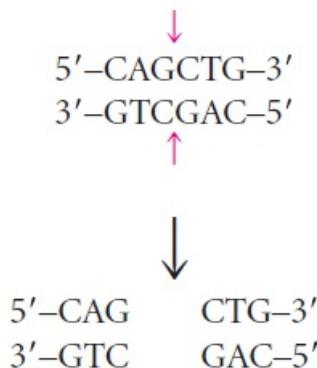
Order of identification-1

The restriction endonucleases cut the DNA in two ways:



- Such ends are called cohesive ends or sticky ends. These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

Some restriction enzymes cut in the middle of its recognition site, producing blunt-ended fragments.



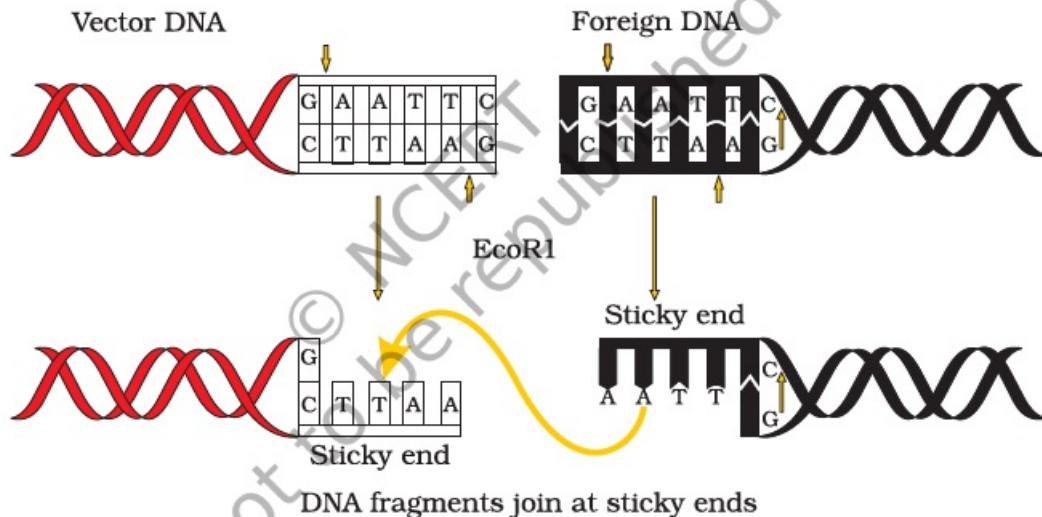
Unless one cuts the vector and the source DNA with the same restriction enzyme, the recombinant vector molecule cannot be created.

Enzyme	Microorganism from Which Enzyme Is Produced	Recognition Sequence	Type of Fragment End Produced
BamHI	<i>Bacillus amyloliquefaciens</i>	5'-GGATCC-3' 3'-CCTAGG-5' ↓ ↑	Cohesive
CofI	<i>Clostridium formicoaceticum</i>	5'-GCGC-3' 3'-CGCG-5' ↓ ↑	Cohesive
EcoRI	<i>Escherichia coli</i>	5'-GAATTC-3' 3'-CTTAAG-5' ↓ ↑	Cohesive
EcoRII	<i>Escherichia coli</i>	5'-CCAGG-3' 3'-GGTCC-5' ↓ ↑	Cohesive

Action of Restriction enzyme

The enzyme cuts both DNA strands at the same site

EcoRI cuts the DNA between bases G and A only when the sequence GAATTC is present in the DNA



Ligases: These are the molecular adhesives.

- These are also known as Synthetase.
- A DNA ligase forms phosphodiester bonds between adjacent molecules.
- It is generally used to join two DNA fragments.
- Most common ligases are DNA ligases.

Polymerase enzyme

- These are the enzymes that catalyze the synthesis of DNA polymers.
- There is also present RNA polymerase which catalyses the synthesis of RNA polymers
- Polymerases responsible for DNA repair function by replacing damaged DNA with a newly synthesized strand to correct the defect.
- There are various types of polymerase such as DNA polymerase I,

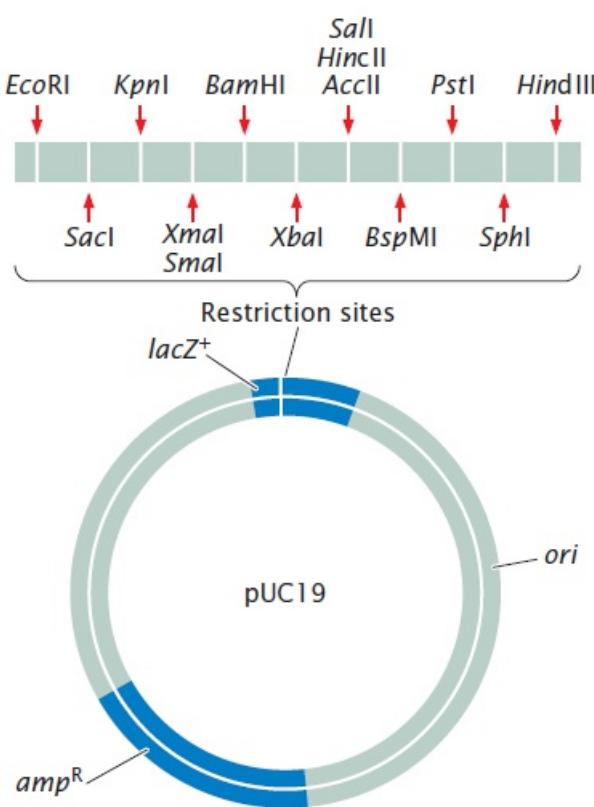
DNA polymerase II, DNA polymerase III, DNA polymerase IV, DNA polymerase V.

Cloning vector:

- A cloning vector is a stable, replicating DNA molecule to which a foreign DNA fragment can be attached for introduction into a cell.
- An effective cloning vector has three important characteristics:
 - **Origin of replication**
 - **Selectable markers**
 - **One or more restriction site**
- **Origin of replication (ori):** It is a particular sequence at which replication is initiated.
- **Selectable markers:** It identifies the bacterial cell that contains the desired recombinant DNA.
- **One or more restriction site** into which a DNA fragment can be inserted. The restriction sites used for cloning must be unique.
- The most commonly used vectors are plasmids.
 - Some other types of vector are bacteriophages, cosmids, phagemid, transposons.

Plasmid

- A plasmid is a small, circular, double-stranded, extra chromosomal DNA molecule that is distinct from a cell's chromosomal DNA.
- It exists naturally in bacteria.
- The plasmid DNA acts as a vector to transfer the piece of DNA attached to it just as the female Anopheles mosquito acts as an insect vector to transfer malarial parasites into the human body.
- In the similar manner a plasmid can be used as a vector to carry an alien piece of DNA into the host organism.



Importance of selectable marker

- If a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into *E. coli* cells, the host cells become transformed into ampicillin-resistant cells.
- If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed recipient cells will die. Since, due to ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a selectable marker.

Host organism

- Since DNA is a hydrophilic molecule, it cannot pass through cell

membranes.

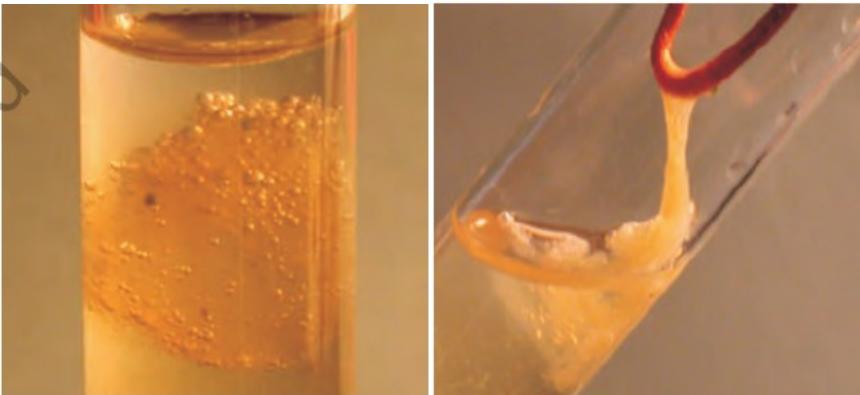
- In order to force bacteria to take up the plasmid, the bacterial cells must first be made ‘competent’ to take up DNA.
- This is done by treating them with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.
- One of the alternative method known as micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell.
- In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biotics or gene gun.

Processes of r-DNA technology

1. Isolation and purification of the genetic material
2. Fragmentation of DNA by a specific restriction endonuclease
3. Separation and isolation of the desired DNA fragment/desired gene by gel electrophoresis
4. Cloning/amplification of the desired DNA fragment/desired gene by PCR
5. Ligation of the desired DNA fragment into a vector by using ligase.
6. Insertion of the recombinant DNA molecule into host organism (transformation)
7. Screening of the transformed host cells. Scaling up and optimization of the process to generate desired product from the expression of desired DNA.
8. Culturing the host cells in a medium at large scale and extraction of the desired product

Isolation of the genetic material

- This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus).

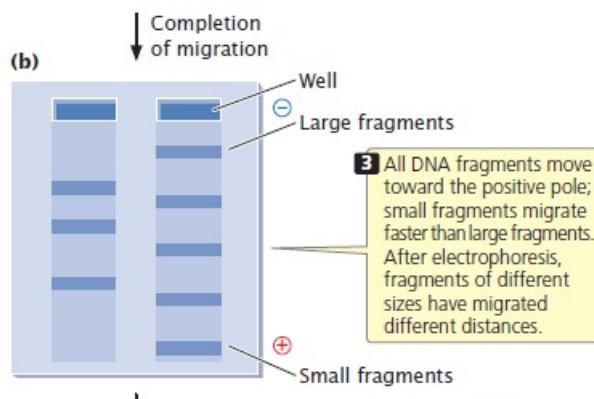
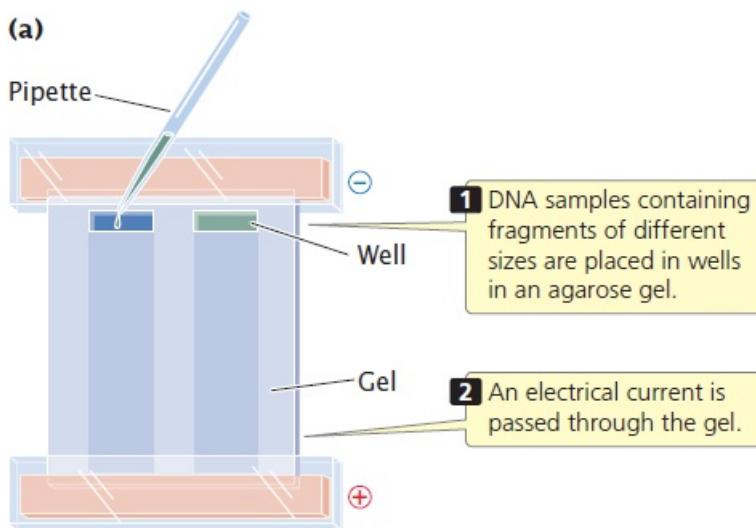


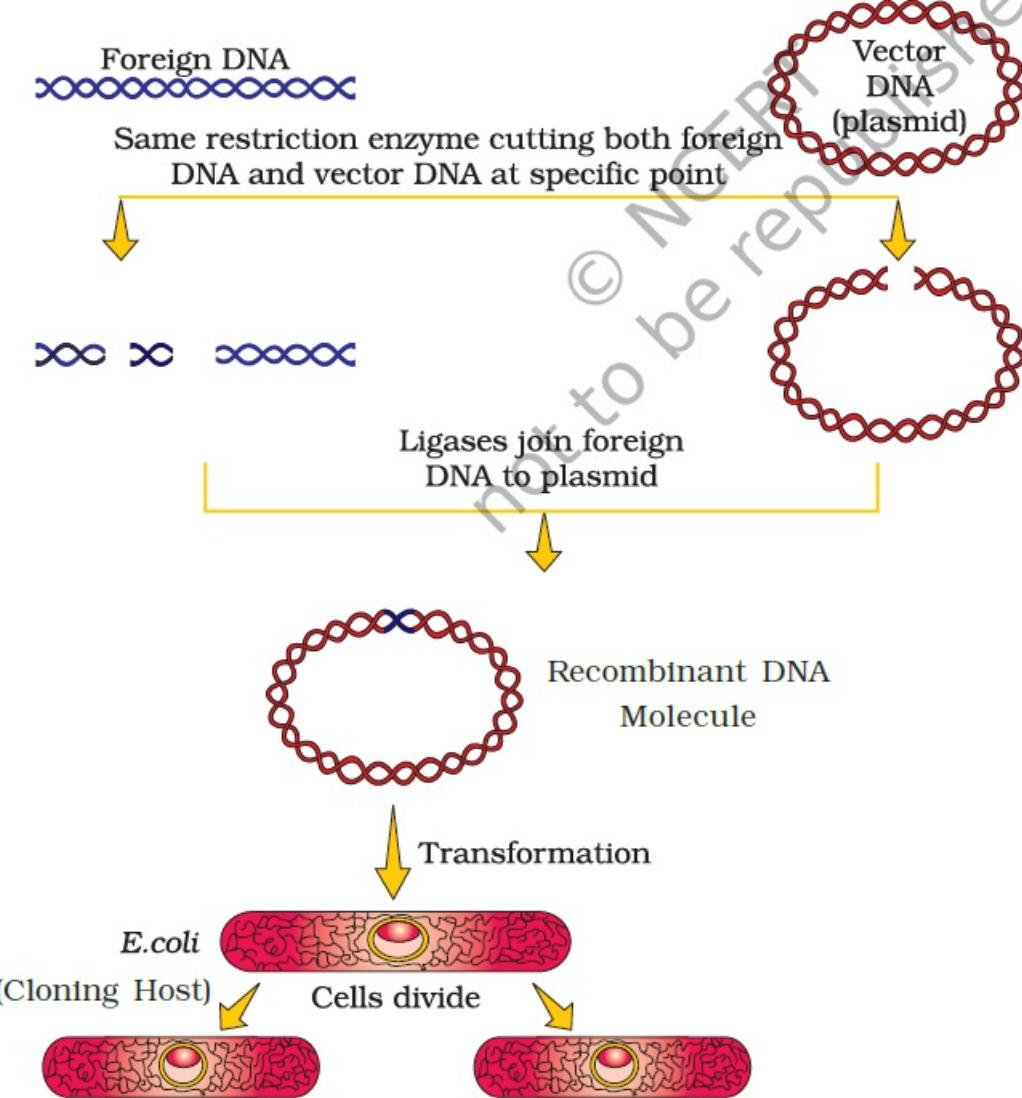
Fragmentation of DNA by restriction endonucleases

- Restriction enzymes are used to cut the DNA molecule with the desired gene.
- Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.

Agarose gel electrophoresis

- The DNA fragments that are formed by the action of restriction endonuclease can be separated by a technique known as gel electrophoresis.
- Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.
- The most commonly used matrix is agarose which is a natural polymer extracted from sea weeds.
- The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel.
- Hence, the smaller the fragment size, the farther it moves.





Amplification of the gene

- One way to amplify a DNA fragment is to clone it in bacterial cells.
- Cloning is labor intensive and requires at least several days to grow the bacteria.
- The alternative method is PCR i.e. polymerase chain reaction.
- PCR makes the amplification of short DNA fragments possible without cloning.
- The segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made!!!

Insertion of the recombinant DNA into host organism

- Recipient cells after making them 'competent' to receive, take up DNA present in its surroundings.
- There are various methods for this such as micro-injection, in which recombinant DNA is directly injected into the nucleus of an animal cell.
- Other methods are using gene gun.

Obtaining the Foreign Gene Product

- In almost all recombinant technologies, the ultimate aim is to produce a desirable protein.
- The protein should be produced in large scale for its purification and application.
- To produce in large quantities, the development of bioreactors, where large volumes (100-1000 litres) of culture can be processed, is required.

Applications of Biotechnology

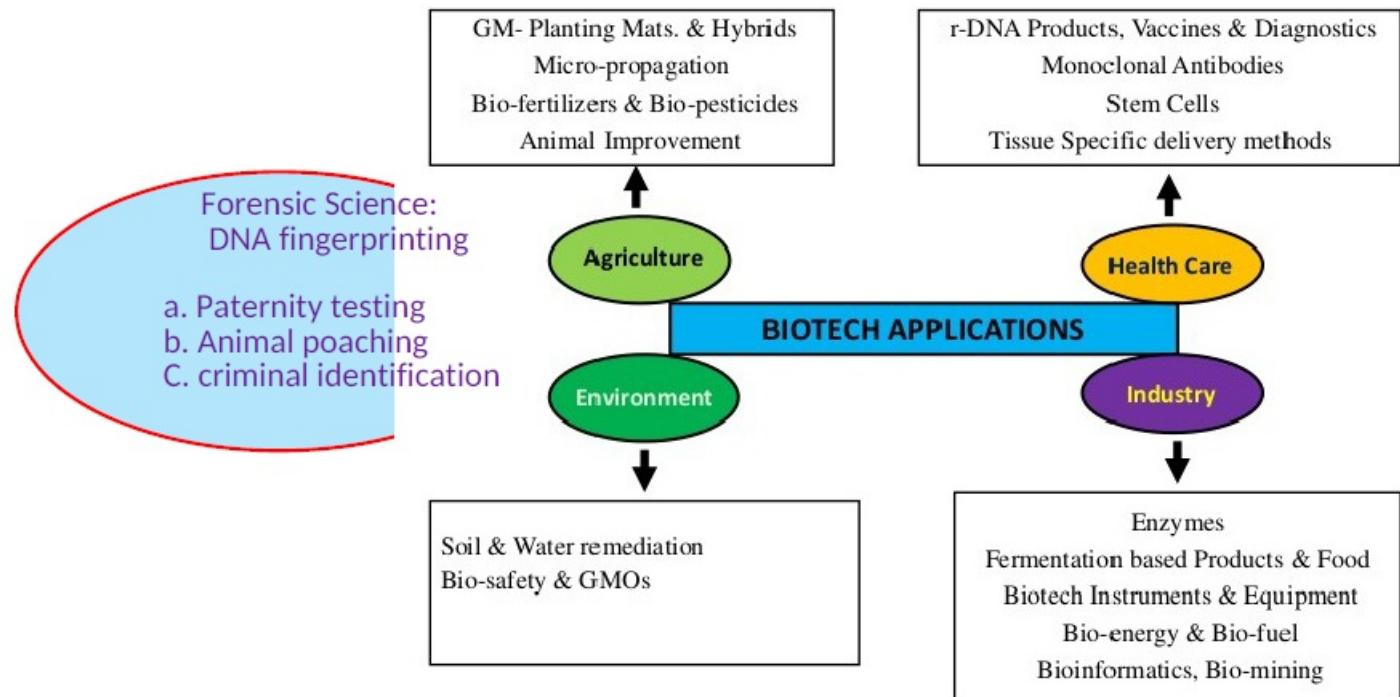
- Application in agriculture
 - Genetically modified crop resistant crops
 - E.g. Bt cotton, Golden rice, Flavr savr tomato etc.
- Application in medicine
 - Genetically engineered insulin
 - Gene therapy to cure various genetic disorders
 - Molecular diagnosis- detection of a particular disease. E.g.-ELISA- Used to detect HIV AIDS

Current used to detect antibodies against novel COVID - 19 virus

- Transgenic animals
 - Transgenic rats, rabbits, pigs, sheep, cows
 - Dolly- the sheep was the 1st mammal to be cloned.
- Biological products

- In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.

- Food industry
 - Production of food constituents such as flavours, aroma, food additives etc.



Plant & Animal tissue culture- Methods and uses in agriculture, medicine and health

Plant and animal tissue culture is a technique used to grow cells, tissues, or organs outside their natural environment in a controlled laboratory setting. Tissue culture has numerous applications in agriculture, medicine, and health.

Plant tissue culture

Methods

The following are the general steps involved in plant tissue culture:

1. Sterilization: The plant tissue is sterilized using an appropriate sterilizing agent, such as ethanol, hypochlorite, or hydrogen peroxide.
2. Explant preparation: A small piece of plant tissue (explant) is cut from a sterile plant and placed onto a culture medium.
3. Culture medium: The culture medium is a nutrient-rich medium that contains the necessary nutrients, vitamins, and hormones to promote cell growth.
4. Growth: The explant is incubated in a controlled environment to promote cell division and growth.
5. Regeneration: After sufficient growth, the cells are stimulated to regenerate into new tissues, organs, or plants.

Uses in agriculture

Plant tissue culture has many uses in agriculture, including:

1. Micropagation: This is the production of large numbers of identical plants through tissue culture.
2. Germplasm preservation: Tissue culture is used to preserve rare and endangered plant species.
3. Crop improvement: Tissue culture is used to develop disease-resistant, drought-tolerant, and high-yielding crop varieties.
4. Production of secondary metabolites: Tissue culture is used to produce valuable secondary metabolites such as alkaloids, flavonoids, and terpenoids.

Animal tissue culture

Methods

The following are the general steps involved in animal tissue culture:

1. Tissue preparation: A small piece of animal tissue is taken from a live or dead animal and placed onto a culture dish.
2. Culture medium: The culture medium is a nutrient-rich medium that contains the necessary nutrients, vitamins, and growth factors to promote cell growth.
3. Incubation: The cells are incubated in a controlled environment to promote cell division and growth.
4. Subculture: The cells are periodically subcultured to maintain the culture.

Uses in medicine and health

Animal tissue culture has many uses in medicine and health, including:

1. Cell-based therapies: Tissue culture is used to grow cells for cell-based therapies such as tissue engineering and regenerative medicine.
2. Drug development: Tissue culture is used to develop and test new drugs.
3. Disease modeling: Tissue culture is used to model human diseases and study disease mechanisms.

4. Toxicology testing: Tissue culture is used to test the toxicity of chemicals and drugs.

In summary, plant and animal tissue culture is a powerful technique with many applications in agriculture, medicine, and health.

Bioindicators

Bioindicators are living organisms or groups of organisms that are used to assess the health and quality of an ecosystem. They can provide valuable insights into environmental conditions and help to detect changes in ecological systems before they become too severe. This can help to inform conservation and management strategies to protect and preserve the health of the environment.

Bioindicators can be found in a wide range of ecosystems, including forests, wetlands, and oceans. They can include plants, animals, and other living organisms and can be studied at different levels, including individual, population, and community levels.

One of the most important benefits of bioindicators is their ability to detect changes in environmental conditions, such as pollution, climate change, and habitat destruction. For example, lichens are often used as bioindicators of air pollution because they are sensitive to changes in air quality. When exposed to pollutants, lichens can change in color or die off completely, providing an indication of the level of pollution in the area.

Similarly, amphibians can be used to monitor changes in water quality because they are sensitive to changes in pH and other water quality parameters. Declines in amphibian populations can indicate problems with water quality or habitat loss.

Other bioindicators include certain species of fish, birds, insects, and microorganisms. For example, some species of fish are used to assess the health of aquatic ecosystems because they are sensitive to changes in water temperature, dissolved oxygen levels, and other factors that can impact the health of the ecosystem. Similarly, birds can be used to monitor changes in the health of forests and wetlands, while insects can be used to assess the health of soil and other ecosystems.

One of the key benefits of bioindicators is their ability to provide information about the health of ecosystems over time. By monitoring bioindicators, researchers can gain insights into changes in environmental conditions and the impacts of human activities on ecosystems. This information can be used to inform conservation and management strategies to protect and preserve the health of the environment.

There are several different approaches to using bioindicators in ecological monitoring. One approach is to use indicator species, which are species that are particularly sensitive to changes in environmental conditions. Indicator species can provide an early warning of changes in environmental conditions, allowing researchers and conservationists to take action before the impacts become too severe.

Another approach to using bioindicators is to use multiple indicators to provide a more comprehensive assessment of ecosystem health. For example, a combination of plant and animal bioindicators could be used to assess the health of a forest ecosystem, providing insights into changes in biodiversity, nutrient cycling, and other ecosystem functions.

The use of bioindicators in ecological monitoring is important for several reasons. First, bioindicators can provide valuable information about the health of ecosystems and the impacts of human activities on the environment. Second, bioindicators can provide an early warning of changes in environmental conditions, allowing researchers and conservationists to take action before the impacts become too severe. Finally, bioindicators can be used to monitor the effectiveness of conservation and management strategies, helping to ensure that these strategies are effective in protecting and preserving the health of the environment.

Hence, bioindicators are an important tool in ecological monitoring and conservation. They can provide valuable insights into the health of ecosystems and help to inform conservation and management strategies to protect and preserve the environment. By monitoring bioindicators over time, researchers can gain insights into changes in environmental conditions and take action to mitigate these impacts, ensuring the long-term health and sustainability of the environment.

Biofuel

Biofuels are renewable energy sources that are produced from organic matter or biomass. They are considered a cleaner alternative to traditional fossil fuels, as they emit less greenhouse gases and pollutants into the atmosphere. In this article, we will explore biofuels in more detail, including their types, benefits, drawbacks, and potential future developments.

Types of Biofuels

There are several types of biofuels, including:

1. Ethanol: Ethanol is produced from corn, sugarcane, and other crops that contain high levels of sugar or starch. It is primarily used as a fuel additive to gasoline to reduce emissions and increase octane ratings.
2. Biodiesel: Biodiesel is produced from vegetable oils, animal fats, and recycled cooking oils. It can be used in diesel engines without any modifications and is typically blended with petroleum diesel.
3. Biogas: Biogas is produced from the anaerobic digestion of organic matter, such as livestock manure, wastewater, and food waste. It is primarily used for electricity generation and heating.
4. Biojet fuel: Biojet fuel is produced from plant oils and animal fats and can be used in aviation as a renewable alternative to traditional jet fuel.

Benefits of Biofuels

Biofuels offer several benefits over traditional fossil fuels, including:

1. Renewable: Biofuels are made from organic matter, which is renewable and can be replenished.
2. Reduced emissions: Biofuels emit fewer greenhouse gases and pollutants than fossil fuels, reducing the negative impact on the environment.
3. Energy security: Biofuels can help reduce reliance on foreign oil imports and provide a domestic source of energy.
4. Rural development: Biofuel production can create jobs and economic opportunities in rural communities.

Drawbacks of Biofuels

Biofuels also have some drawbacks that need to be considered, including:

1. Land use: Biofuel production requires significant amounts of land, which can compete with food production and natural habitats.
2. Water use: Biofuel production requires large amounts of water, which can lead to water scarcity in some regions.
3. Energy balance: The energy required to produce biofuels can sometimes be greater than the energy obtained from using them, reducing their overall efficiency.
4. Food prices: Biofuel production can increase food prices by diverting crops from food production to fuel production.

Future of Biofuels

The future of biofuels looks promising, as research and development efforts are focused on improving their efficiency, reducing their environmental impact, and finding new and innovative ways to produce them. Some potential future developments in biofuels include:

1. Advanced biofuels: Advanced biofuels are produced from non-food sources, such as agricultural waste and algae, which do not compete with food production and have a lower environmental impact.
2. Bioenergy with carbon capture and storage (BECCS): BECCS involves capturing carbon dioxide emitted from biofuel production and storing it underground, reducing greenhouse gas emissions.
3. Synthetic biology: Synthetic biology involves engineering microbes to produce biofuels more efficiently and sustainably.

Conclusion

Biofuels are a renewable energy source that offers several benefits over traditional fossil fuels, including reduced emissions and energy security. However, they also have drawbacks, such as land and water use, energy balance, and food prices. The future of biofuels looks promising, with research and development efforts focused on improving their efficiency, reducing their environmental impact, and finding new and innovative ways to produce them.

Biochips

Biochips, also known as microarrays, are small devices that are used for detecting and analyzing biological molecules such as DNA, RNA, and proteins. They are based on the principles of microfabrication and microelectronics, and they have revolutionized the way researchers study and understand biological systems.

Biochips are essentially miniature laboratories that can perform a variety of biological experiments in a short amount of time. They consist of a solid substrate, usually made of glass or silicon, that is covered with a layer of material that can selectively bind to biological molecules. This layer is then patterned with a series of probes, which are small fragments of DNA, RNA, or protein that are complementary to specific target molecules.

When a sample containing the target molecules is applied to the biochip, the probes on the surface of the chip selectively bind to the target molecules. The chip is then washed to remove any non-specifically bound molecules, and the bound molecules are detected using a variety of methods, including fluorescence, chemiluminescence, and electrochemical detection.

Biochips can be used for a wide variety of applications, including gene expression analysis, protein detection, and DNA sequencing. They are also used in clinical settings for disease diagnosis and monitoring, and in the development of new drugs and therapies.

One of the most common types of biochips is the DNA microarray. This type of biochip consists of thousands of different probes that are arranged in a grid pattern on a small glass slide. Each probe is designed to detect a specific sequence of DNA, and the entire chip can be used to measure the expression of thousands of genes simultaneously.

DNA microarrays have been used in a wide range of biological studies, including cancer research, drug discovery, and environmental monitoring. They have also been used in clinical settings for disease diagnosis and personalized medicine.

Another type of biochip is the protein microarray. This type of biochip consists of a solid substrate that is coated with a variety of different proteins. When a sample containing a specific protein is applied to the chip, the protein binds to its corresponding partner on the chip. This binding event can be detected using a variety of methods, and can be used to identify specific protein-protein interactions.

Protein microarrays have been used in a variety of biological studies, including the identification of protein-protein interactions, the discovery of new drugs and therapies, and the development of new diagnostic tests.

In addition to DNA and protein microarrays, there are also other types of biochips that are used for a variety of applications. For example, RNA microarrays are used to study gene expression at the level of RNA, while tissue microarrays are used to analyze the expression of proteins in different tissues.

There are also biochips that are designed to be used in clinical settings. For example, there are biochips that are used for the diagnosis of infectious diseases, such as HIV and hepatitis, and there are biochips that are used for the detection of cancer biomarkers.

The development of biochips has been a major technological advance in the field of biology and medicine. They have allowed researchers to analyze biological systems in ways that were previously impossible, and they have the potential to revolutionize the way that diseases are diagnosed and treated.

However, there are also some challenges associated with the use of biochips. For example, the development of biochips requires a high level of expertise in microfabrication and microelectronics, and the cost of producing biochips can be prohibitive for some applications.

In addition, the analysis of biochip data can be complex, and requires specialized software and algorithms to identify meaningful patterns and correlations in the data.

Despite these challenges, biochips have already had a major impact on the field of biology and medicine.

Nanoshells

Nanoshells are a type of nanomaterial composed of a dielectric core surrounded by a thin metallic shell. They are typically fabricated using a technique called electroless plating, in which metal ions are reduced onto the surface of the dielectric core. The resulting structure is a hollow shell with a thickness on the order of tens of nanometers.

Nanoshells have unique optical properties that make them useful in a variety of applications, including biomedical imaging and cancer therapy. Specifically, the metallic shell of a nanoshell can be designed to resonate with light of a specific wavelength, allowing for efficient absorption or scattering of light. This property can be used to enhance contrast in medical imaging or to selectively heat cancer cells for targeted therapy.

Overall, nanoshells represent a promising area of research for the development of new materials with unique optical properties and potential applications in various fields.

Nano Biomolecules

Nano biomolecules refer to very small biological molecules that have dimensions in the range of nanometers (nm). These molecules have unique properties that make them highly attractive for a wide range of applications in medicine, biotechnology, and nanotechnology.

Examples of nano biomolecules include:

1. Nanoparticles: These are particles with a size range of 1-100 nm. They can be made from a variety of materials, such as metals, polymers, and lipids. Nanoparticles have a large surface area-to-volume ratio, which makes them ideal for drug delivery, imaging, and sensing applications.
2. Nanotubes: These are cylindrical structures with a diameter of a few nanometers. Carbon nanotubes are a well-known example of nanotubes. They have unique mechanical, thermal, and electrical properties, which make them useful in applications such as drug delivery, tissue engineering, and biosensors.
3. Peptides: These are short chains of amino acids with a size range of 1-10 nm. Peptides have a wide range of biological activities and are used in drug development, imaging, and biosensing.
4. Proteins: These are large biomolecules with a size range of 2-50 nm. Proteins have a variety of functions in the body, and can be used in drug delivery, imaging, and biosensing applications.

The unique properties of nano biomolecules make them highly promising for a wide range of applications. However, their small size also makes them difficult to work with, and there are still many challenges to be overcome before they can be widely used in practical applications.

Note: Study Materials and Images have been used from the online resources and NCERT Biology books of 11th and 12th.