

BASICS OF CELL

All living things are made from one or more cells. A cell is the simplest unit of life and they are responsible for keeping an organism alive and functioning. This introduction to cells is the starting point for the area of biology that studies the various types of cells and how they work.

Cell, in biology, the basic membrane-bound unit that contains the fundamental molecules of life and of which all living things are composed. A single cell is often a complete organism in itself, such as a bacterium or yeast. Other cells acquire specialized functions as they mature. These cells cooperate with other specialized cells and become the building blocks of large multicellular organisms, such as humans and other animals. Although cells are much larger than atoms, they are still very small. The smallest known cells are a group of tiny bacteria called mycoplasmas; some of these single-celled organisms are spheres as small as 0.2 μm in diameter (1 μm = about 0.000039 inch), with a total mass of 10–14 gram—equal to that of 8,000,000,000 hydrogen atoms. Cells of humans typically have a mass 400,000 times larger than the mass of a single mycoplasma bacterium, but even human cells are only about 20 μm across. It would require a sheet of about 10,000 human cells to cover the head of a pin, and each human organism is composed of more than 30,000,000,000,000 cells.

There is a massive variety of different types of cells but they all have some common characteristics. Almost every different type of cell contains genetic material, a membrane and cytoplasm. Cells also have many other features such as organelles and ribosomes that perform specific functions.

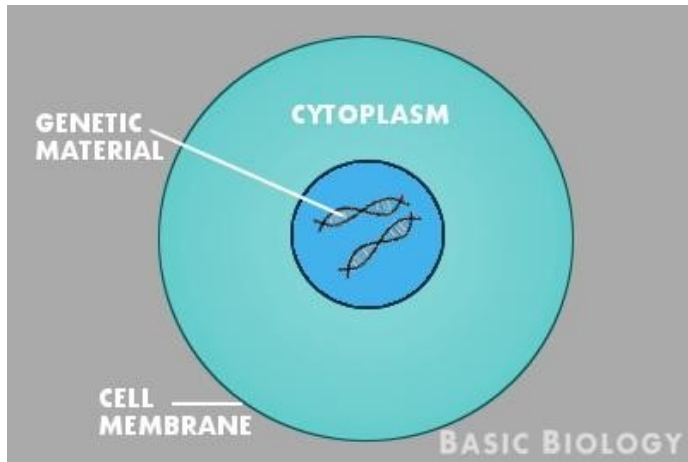
Many different organisms on the tree of life contain only one cell and are known as single-celled or unicellular organisms. Their single cell performs all the necessary functions to keep the organism alive. All species of bacteria and archaea are single-celled organisms. On the other hand, large organisms like humans are made from many trillions of cells that work together to keep the organism alive.

Cells are the basic unit of life.

The Cell Theory states that:

- 1) All organisms are made up of one or more cells and the products of those cells.
- 2) All cells carry out life activities (require energy, grow, have a limited size).
- 3) New cells arise only from other living cells by the process of cell division.

STRUCTURE OF CELL



Every cell is different but there is a basic structure that is common to all cells. A cell is essentially genetic material in a gel-like substance surrounded by a membrane.

The genetic material of cells is found as molecules called DNA. The DNA of a cell holds all the information that a cell needs to keep itself alive. A DNA molecule contains a code that can be translated by a cell and tells it how to perform different tasks. A gene is a specific segment of a DNA molecule and each gene tells a cell how to perform one specific task.

The gel-like substance that the genetic material is found in is called the cytoplasm. The cytoplasm fills a cell and gives it its shape. The cytoplasm also allows for different materials to move around the cell. All cells have other structures in their cytoplasm that help the cell stay alive.

The cytoplasm of all cells is surrounded by a membrane called the plasma membrane. The plasma membrane separates the cell from the outside world and keeps the contents of the cell together. The plasma membrane provides a barrier that substances have to pass through before they can enter or exit a cell.

EUKARYOTIC CELLS VS. PROKARYOTIC CELLS

The most basic categorisation of Earth's organisms is determined by different types of cells. All cells can be divided into one of two classifications: prokaryotic cells and eukaryotic cells. Prokaryotic cells are found in bacteria and archaea. Eukaryotic cells are found in organisms from the domain Eukaryota which includes animals, plants, fungi and protists.

The main difference between prokaryotic cells and eukaryotic cells is the presence of a nucleus and organelles. Prokaryotic cells do not have either a nucleus or organelles. The word prokaryotic can be translated to mean 'before nucleus'.

Eukaryotic cells have both a nucleus and a range of different organelles. The nucleus is a structure found in eukaryotic cells that contains the cell's DNA. Organelles are cellular 'factories' that perform important functions such as building different molecules of life, removing wastes and breaking down sugars.

Having organelles makes eukaryotic cells much more efficient at completing important cellular functions. Because they are more efficient, eukaryotic cells can grow much larger than prokaryotic cells.

For a cellular structure to be considered an organelle it must be surrounded by a membrane just as the nucleus is. Prokaryotic cells contain various structures that help with certain functions, such as ribosomes, but these structures are not encapsulated by membranes and are therefore not considered organelles.

Eukaryotic cells have evolved into multicellular organisms. By specializing into different types of cells, they are able to perform functions even more efficiently and are able to keep large, multicellular organisms alive.

CELL ORGANELLES

An organelle is a membrane bound structure found within a cell. Just like cells have membranes to hold everything in, these mini-organs are also bound in a double layer of phospholipids to insulate their little compartments within the larger cells.

A small organ-like structure present inside the cell is called a cell organelle. It has a particular structural makeup and performs a specific function. Depending upon the presence or absence of membrane, cell organelles can be classified into three categories, namely:

Without membrane: Some cell organelles like ribosomes are not bounded by any membrane. They are present in prokaryotic as well as eukaryotic organisms.

Single membrane-bound: Some organelles are bounded by a single membrane. For example, vacuole, lysosome, Golgi Apparatus, Endoplasmic Reticulum etc. They are present only in a eukaryotic cell.

Double membrane-bound: Cell organelles like mitochondria and chloroplast are double membrane-bound organelles. They are present only in a eukaryotic cell.

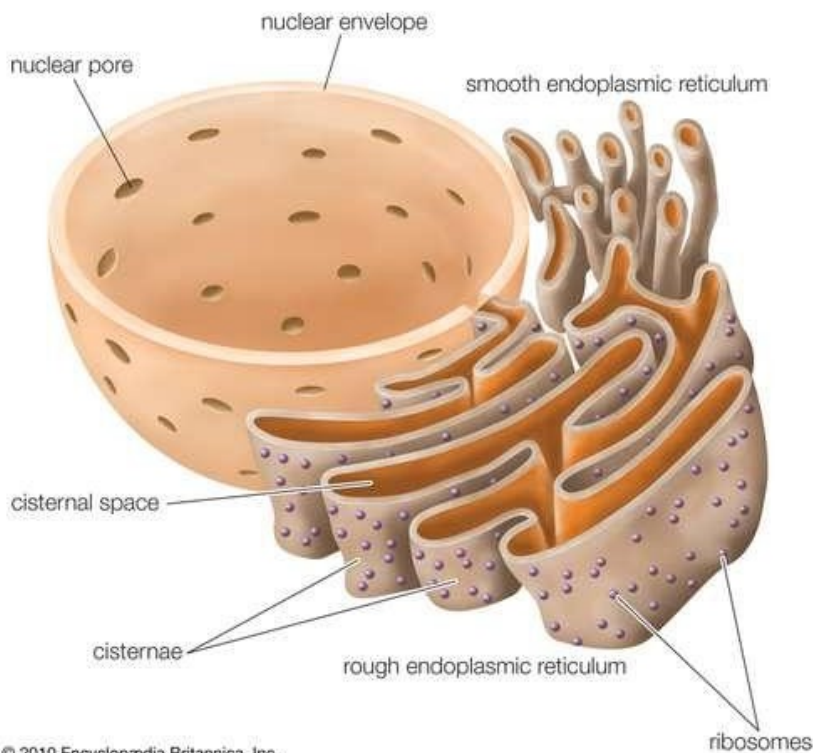
ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is the largest, membrane-bound intracellular organelle found in eukaryotic cells (prokaryotes lack membrane-bound organelles). It is a highly dynamic organelle that radiates from the nuclear envelope towards the plasma membrane.

Endoplasmic reticulum transpires in two forms: a type with ribosome-studded surface and another with a smooth surface. The latter is called smooth endoplasmic reticulum, and the former is called rough endoplasmic reticulum. These membranes form continuous folds, eventually joining the outer layer of the nuclear membrane. Except for sperm cells and red blood cells, the endoplasmic reticulum is observed in every other type of eukaryotic cell. Rough endoplasmic reticulum has ribosomes embedded within its structure, giving a -rough appearance. Smooth endoplasmic reticulum does not have these ribosomes, hence appear -smooth.

Structure of endoplasmic reticulum

Endoplasmic reticulum



The structure of endoplasmic reticulum is shaped like a sac. Since ER is of two types, each has its own distinguishing features:

Rough Endoplasmic Reticulum Structure

- The rough endoplasmic reticulum is named so because of its appearance.
- It is a series of connected flattened sacs that have many ribosomes on their outer surface, hence the name.
- It synthesises and secretes proteins in the liver, hormones and other substances in the glands.
- Rough ER is prominent in cells where protein synthesis happens (such as hepatocytes)

Smooth Endoplasmic Reticulum Structure

- The smooth endoplasmic reticulum, on the other hand, does not have ribosomes.
- The smooth endoplasmic reticulum has a tubular form.
- It is involved in the synthesis of phospholipids, the main lipids in cell membranes and are essential in the process of metabolism.
- Smooth ER transports the products of the rough ER to other cellular organelles, especially the Golgi apparatus.

Endoplasmic reticulum function

As stated above, the endoplasmic reticulum is categorised into two types, and both these types of ER perform specific functions:

Smooth Endoplasmic Reticulum Function:

- Smooth ER is responsible for the synthesis of essential lipids such as phospholipids and cholesterol.
- Smooth ER is also responsible for the production and secretion of steroid hormones.
- It is also responsible for the metabolism of carbohydrates.
- The smooth ER store and release calcium ions. These are quite important for the nervous system and muscular system.

Rough Endoplasmic Reticulum Function:

- The majority of the functions of rough ER plays is associated with protein synthesis.
- Rough endoplasmic reticulum also plays a vital role in protein folding.
- Also ensures quality control (regarding correct protein folding).
- The second most important function after protein synthesis and protein folding is protein sorting.

GOLGI COMPLEX

In the animal and plant cells clusters of fat filled structures are present. They are called Golgi apparatus or complex. In 1898 Camillo Golgi' recognised it in the nerve cell of the owl. The Golgi complex of invertebrates is called dictyo•some.

Occurrence: Golgi complex is seen in all eukaryotic cells. Golgi complex is not seen in mature sperm, red blood cell and prokaryotes.

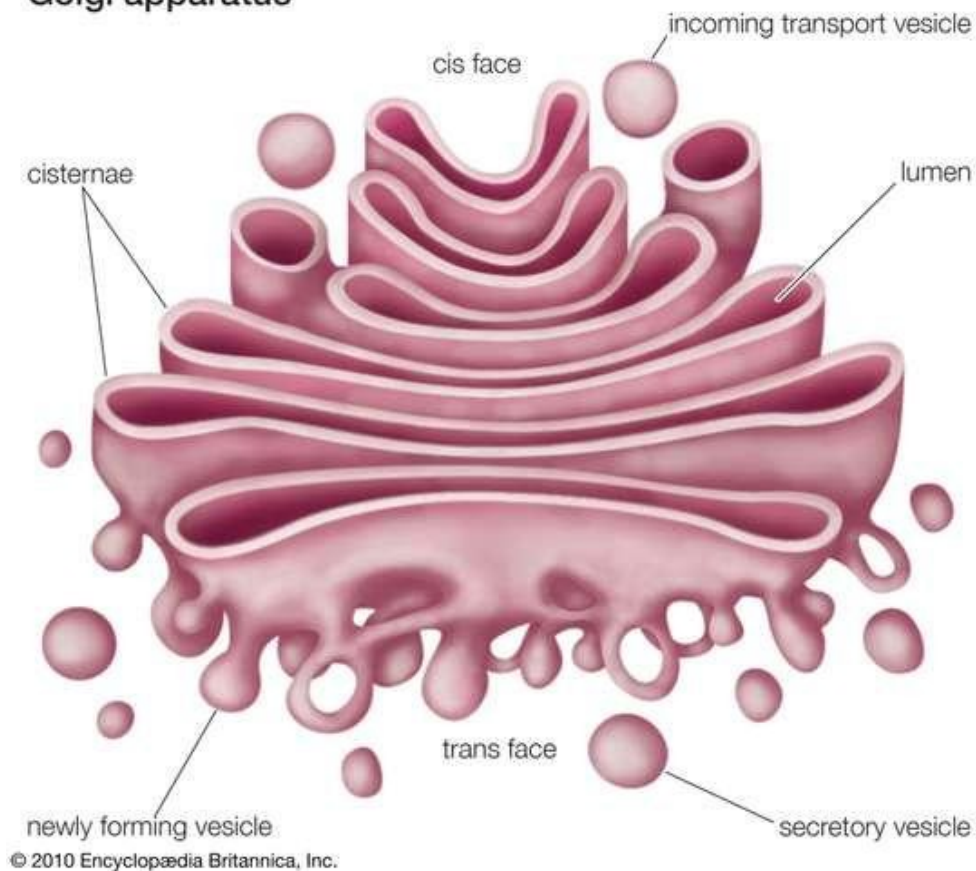
Golgi complex occurs in two forms:

a) Localized form: Golgi complex occurs singly and has a fixed position. (In between nucleus and secretory pore)

b) Diffused form: In the nerve and liver cells Golgi complex is scattered, in it each unit is called dictyosome.

Structure:

Golgi apparatus



Golgi body is seen in the form of three components.

1. Cisternae: These are tubular, flat, fluid filled sacs. They show 200 to 300Å width. Each sac is covered by two membranes. In a dictyosome 3 to 7 cisternae are present. They are arranged one above the other. Their convex side is towards nucleus and their concave surface is towards plasma membrane. The convex side of the cisternae is called forming face. The concave surface is called maturing face. It shows big secreting vesicles. These secretory vesicles store secretory substances. They may develop into lysosomes.

Polarity of cisternae: The cisternae shows maturing face and forming face. Forming face is convex and towards nucleus. The smooth E.R. gives vesicles. They unite to form cisternae.

2. Golgi vesicles: On the forming face of golgi cisternae small vesicles are present. They are 400 Å width. They usually develop from E.R.

3. Secretory vesicles: On the maturing face of golgi cisternae secretory vesicles are present. They contain secretory products of golgi. They finally change into lysosomes.

Chemical Composition:

Golgi complex will be rich in chemical substances.

1. Phospholipids: These substances have a composition which is in between the structure of phospholipids of endoplasmic reticulum and plasma membrane.
2. Enzymes: ATP-ase, CPT-ase, transphorases, etc. enzymes are. present.
3. Carbohydrates: Glucose, manose, galactose carbohydrates are seen.

Golgi Apparatus Functions

The Golgi apparatus has many discrete functions. But, all functions are associated with moving molecules from the endoplasmic reticulum to their final destination and modifying certain products along the way. The multiple sacs of the Golgi serve as different chambers for chemical reactions. As the products of the endoplasmic reticulum move through the Golgi apparatus, they are continuously transferred into new environments, and the reactions that can take place are different.

In this way, a product can be given modifications, or multiple products can be combined to form large macromolecules. The many sacs and folds of the Golgi apparatus allow for many reactions to take place at the same time, increasing the speed at which an organism can produce products.

The main function of the Golgi apparatus is the ability to deliver vesicles, or packets of various cell products, to different locations throughout the cell. The Golgi also has important functions in tagging vesicles with proteins and sugar molecules, which serve as identifiers for the vesicles so

they can be delivered to the proper target. The organelle is also called the Golgi complex or Golgi body.

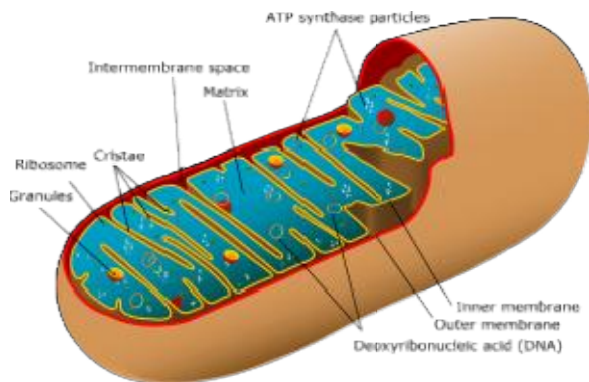
Typically, proteins and cellular products are manufactured in the endoplasmic reticulum. The rough endoplasmic reticulum has a number of ribosomes, which assemble proteins from instructions contained in messenger RNA. Throughout the rest of the endoplasmic reticulum, these protein products are folded and modified. As they reach the Golgi apparatus, more modifications are made. Finally, the products are packaged within vesicles which are labeled by other proteins and molecules. The vesicles are released and based on their tags or labels they are carried to the appropriate location within the cell by the cytoskeleton.

MITOCHONDRIA

Popularly known as the "Powerhouse of the cell," mitochondria (singular: mitochondrion) are a double-membrane-bound organelle found in most of the eukaryotic organisms. They are found inside the cytoplasm and essentially function as the cell's digestive system.

They play a major role in breaking down the nutrients and generating energy-rich molecules for the cell. Many of the biochemical reactions involved in cellular respiration take place within the mitochondria. The term "mitochondrion" is derived from the Greek words "mitos" and "chondrion" which means "thread" and "granules-like" respectively. It was first described by a German pathologist named Richard Altmann in the year 1890.

Structure of Mitochondria



The mitochondrion is a double-membraned, rod-shaped structure found in both plant and animal cell. Its size ranges from 0.5 to 1.0 micrometre in diameter. The structure comprises an outer membrane, an inner membrane, and a gel-like material called the matrix. The outer membrane and the inner membrane are made of proteins and phospholipid layers separated by the intermembrane space.

The outer membrane covers the surface of the mitochondrion and has a large number of special proteins known as porins. It is freely permeable to ions, nutrient molecules, energy molecules like the ADP and ATP molecules.

Cristae

The inner membrane of mitochondria is rather complex in structure. It has many folds that form a layered structure called cristae and this helps in increasing the surface area inside the organelle. The cristae and the proteins of the inner membrane aids in the production of ATP molecules. The inner membrane is strictly permeable only to oxygen and to ATP molecules. A number of chemical reactions take place within the inner membrane of mitochondria.

Mitochondrial Matrix

The mitochondrial matrix is a viscous fluid that contains a mixture of enzymes and proteins. It also comprises ribosomes, inorganic ions, mitochondrial DNA, nucleotide cofactors, and organic molecules. The enzymes present in the matrix play an important role in the synthesis of ATP molecules.

Function of Mitochondria

The most important function of mitochondria is to produce energy through the process of oxidative phosphorylation. It is also involved in the following process:

- Regulates the metabolic activity of the cell
- Promotes the growth of new cells and cell multiplication
- Helps in detoxifying ammonia in the liver cells
- Plays an important role in apoptosis or programmed cell death
- Responsible for building certain parts of the blood and various hormones like testosterone and oestrogen
- Helps in maintaining an adequate concentration of calcium ions within the compartments of the cell
- It is also involved in various cellular activities like cellular differentiation, cell signalling, cell senescence, controlling the cell cycle and also in cell growth.

Disorders Associated With Mitochondria

Any irregularity in the way mitochondria functions can directly affect human health, but often, it is difficult to identify because symptoms differ from person to person. Disorders of the mitochondria can be quite severe, it can eventually cause an organ to fail.

Mitochondrial diseases: Alpers Disease, Barth Syndrome, Kearns-Sayre syndrome (KSS)

CHLOROPLAST

A chloroplast is an organelle unique to plant cells that contains chlorophyll (which is what makes plants green) and is responsible for enabling photosynthesis to occur so that plants can convert sunlight into chemical energy. Chloroplasts are the site of photosynthesis in eukaryotic cells. They are only present in photosynthetic cells like plant cells and algae. There are no chloroplasts in animal or bacterial cells.

The chloroplast, found only in algal and plant cells, is a cell organelle that produces energy through photosynthesis. The word chloroplast comes from the Greek words khloros, meaning –green, and plastes, meaning –formed. It has a high concentration of chlorophyll, the molecule that captures light energy, and this gives many plants and algae a green color. Like the mitochondrion, the chloroplast is thought to have evolved from once free-living bacteria.

Chloroplasts are the part of plant and algal cells that carry out photosynthesis, the process of converting light energy to energy stored in the form of sugar and other organic molecules that the plant or alga uses as food. Photosynthesis has two stages. In the first stage, the light-dependent reactions occur. These reactions capture sunlight through chlorophyll and carotenoids to form adenosine triphosphate (ATP, the energy currency of the cell) and nicotinamide adenine dinucleotide phosphate (NADPH), which carries electrons. The second stage consists of the light-independent reactions, also known as the Calvin cycle. In the Calvin cycle, the electrons carried by NADPH convert inorganic carbon dioxide and to an organic molecule in the form of a carbohydrate, a process known as CO₂ fixation. Carbohydrates and other organic molecules can be stored and used at a later time for energy.

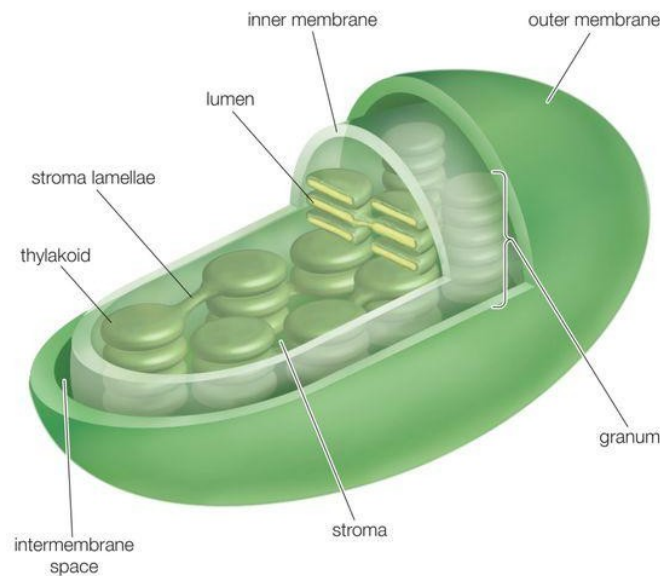
Chloroplasts are essential for the growth and survival of plants and photosynthetic algae. Like solar panels, chloroplasts take light energy and convert it into a usable form that powers activities. However, a few plants no longer have chloroplasts. One example is the parasitic plant genus *Rafflesia*, which obtains its nutrients from other plants—specifically, *Tetrastigma* vines. Since *Rafflesia* gets all of its energy from parasitizing another plant, it no longer needs its chloroplasts, and has lost the genes coding for the development of the chloroplast over a long period of evolutionary time. *Rafflesia* is the only genus of land plant known to be lacking chloroplasts.

Structure of Chloroplasts

- Chloroplasts found in higher plants are generally biconvex or planoconvex shaped.
- Chloroplasts can be found in the cells of the mesophyll in plant leaves.
- In different plants chloroplasts have different shapes, they vary from spheroid, filamentous saucer-shaped, discoid or ovoid shaped.
- They are vesicular and have a colorless center.
- Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll.

- In algae a single huge chloroplast is seen that appears as a network, a spiral band or a stellate plate.
- The size of the chloroplast also varies from species to species and it is constant for a given cell type.
- In higher plants, the average size of chloroplast is 4-6 μm in diameter and 1-3 μm in thickness.

Chloroplast



Parts of Chloroplasts

- **Outer membrane** – It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.
- **Intermembrane Space** – It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.
- **Inner membrane** – The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.
- **Stroma**- Stroma is a alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA, chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.
- **Thylakoid System**- The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the site for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. Each granum contains around 10-20 thylakoids.

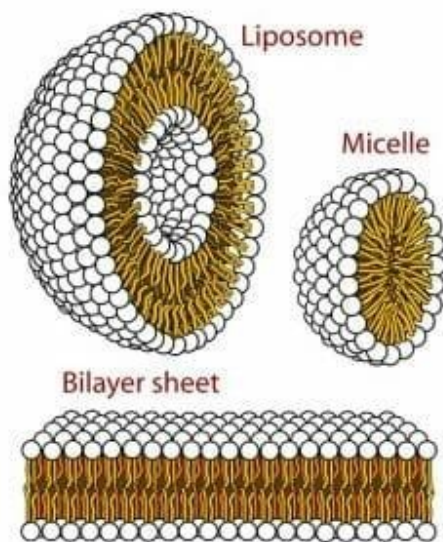
LYSOSOMES

Lysosomes are an important cell organelle found within eukaryotic animal cells. Due to their peculiar function, they are also known as the –suicide bags of the cell. The term was coined by Christian de Duve, a Belgian biologist, who discovered it.

–Lysosomes are membranous organelles whose specific function is to breakdown cellular wastes and debris by engulfing it with hydrolytic enzymes.‡

Lysosome Structure

Lysosomes are generally very small, ranging in size from 0.1-0.5 μm , though they can reach up to 1.2 μm . They have a simple structure; they are spheres made up of a lipid bilayer that encloses fluid that contains a variety of hydrolytic enzymes. The lipids that make up the bilayer are phospholipids, which are molecules that have hydrophilic phosphate group heads, a glycerol molecule, and hydrophobic fatty acid tails. Due to these differences in properties, phospholipids naturally form double-layered membranes when placed in a solution containing water. The phosphate group heads move to the outside of the layer, while the fatty acid tails move to the inside of the layer to be away from water. Phospholipids make up many other membranes in the cell, such as the cell membrane which surrounds the entire cell, the nuclear membrane (or nuclear envelope) that surrounds the nucleus, the Golgi apparatus, and the endoplasmic reticulum.



Lysosomes are formed by budding off of the Golgi apparatus, and the hydrolytic enzymes within them are formed in the endoplasmic reticulum. The enzymes are tagged with the molecule mannose-6-phosphate, transported to the Golgi apparatus in vesicles, and then packaged into the lysosomes.

There are many different types of enzymes in lysosomes including proteases, amylases, nucleases, lipases, and acid phosphatases, among many others. Enzymes are usually named for the molecules that they break down; for example, proteases break down proteins, and nucleases break down nucleic acids. Amylases break down starches into sugars.

Function

Lysosomes play an important role in phagocytosis. When macrophages phagocytose foreign particles, they contain them within a phagosome. The phagosome will then bind with a lysosome to form a phagolysosome. The hydrolytic enzymes contained within the lysosome allow foreign particles to be destroyed.

The key function of lysosomes is digestion and removal of waste. Cellular debris or foreign particles are pulled in to the cell through the process of endocytosis. The process of endocytosis happens when the cell membrane falls in on itself (invagination), creating a vacuole or a pouch around the external contents and then bringing those contents into the cell.

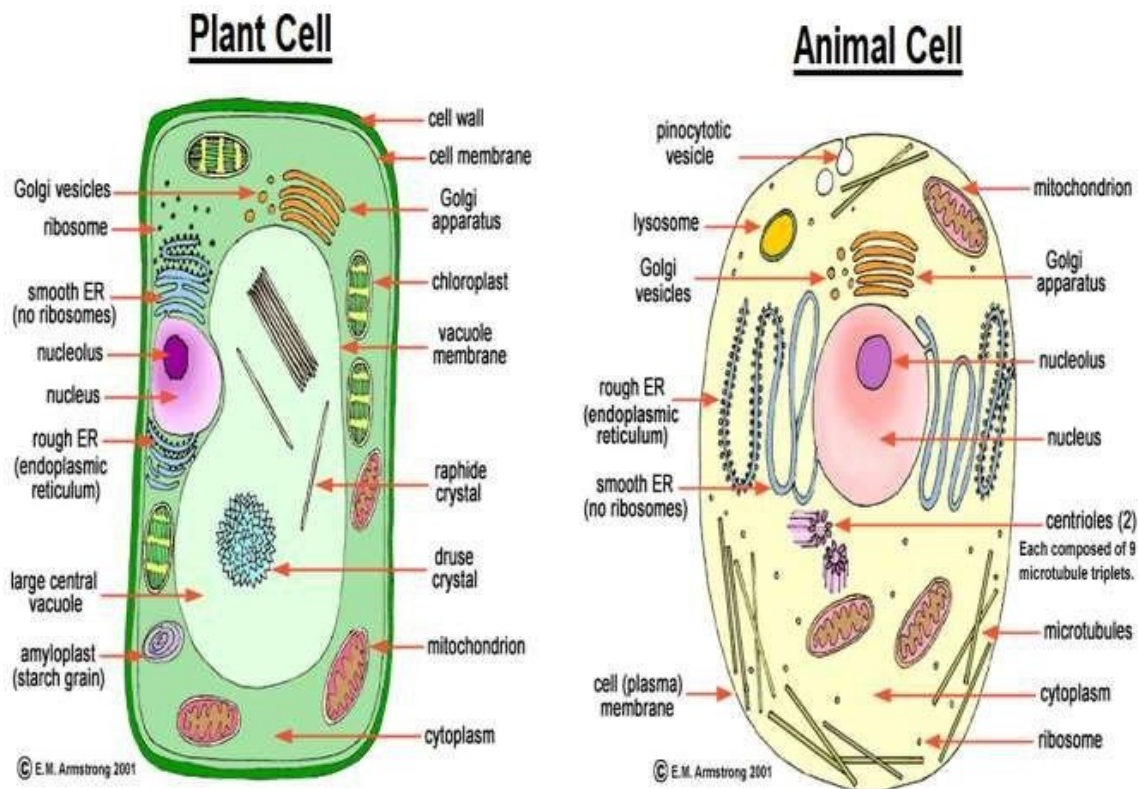
On the other hand, discarded wastes and other substances originating from within the cell is digested by the process of autophagocytosis or autophagy. The process of autophagy involves disassembly or degradation of the cellular components through a natural, regulated mechanism.

Why are Lysosomes known as Suicidal Bags?

As stated before, lysosomes work as the waste discarding structures of the cell by processing undesirable materials and degrading them, both from the exterior of the cell and waste constituents inside the cell. But sometimes, the digestive enzymes may end up damaging the lysosomes themselves, and this can cause the cell to die. This is termed as autolysis, where -auto- means -self- and -lysis- means -the disintegration of the cell by the destruction of its cell membrane-. Hence, lysosomes are known as -Suicidal Bags- of the cell.

PLANT CELL AND ANIMAL CELL

Plant and animal cells have several differences and similarities. The plant cell and the animal cell can be differentiated by the presence of organelles in them. Although both are classified as Eukaryotes, the presence of the cell wall, vacuoles, and chloroplasts are the most remarkable and distinguishing components of the plant cells which are absent in the animal cells. Even the size of the animal cell is smaller than the plant cell.



Plant Cell

Mainly Kingdom Plantae consists of multi-cellular eukaryotes living things, which are autotrophic by nature. As we discussed above that the organelles in plant cell like – chloroplast, cell wall, and vacuoles distinguishes them from the animal cells. Till yet around 400,000 number of plants species have been identified, and there is the lot remain undiscovered.

Normally the range of plant cells varies from 10-100 μm in size. Plant cell carries out the function of photosynthesis, due to which the green plants are called as autotrophs. This is done by the presence of chlorophyll in the chloroplast of the plant cells. The cell wall is made up of cellulose, which provides support and rigidity to the cells.

Functions of the few important cell organelles:

Plasma Membrane – It controls the movement of the molecules in and out of the cell and function in adhesion and signaling also.

Cell Wall – The cell wall is usually rigid, non-living and permeable component surrounding the plasma membrane. They are two types: The primary cell wall and the secondary cell wall. The primary cell wall is made up of cellulose and is formed at the time of cell division. The secondary cell wall is made up of lignin and cellulose and helps giving shape and size to the cell.

Chloroplasts – This is the unique features found in plant cells, which help in the preparation of the food at the site of photosynthesis. Plastids are the term used collectively to represent chloroplasts (green plastids containing chlorophylls), chromoplast (yellow to reddish color plastids) and leucoplast (colorless plastids). Chloroplast contains the other parts like thylakoids and stroma, which helps in capturing sunlight, helping in the synthesis of food.

Vacuoles – Vacuoles occupy 90% of the total cell volume. These are the membrane-bound, liquid filled vesicles. Vacuoles contain the high range of dissolved salts, sugars, pigments and other toxic waste. They also provide physical support and contribute in giving color to the leaves and the flowers.

Types of Plant Cells:

1. **Parenchyma** – These are the structurally simplest cells, and have thin walls. They are used for storage of organic products.
2. **Collenchyma** – These have thin walls, with thickening at some parts of the cell. These cells provide structural support to the cell.
3. **Sclerenchyma** – The cell wall of this cell are embedded with lignin.
4. **Water Conducting Cells** – The vascular tissue in plants known as Xylem, helps in transmitting water from roots to other parts of the plants.
5. **Sieve Tube Members** – The another plant tissue known as Phloem, helps in transporting food and nutrients. This (food) is prepared in the green leaves by the process of photosynthesis.

Animal Cell

The three-fourth part among all the species is taken by the Kingdom Animalia on the planet. The human body is composed of 10¹⁴ cells, whose size varies from 10-30 μm in diameter. Animal cells do not have cell wall and chloroplast which mainly distinguish them from the plant cells.

It is believed that cell wall disappeared with the evolution and the animal cells developed with the more advanced cells, tissues, and organs which are more specified in their function. Nerves and muscles are such kinds which help in locomotion, mobility and in performing other functions too.

Functions of some important organelles:

Plasma Membrane – As discussed above that it controls the movement of the molecules in and out of the cell and function in cell-cell signaling and cell adhesion. It is the outermost layer of the cell and the protect the internal organelles also.

Mitochondria – It is called as ‘the powerhouse of the cell’ as ATP (adenosine triphosphate) is generated by oxidation of glucose and fatty acids.

Lysosomes – It has the acidic lumen which degrades material engulfed by the cell, and worn out cellular membranes and organelles. They are regarded as the digestive tract of the cell.

Nuclear envelope – This is the double layer membrane, protecting the contents of the nucleus.

Nucleus – It contains the hereditary material and is filled with chromatin made up of DNA and proteins.

Endoplasmic reticulum (ER) – It is of two types Smooth endoplasmic reticulum and Rough endoplasmic reticulum. In Smooth endoplasmic reticulum, lipids are synthesized, and detoxification occurs of the hydrophobic compounds. In Rough endoplasmic reticulum protein synthesis, processing takes place.

Golgi Complex – This organelle processes and sorts lysosomal proteins, secreted proteins and membrane proteins synthesized on the rough endoplasmic reticulum.

Secretory vesicles – It stores secreted proteins and fuse with the plasma membrane to release their content.

Peroxisomes – Also known as microbodies and are the single membrane cellular bodies. They are oval or spherical and contain the enzyme catalase. Peroxisomes detoxify the molecules and break down the fatty acids to produce acetyl groups for biosynthesis.

Cytoskeletal fibers – It forms the network and bundles that support cellular membrane, and help organize organelles and supports the cell movement. The cellular matrix is collectively referred to as cytosol. The cytosol is a compartment containing several metabolites, enzymes, and salts in an aqueous gel like the medium.

Microvilli – It increases the surface area for absorption of the nutrients from surrounding medium.

Some Common types of Animal Cells:

1. **Skin Cells** – These are found in the dermal and epidermal layer, the skin works in the protection of the internal parts, prevent the excess loss of water through dehydration, perception and in the transmission of sensation.

2. **Bone Cells** – Bone cells are responsible for making bones and skeleton of animals. There are many types of bone cell, and the primary function is to provide the structural support and helps in the movement of the body.

3. **Muscle Cells** – Muscles cells or myocytes functions for the movement of the body. They also help in the protection of the delicate organs of the body.

4. **Blood Cells** – They work as the transporter in the body which carry hormones and nutrients. Mainly the blood carries oxygen to different tissues of the body and also helps in taking back the carbon dioxide from them. Blood cells are also known as hematopoietic cells.

5. **Nerve Cells** – These are specialized cells, appointed to send impulses or information. These are signals or messages help the body to connect and perform the function in the synchronized manner and according to the outer environment. These electrochemical signals are sent from central nervous system and the sensory receptors.

Characteristic	Plant Cell	Animal Cell
Cell shape	Has distinct edges, usually square or rectangular in shape.	Is irregular and round in shape.
Cell wall	Present	Absent
Plasma membrane	Present	Present
Endoplasmic reticulum	Present	Present
Nucleus	Present and lies on one side of the cell	Present and lies in the centre of the cell
Lysosomes	Present but are very rare	Present
Centrosomes	Absent	Present
Golgi apparatus	Present	Present
Cytoplasm	Present	Present
Ribosome	Present	Present
Plastids	Present	Absent
Ribosomes	Present	Present
Vacuoles	Few large or a single, centrally positioned vacuole	Usually small and numerous

Cilia	Absent	Most of the animal cells consist of cilia
Mitochondria	Present, but fewer in number	Are present and are numerous
Essential nutrients	The plant cell can synthesize amino acids, vitamins and coenzymes	The animal cell cannot synthesize amino acids, vitamins, & coenzymes

Similarities

The plant cells and animals cells, though are different in many ways but they share few similarities also, like:

- Plant and Animal cells are eukaryotic cells.
- The both have the cell membrane.
- Well- defined nucleus is present.
- Both the cells contain Golgi apparatus.
- One of the most important parts of the cell is the Cytoplasm, which is also the present in both.
- Ribosomes are also found in both the plant cells and the animal cells.

AMINO ACIDS

Amino acids are organic molecules that, when linked together with other amino acids, form a protein. Amino acids are essential to life because the proteins they form are involved in virtually all cell functions. Some proteins function as enzymes, some as antibodies, while others provide structural support. Although there are hundreds of amino acids found in nature, proteins are constructed from a set of 20 amino acids.

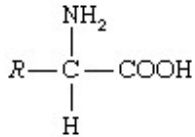
STRUCTURE

Generally, amino acids have the following structural properties:

- A carbon (the alpha carbon)
- A hydrogen atom (H)
- A Carboxyl group (-COOH)

- An Amino group (-NH₂)
- A "variable" group or "R" group

The formula of a general amino acid is:



Hence, amino acids, are a class of important biomolecules, that consist of a basic amino group (—NH₂), an acidic carboxyl group (—COOH), and an organic R group (or side chain) that is unique to each amino acid. The term amino acid is short for α -amino [alpha-amino] carboxylic acid. Each molecule contains a central carbon (C) atom, called the α -carbon, to which both an amino and a carboxyl group are attached. The remaining two bonds of the α -carbon atom are generally satisfied by a hydrogen (H) atom and the R group.

CLASSIFICATION

Amino acid can be classified **on the basis of their need to the human body and their availability** in the human body.

1] Essential Amino Acids

These are the acids that cannot be synthesized in our bodies. We must rely on food sources to obtain these amino acids. They are:

Leucine, Isoleucine, Lysine, Theorine, Methionine, Phenylalanine, Valine, Tryptophan, and Histidine (conditionally essential).

2] Non-Essential Amino Acids

These acids are synthesized in our bodies itself and we need not rely on outside sources for them. They are either produced in our bodies or obtained from protein breakdowns. They are:

Arginine, glutamine, tyrosine, cysteine, glycine, proline, serine, ornithine, alanine, asparagine, and aspartate.

Amino Acid can also **be classified based on their structure** and the structure of their side chains i.e. the R chains. Now two basic subcategories are

1] Non-Polar Amino Acids

These are also known as Hydrophobic. The R group can be either of Alkyl groups (with an alkyl chain) or Aromatic groups. The acids falling in this group are stated below. Numbers one to seven are Alkyl and the last two are aromatic.

The non polar amino acids are:

Glycine (H), Alanine (CH₃), Valine (CH (CH₃)₂), Methionine (CH₂CH₂SCH₃), Leucine (CH₂CH(CH₃)₂), Isoleucine (-CH(CH₃)CH₂CH₃), Proline (special structure), Phenylalanine.

2] Polar Amino Acids

If the side chains of amino acid contain different polar groups like amines, alcohols or acids they are polar in nature. These are also known as Hydrophilic Acids.

These are further divided into three further categories.

a) Acidic: If the side chain contains an extra element of carboxylic acid component these are acid-polar amino acids. They tend to donate their hydrogen atom. These are:

Aspartic Acid (CH₂COOH), Glutamic Acid (CH₂CH₂COOH)

b) Basic: These have an extra nitrogen group that tend to attract a hydrogen atom. The three basic polar amino acids are

Histidine, Lysine (CH₂(CH₂)₂NH₂), Arginine

c) Neutral: These are neither acidic nor basic. They have an equal number of amino and carboxyl groups. Also, they have at least one hydrogen component connected to electronegative atoms. Some of these neutral acids are:

Serine (CH₂OH), Threonine (CH(OH)CH₃), Asparagine (CH₂OHNH₂), Glutamine (CH₂CH₂CONH₂), Cysteine (CH₂SH), Tyrosine.

PROTEINS

Proteins are large, complex molecules that play many critical roles in the body. They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs. Proteins are made up of hundreds or thousands of smaller units called amino acids, which are attached to one another in long chains.

Composition of Proteins:

Proteins are organic compounds which are polymers of amino acids.

There are 20 different amino acids which make up proteins. Each amino acid consists of a central carbon. The central carbon is bonded to an amine group (NH₂), a carboxyl group (COOH), a hydrogen atom and an R group.

Amino acids can be linked together when the amine group of one amino acid is bonded to the carboxyl group of a different amino acid. The COOH donates an OH group and the NH₂ donates a H. The OH and H come together to form a water molecule, so this process is called dehydration synthesis - water is removed to form something new. The bond which holds the amino acids together is called a polypeptide.

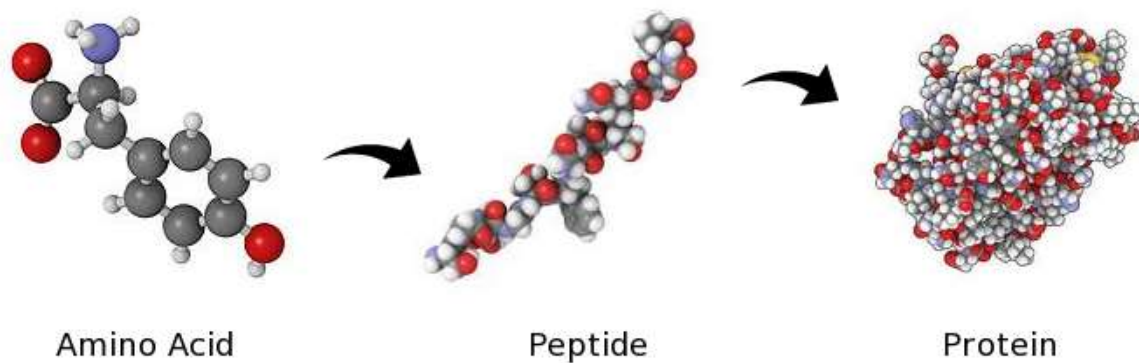
PROTEINS AND PEPTIDES

Proteins and peptides are fundamental components of cells that carry out important biological functions. Proteins give cells their shape, for example, and they respond to signals transmitted from the extracellular environment. Certain types of peptides play key roles in regulating the activities of other molecules. Structurally, proteins and peptides are very similar, being made up of chains of amino acids that are held together by peptide bonds (also called amide bonds). So, what distinguishes a peptide from a protein?

The basic distinguishing factors are size and structure. Peptides are smaller than proteins. Traditionally, peptides are defined as molecules that consist of between 2 and 50 amino acids, whereas proteins are made up of 50 or more amino acids. **In addition, peptides tend to be less well defined in structure than proteins, which can adopt complex conformations known as secondary,**

tertiary, and quaternary structures. Functional distinctions may also be made between peptides and proteins.

Peptides, however, may be subdivided into oligopeptides, which have few amino acids (e.g., 2 to 20), and polypeptides, which have many amino acids. Proteins are formed from one or more polypeptides joined together. Hence, proteins essentially are very large peptides. In fact, some researchers use the term peptide to refer specifically to oligopeptides, or otherwise relatively short amino acid chains, with the term polypeptide being used to describe proteins, or chains of 50 or more amino acids.



STRUCTURE OF PROTEIN

The shape of a protein is critical to its function because it determines whether the protein can interact with other molecules. Protein structures are very complex, and researchers have only very recently been able to easily and quickly determine the structure of complete proteins down to the atomic level. (The techniques used date back to the 1950s, but until recently they were very slow and laborious to use, so complete protein structures were very slow to be solved.) Early structural biochemists conceptually divided protein structures into four “levels” to make it easier to get a handle on the complexity of the overall structures. To determine how the protein gets its final shape or conformation, we need to understand these four levels of protein structure: primary, secondary, tertiary, and quaternary.

Primary Structure

A protein's primary structure is the unique sequence of amino acids in each polypeptide chain that makes up the protein. Really, this is just a list of which amino acids appear in which order in a polypeptide chain, not really a structure. But, because the final protein structure ultimately depends on this sequence, this was called the primary structure of the polypeptide chain. For example, the pancreatic hormone insulin has two polypeptide chains, A and B.

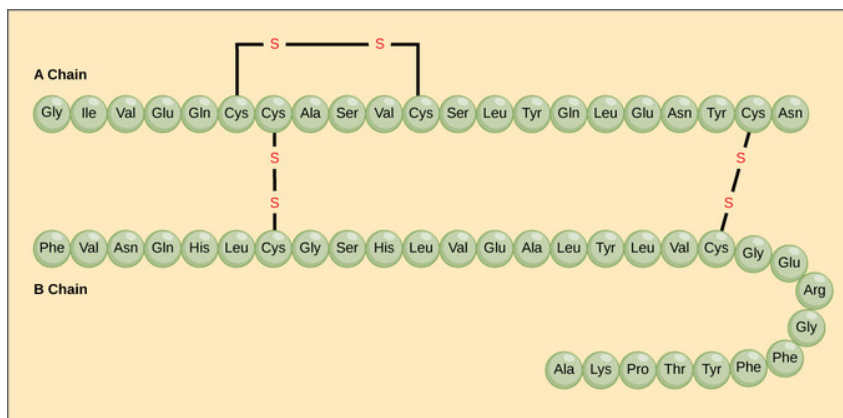


Fig. Primary structure. The A chain of insulin is 21 amino acids long and the B chain is 30 amino acids long, and each sequence is unique to the insulin protein.

The gene, or sequence of DNA, ultimately determines the unique sequence of amino acids in each peptide chain. A change in nucleotide sequence of the gene's coding region may lead to a different amino acid being added to the growing polypeptide chain, causing a change in protein structure and therefore function.

The oxygen-transport protein hemoglobin consists of four polypeptide chains, two identical α chains and two identical β chains. In sickle cell anemia, a single amino substitution in the hemoglobin β chain causes a change the structure of the entire protein. When the amino acid glutamic acid is replaced by valine in the β chain, the polypeptide folds into a slightly-different shape that creates a dysfunctional hemoglobin protein. So, just one amino acid substitution can cause dramatic changes. These dysfunctional hemoglobin proteins, under low-oxygen conditions, start associating with one another, forming long fibers made from millions of

aggregated hemoglobins that distort the red blood cells into crescent or “sickle” shapes, which clog arteries . People affected by the disease often experience breathlessness, dizziness, headaches, and abdominal pain.

Secondary Structure

A protein’s secondary structure is whatever regular structures arise from interactions between neighboring or near-by amino acids as the polypeptide starts to fold into its functional three-dimensional form. Secondary structures arise as H bonds form between local groups of amino acids in a region of the polypeptide chain. Rarely does a single secondary structure extend throughout the polypeptide chain. It is usually just in a section of the chain. The most common forms of secondary structure are the α -helix and β -pleated sheet structures and they play an important structural role in most globular and fibrous proteins.

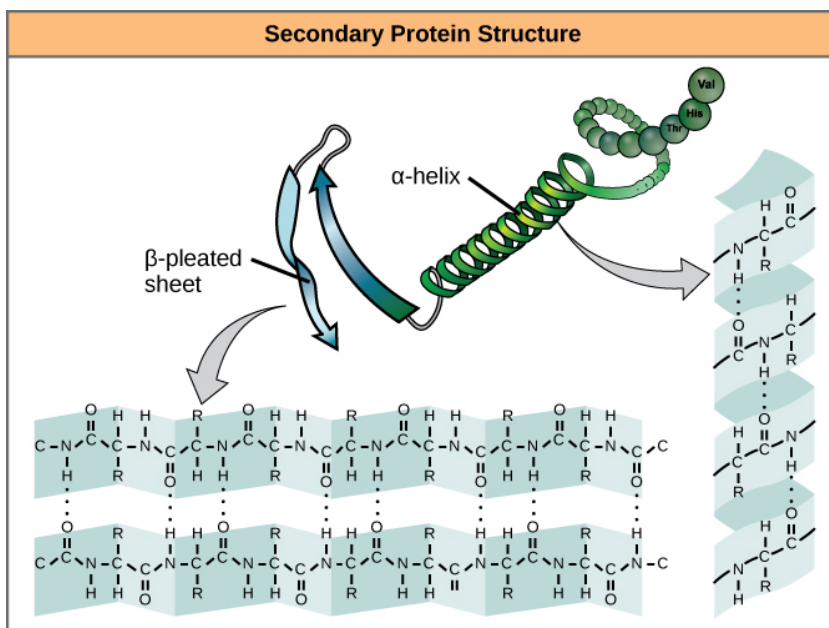


Fig.Secondary structure. The α -helix and β -pleated sheet form because of hydrogen bonding between carbonyl and amino groups in the peptide backbone. Certain amino acids have a propensity to form an α -helix, while others have a propensity to form a β -pleated sheet.

In the α -helix chain, the hydrogen bond forms between the oxygen atom in the polypeptide backbone carbonyl group in one amino acid and the hydrogen atom in the polypeptide backbone amino group of another amino acid that is four amino acids farther along the chain. This holds the stretch of amino acids in a right-handed coil. Every helical turn in an alpha helix has 3.6 amino acid residues. The R groups (the side chains) of the polypeptide protrude out from the α -helix chain and are not involved in the H bonds that maintain the α -helix structure.

In β -pleated sheets, stretches of amino acids are held in an almost fully-extended conformation that “pleats” or zig-zags due to the non-linear nature of single C-C and C-N covalent bonds. β -pleated sheets never occur alone. They have to be held in place by other β -pleated sheets. The stretches of amino acids in β -pleated sheets are held in their pleated sheet structure because hydrogen bonds form between the oxygen atom in a polypeptide backbone carbonyl group of one β -pleated sheet and the hydrogen atom in a polypeptide backbone amino group of another β -pleated sheet. The β -pleated sheets which hold each other together align parallel or antiparallel to each other. The R groups of the amino acids in a β -pleated sheet point out perpendicular to the hydrogen bonds holding the β -pleated sheets together, and are not involved in maintaining the β -pleated sheet structure.

Tertiary Structure

The tertiary structure of a polypeptide chain is its overall three-dimensional shape, once all the secondary structure elements have folded together among each other. Interactions between polar, nonpolar, acidic, and basic R groups within the polypeptide chain create the complex three-dimensional tertiary structure of a protein. When protein folding takes place in the aqueous environment of the body, the hydrophobic R groups of nonpolar amino acids mostly lie in the interior of the protein, while the hydrophilic R groups lie mostly on the outside. Cysteine side chains form disulfide linkages in the presence of oxygen, the only covalent bond forming during protein folding. All of these interactions, weak and strong, determine the final three-dimensional shape of the protein. When a protein loses its three-dimensional shape, it will no longer be functional.

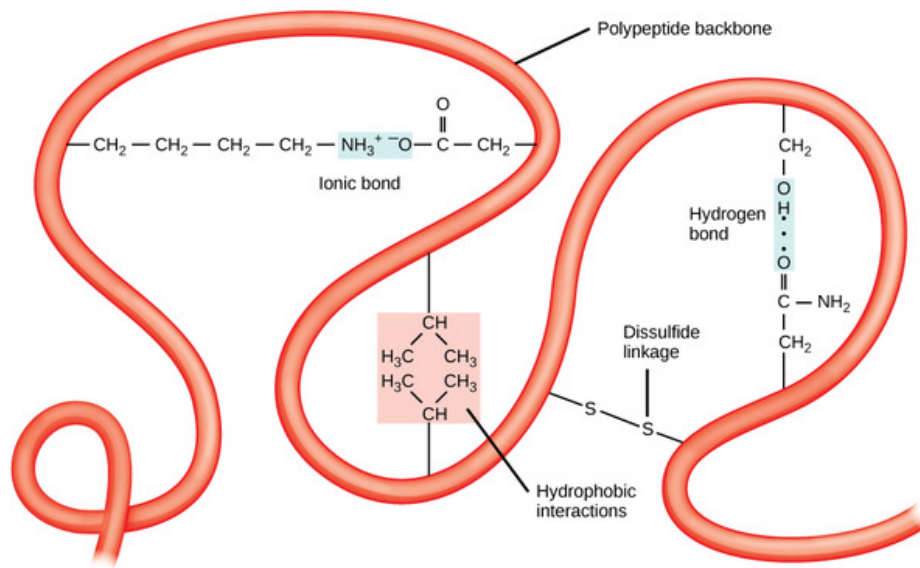


Fig. Tertiary structure-The tertiary structure of proteins is determined by hydrophobic interactions, ionic bonding, hydrogen bonding, and disulfide linkages.

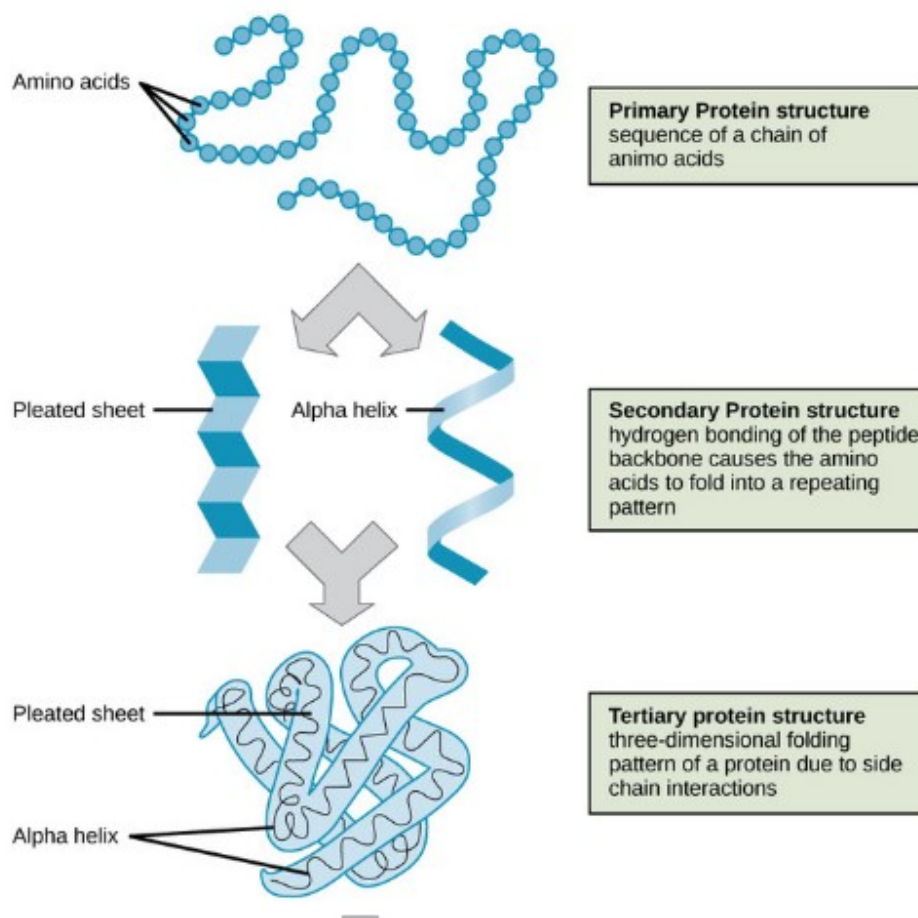


Fig. The three levels of protein structure can be observed in these illustrations.

ENZYMES

“Enzymes can be defined as biological polymers that catalyze biochemical reactions.”

Enzymes play an important role in all living organisms by regulating all the biological processes. The vast majority of enzymes are proteins with catalytic capabilities that are essential for maintaining various life processes. Metabolic processes and other chemical reactions in the cell are carried out by a set of enzymes that are necessary to sustain life.

The initial stage of metabolic process depends upon the enzymes, which react with a molecule and is called the substrate. Enzymes convert the substrates into other distinct molecules and are called the products.

Enzymes catalyze all kinds of chemical reactions that are involved in growth, blood coagulation, healing, diseases, breathing, digestion, reproduction, and many other biological activities. On biological aspects, enzymes are instrumental substances to many functions in living organisms.

Functions of Enzymes

The enzymes perform a number of functions in our body. These include:

1. Enzymes help in signal transduction. The most common enzyme used in the process includes protein kinase that catalyzes the phosphorylation of proteins.
2. They breakdown large molecules into smaller substances that can be easily absorbed by the body.
3. They help in generating energy in the body. ATP synthases are the enzymes involved in the synthesis of energy.
4. Enzymes are responsible for the movement of ions across the plasma membrane.
5. Enzymes perform a number of biochemical reactions, including oxidation, reduction, hydrolysis, etc. to eliminate the non-nutritive substances from the body.
6. They function to reorganize the internal structure of the cell to regulate cellular activities.

Mechanism of Enzyme Reaction

Any two molecules have to collide for the reaction to occur along with the right orientation and a sufficient amount of energy. The energy between these molecules needs to overcome the barrier in the reaction. This energy is called activation energy.

Enzymes are said to possess an active site. The active site is a part of the molecule that has a definite shape and the functional group for the binding of

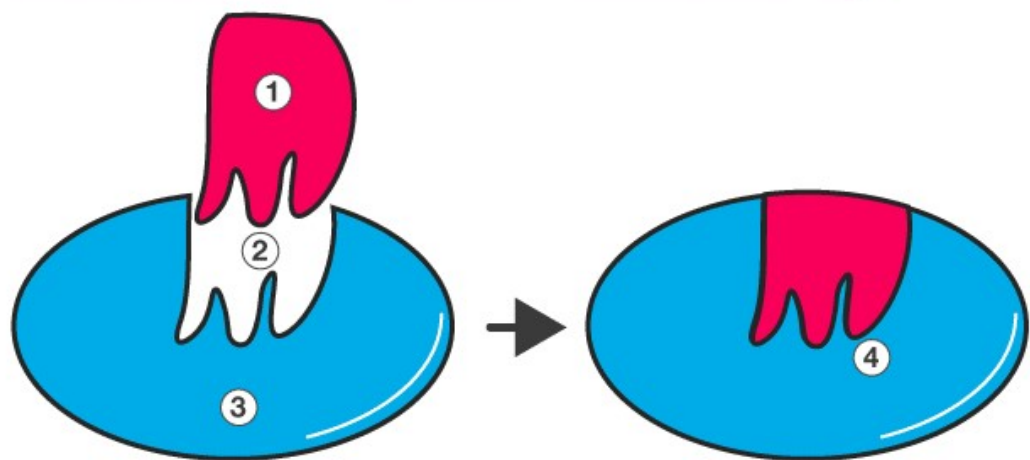
reactant molecules. The molecule binding with the enzyme is called the substrate group. The substrate and the enzyme form an intermediate reaction with low activation energy without any catalysts.

reactant(1)+reactant(2) → product

reactant(1)+enzyme → intermediate

intermediate+ reactant(2) → product+ enzyme

MECHANISM OF ENZYME REACTION



① Substrate | ② Active site | ③ Enzyme | ④ Enzyme-Substrate complex

The basic mechanism of enzyme action is to catalyze the chemical reactions, which begins with the binding of the substrate with the active site of the enzyme. This active site is a specific area that combines with the substrate.

Enzyme-Substrate Interactions

Enzymes are the biocatalysts with high molecular weight proteinous compound. It enhances the reactions which occur in the body during various life processes. It helps the substrate by providing the surface for the reaction to occur. The enzyme comprises hollow spaces occupying groups such as -SH, -COOH, and others on the outer surface. The substrate which has an opposite charge of the enzyme fits into these spaces just like a key fits into a lock. This substrate binding site is called the active site of an enzyme (E).

CARBOHYDRATES

Carbohydrates are one of the most important components of the biological world in addition to being one of the most abundant classes of biological molecules. The word 'carbohydrate' is derived from the Greek word 'sakcharon' meaning 'sugar'. Carbohydrates are nothing but aldehyde or ketone compounds with multiple hydroxyl groups. The literal meaning of carbohydrates is 'carbon hydrates' which originates from their chemical composition. The chemical composition of carbohydrates or saccharides is $(CH_2O)_n$ where $n > 3$ or $n = 3$.

It is generally a group of organic compounds occurring in living tissues and foods in the form of starch, cellulose, and sugars. The ratio of oxygen and hydrogen in carbohydrates is the same as in water i.e. 2:1. It typically breaks down in the animal body to release energy.

- Carbohydrates consist of carbon, hydrogen, and oxygen.
- The general empirical structure for carbohydrates is $(CH_2O)_n$.
- They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain.
- The building blocks of all carbohydrates are simple sugars called monosaccharides.
- A monosaccharide can be a polyhydroxy aldehyde (aldose) or a polyhydroxy ketone (ketose).

Biological Importance/ Role of Carbohydrates

- Living organisms use carbohydrates as accessible energy to fuel cellular reactions. They are the most abundant dietary source of energy (4kcal/gram) for all living beings.
- Carbohydrates along with being the chief energy source, in many animals, are instant sources of energy. Glucose is broken down by glycolysis/ Krebs' cycle to yield ATP.
- Serve as energy stores, fuels, and metabolic intermediates. It is stored as glycogen in animals and starch in plants.
- Stored carbohydrates act as an energy source instead of proteins.

- They form structural and protective components, like in the cell wall of plants and microorganisms. Structural elements in the cell walls of bacteria (peptidoglycan or murein), plants (cellulose) and animals (chitin).
- Carbohydrates are intermediates in the biosynthesis of fats and proteins.
- Carbohydrates aid in the regulation of nerve tissue and is the energy source for the brain.
- Carbohydrates get associated with lipids and proteins to form surface antigens, receptor molecules, vitamins, and antibiotics.
- Formation of the structural framework of RNA and DNA (ribonucleic acid and deoxyribonucleic acid).
- They are linked to many proteins and lipids. Such linked carbohydrates are important in cell-cell communication and in interactions between cells and other elements in the cellular environment.
- In animals, they are an important constituent of connective tissues.
- Carbohydrates that are rich in fiber content help to prevent constipation.
- Also, they help in the modulation of the immune system.

CLASSIFICATION

Carbohydrates are classified into three subtypes:

- 1) Monosaccharides,
- 2) Disaccharides,
- 3) Polysaccharides.

Monosaccharides

Monosaccharides (mono- = “one”; sacchar- = “sweet”) are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbons usually ranges from three to seven. 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, and if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose. Depending on the number of carbons in the sugar, they also may be known as trioses (three

carbons), pentoses (five carbons), and or hexoses (six carbons). See Fig 1 for an illustration of the monosaccharides.

MONOSACCHARIDES

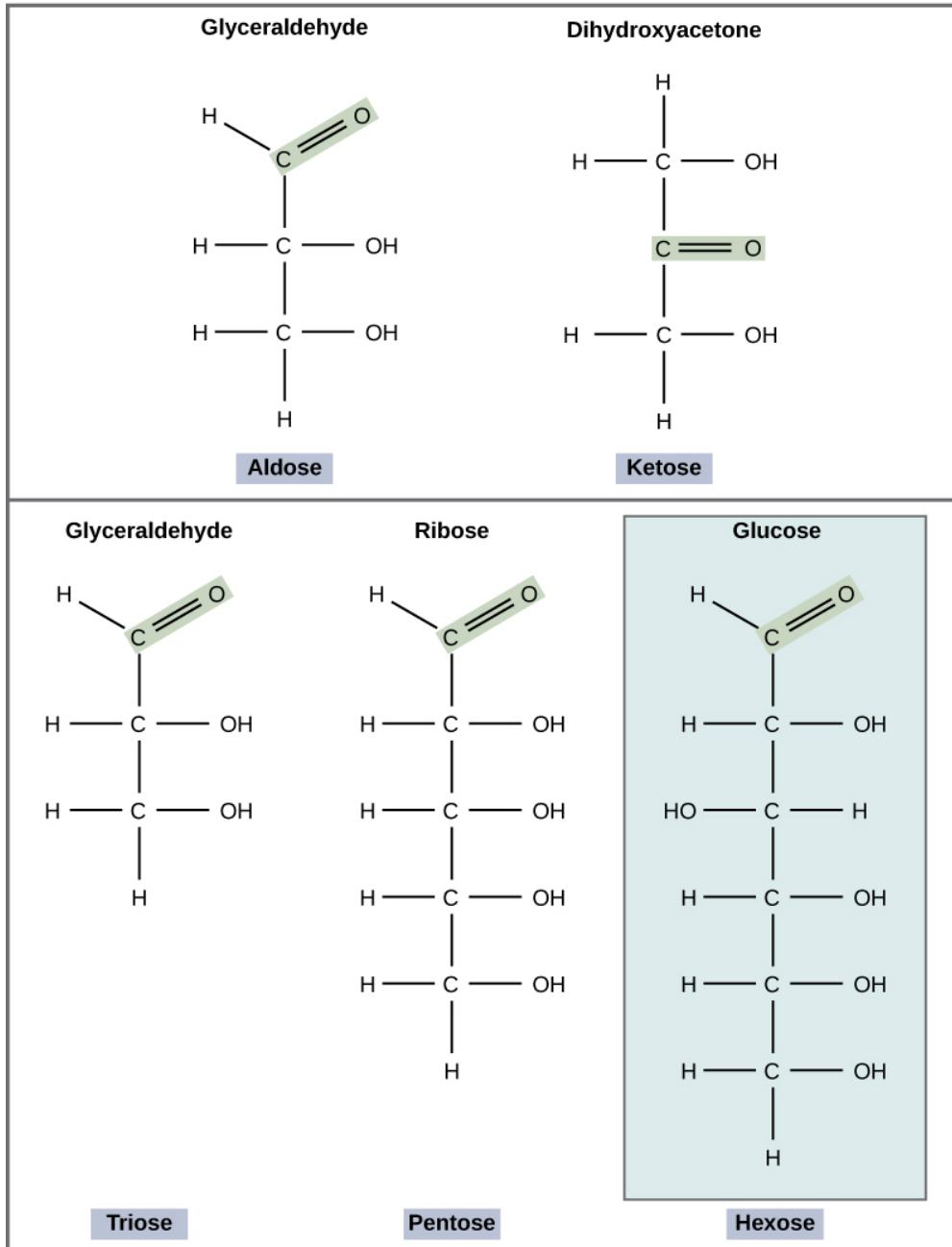


Figure 1. Monosaccharides are classified based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the end of the carbon chain, and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three, five, and six carbon backbones, respectively.

Disaccharides

Disaccharides (di = “two”) form when two monosaccharides undergo a dehydration reaction (also known as a condensation reaction or dehydration synthesis). During this process, the hydroxyl group of one monosaccharide combines with the hydrogen of another monosaccharide, releasing a molecule of water and forming a covalent bond. A covalent bond formed between a carbohydrate molecule and another molecule (in this case, between two monosaccharides) is known as a glycosidic bond (Figure 2). Glycosidic bonds (also called glycosidic linkages) can be of the alpha or the beta type.

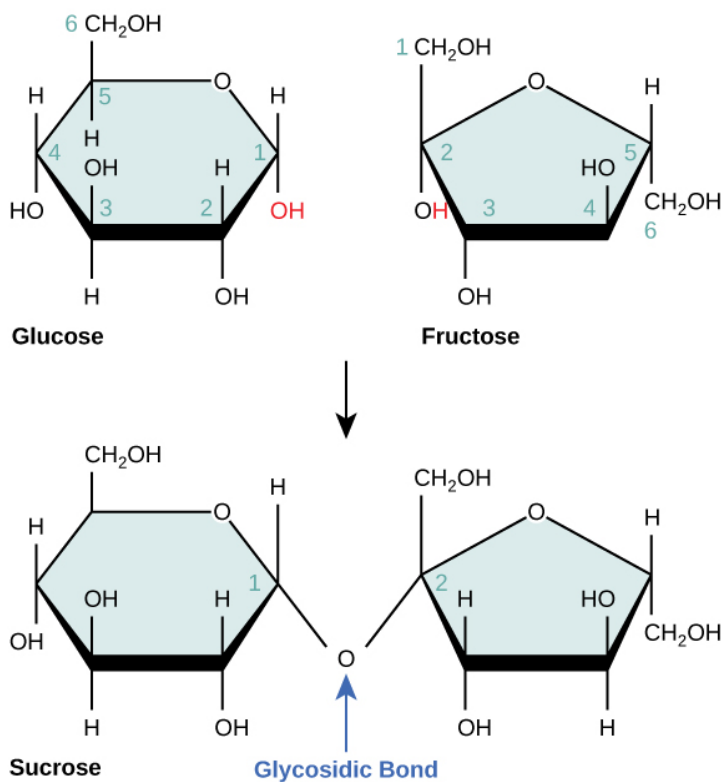


Figure 2. Sucrose is formed when a monomer of glucose and a monomer of fructose are joined in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage is formed between carbon 1 in glucose and carbon 2 in fructose.

Common disaccharides include lactose, maltose, and sucrose. Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is known as a polysaccharide (poly- = “many”). The chain may be branched or unbranched, and it may contain different types of monosaccharides. The molecular weight may be 100,000 daltons or more depending on the number of monomers joined. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Starch is the stored form of sugars in plants and is made up of a mixture of amylose and amylopectin (both polymers of glucose). Plants are able to synthesize glucose, and the excess glucose, beyond the plant’s immediate energy needs, is stored as starch in different plant parts, including roots and seeds. The starch in the seeds provides food for the embryo as it germinates and can also act as a source of food for humans and animals. The starch that is consumed by humans is broken down by enzymes, such as salivary amylases, into smaller molecules, such as maltose and glucose. The cells can then absorb the glucose.

Starch is made up of glucose monomers 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. As illustrated in Figure 3, 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 is the storage form of glucose in humans and other vertebrates and is made up of monomers of glucose. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever blood glucose levels decrease, glycogen is broken down to release glucose in a process known as glycogenolysis.

Cellulose is the most abundant natural biopolymer. The cell wall of plants is mostly made of cellulose; this provides structural support to the cell. Wood and paper are mostly cellulosic in nature. Cellulose is made up of glucose monomers that are linked by β 1-4 glycosidic bonds.

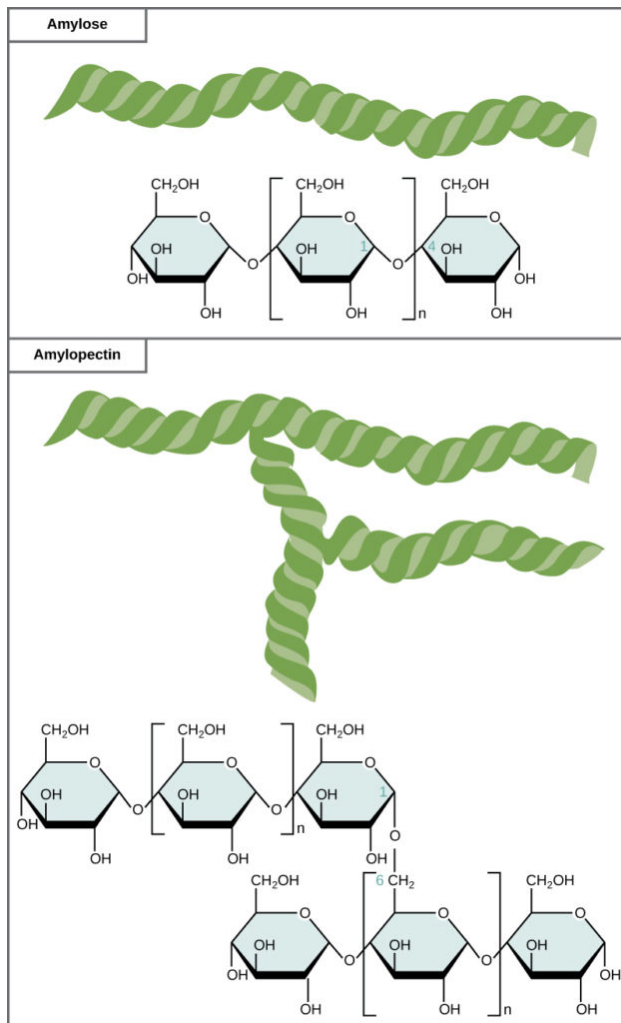


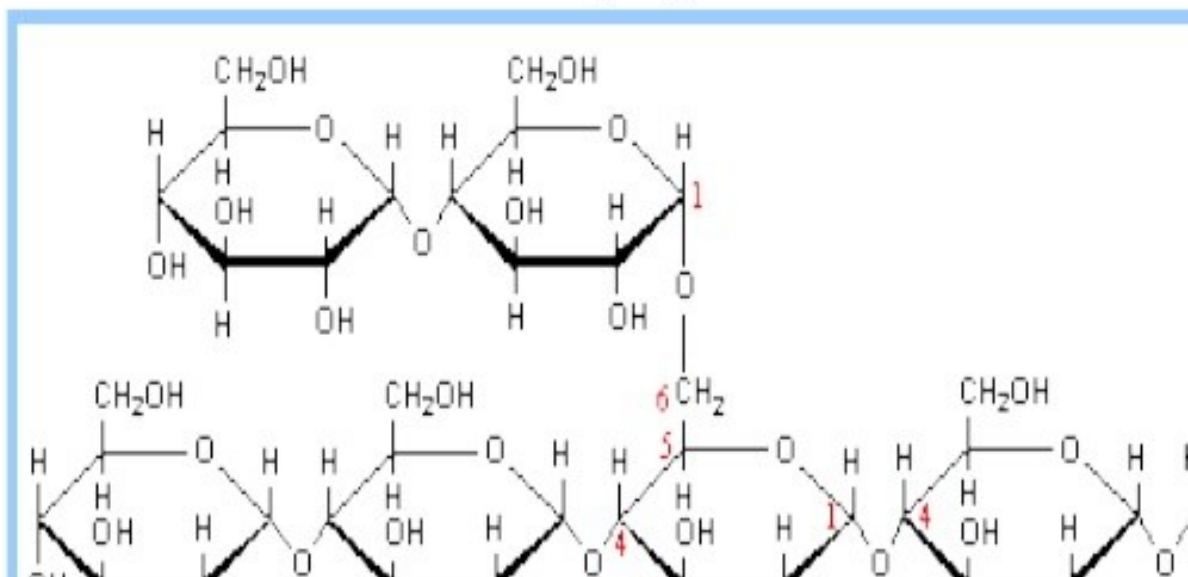
Figure 3. Amylose and amylopectin are two different forms of starch. Amylose is composed of unbranched chains of glucose monomers connected by α 1,4 glycosidic linkages. Amylopectin is composed of branched chains of glucose monomers connected by α 1,4 and α 1,6 glycosidic linkages. Because of the way the subunits are joined, the glucose chains have a helical structure. Glycogen (not shown) is similar in structure to amylopectin but more highly branched.

GLYCOGEN

Glycogen is a large, branched polysaccharide that is the main storage form of glucose in animals and humans. Glycogen is as an important energy reservoir; when energy is required by the body, glycogen is broken down to glucose, which then enters the glycolytic or pentose phosphate pathway or is released into the bloodstream. Glycogen is also an important form of glucose storage in fungi and bacteria.

Glycogen is a branched polymer of glucose. Glucose residues are linked linearly by α -1,4 glycosidic bonds, and approximately every ten residues a chain of glucose residues branches off via α -1,6 glycosidic linkages. The α -glycosidic bonds give rise to a helical polymer structure.

Structure of Glycogen molecule



GLYCOSAMINOGLYCON

Glycosaminoglycans (GAGs) are large linear polysaccharides constructed of repeating disaccharide units with the primary configurations containing an amino sugar (either GlcNAc or GalNAc) and an uronic acid (either glucuronic acid and/or iduronic acid). There are five identified glycosaminoglycan chains.

- 1) Hyaluronan
- 2) Chondroitin
- 3) Dermatan
- 4) Heparin/heparan
- 5) Keratan

Hyaluronan is not sulfated, but the other glycosaminoglycan chains contain sulfate substituents at various positions of the chain. The sulfate groups as well as the uronic acids result in the glycosaminoglycan chains having a negative charge.

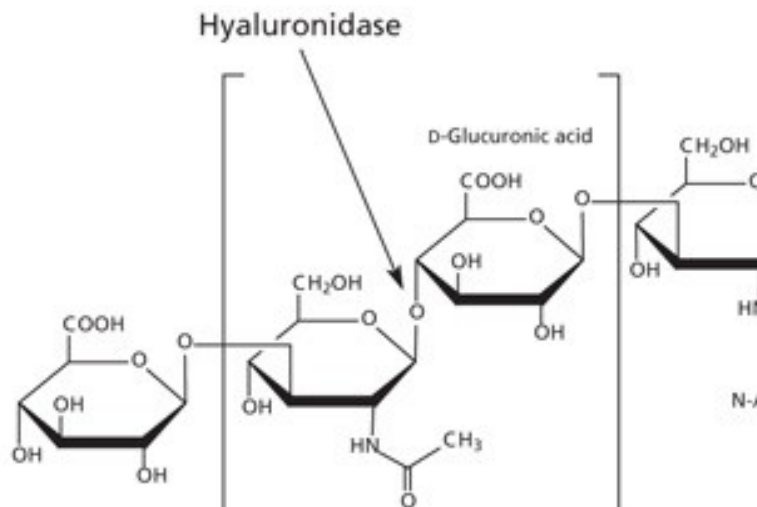


Figure. Hyaluronic acid is composed of alternating residues of β -D-(1-3) glucuronic acid and β -D-(1-4)-N-acetylglucosamine

LIPIDS

Lipids are a group of biological molecules that include fats, oils and some steroids. They are built from fatty acids bonded to a wide range of other compounds.

Their importance in the biological world is immense. They fill a number of important roles in the cells of all of Earth's organisms. Of the four molecules of life, lipids arguably have the greatest variation in their basic structure and are far more difficult to define than proteins, carbohydrate and nucleic acids.

Almost all lipids are insoluble in water. They are known as hydrophobic molecules because they are repelled by water.

- Arguably the most important function lipids perform is as the building blocks of cellular membranes.
- Other functions include energy storage, insulation, cellular communication and protection.
- Lipids serve as metabolic fuels alternative to glucose
- Lipids are a component of cell membranes
- They are very good insulators (subcutaneous fat, tunics of nerve conduction)

FATS AND FATTY ACIDS

Fats are a subgroup of compounds known as lipids that are found in the body and have the general property of being hydrophobic (meaning they are insoluble in water). Fats are also known as triglycerides, molecules made from the combination of one molecule of glycerol with three fatty acids (Figure 1).

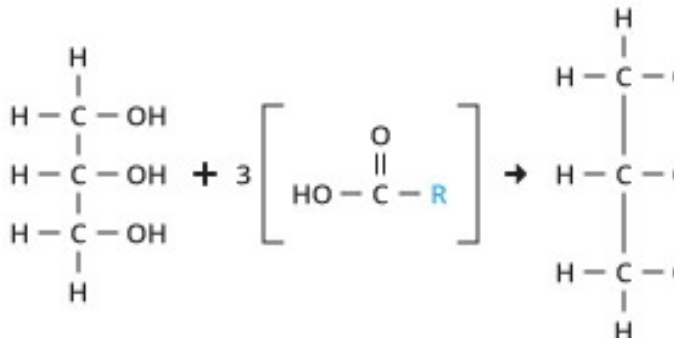


Figure 1: A fat molecule. The R in the three fatty acids represents a long C-C-C chain. In the triglyceride, the Rs may or may not be the same.

The main purpose of fats in the body is to serve as a storage system and reserve supply of energy. During periods of low food consumption, fat reserves in the body can be mobilized and broken down to release energy. Fats serve as an insulation material to allow body heat to be conserved and fats line and protect delicate internal organs from physical damage. Fats in the diet can be converted to other lipids that serve as the main structural material in the membranes surrounding our cells. Fats are also used in the manufacture of some steroids and hormones that help regulate proper growth and maintenance of tissue in the body.

Fats are a group of chemical compounds that contain fatty acids. Energy is stored in the body mostly in the form of fat. Fat is needed in the diet to supply essential fatty acids, substances essential for growth but not produced by the body itself.

Fatty acids can be classified as either saturated or unsaturated depending on the structure of the long carbon-carbon chains in the fatty acids :

Saturated Fatty acids: Fats that contain no double bonds in their fatty acid chains are referred to as saturated fatty acids. These fats tend to be solid at room temperature, such as butter or animal fat. The consumption of saturated fats carries

some health risks in that they have been linked to arteriosclerosis (hardening of the arteries) and heart disease.

Unsaturated Fatty acids: Unsaturated fatty acids contain some number of double bonds in their structure. These fats are generally liquid at room temperature (fats that are liquid at room temperature are referred to as oils). Unsaturated fatty acids can be either polyunsaturated (many double bonds) or monounsaturated fatty acids (one or few double bonds). Recent research suggests that the healthiest of the fats in the human diet are the monounsaturated fats, such as olive oil and canola oil, because they appear to be beneficial in the fight against heart disease.

OILS AND FAT

Oils

A mixture of triglycerols triglycerols that is liquid because it contains a high proportions of unsaturated fatty acids

Fat

A mixture of triglycerols that is solid because it contains a high proportions of saturated fatty acids.

COMPOUND LIPIDS

Lipids can be categorized as simple lipid, compound lipid and derived lipids. Compound lipids are Esters of fatty acids with alcohol and posses additional group. Compound lipids contain certain chemical groups in addition to alcohol and fatty acids. These group of lipids include glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins.

Compound lipids can be further divided into 2:

- Phospholipids
- Glycolipids

CHOLESTEROL

Cholesterol is a steroid lipid, found in the body tissues (and blood plasma) of vertebrates. It can be found in large concentrations within the liver, spinal cord, and brain. It plays a central role in many biochemical processes, but is best known for causing cardiovascular disease when present in elevated levels.

Properties

Mostly insoluble in water, it travels in the blood stream in the form of lipoproteins. Initially, it is carried from the intestinal mucosa to the liver in chylomicrons. In the liver it is converted into low-density lipoprotein (LDL) to carries cholesterol to the body cells, while high-density lipoprotein (HDL) carries it back to the liver for excretion.

It is interesting to note that the cholesterol in LDL cholesterol and the cholesterol in HDL cholesterol are identical. The only difference between the two is the carrier molecule (i.e. the lipoprote.)

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Functions

Cholesterol serves a variety of functions in human body. This includes:

- The manufacture of steroids, or cortisone-like hormones, including vitamin D and the sex hormones testosterone, estrogen and cortisone. This in turn controls a myriad of bodily functions.

- Assisting the liver in the manufacture of bile acids, which is essential for digestion and absorption of fat-soluble vitamins such as vitamin A, D, E and K.
- Formation of the myelin sheath, a neuron consisting of fat-containing cells that insulate the axon from electrical activity. This ensures proper function of our brains by aiding route of electrical impulses. The absence of cholesterol might lead to loss of memory and difficulty in focusing.
- As a cell to interconnect "lipid molecules", which are needed to stabilize our cell membranes.
- As a source of energy
- Maintenance of our body temperature
- Protection of internal organs
- Modulation the fluidity of cell membranes

BILE SALTS

Bile salts are one of the primary components of bile. Bile is a greenish-yellow fluid made by the liver and stored in our gallbladder.

Bile salts help with the digestion of fats in our bodies. They also help us to absorb fat-soluble vitamins like A, D, E, and K.

Bile salts are produced by the hepatocyte cells in the liver and are derived from cholesterol. When an alkaline substance meets an acid, it causes a neutralizing reaction. This reaction produces water and the chemical salts called bile salts.

Functions:

The role of bile (and bile salts) in the body is to:

- Aid digestion by breaking down fats
- Help absorb fat-soluble vitamins
- Eliminate waste products

Bile and bile salts are made in the liver and stored in the gallbladder between meals. After we eat and there are fats present in our digestive tracts, our hormones send a signal to our gallbladders to release bile.

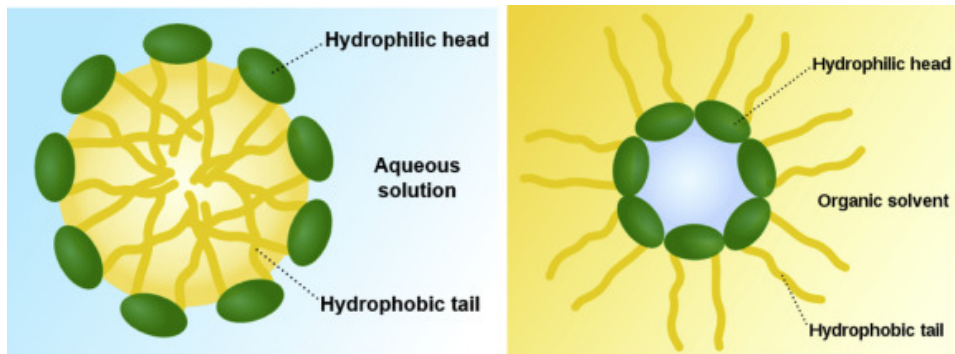
The bile is released into the first part of our small intestine called the duodenum. This is where most of the digestion happens. The bile helps to process and digest the fats.

Another primary function of bile is to remove toxins. Toxins are secreted into the bile and eliminated in feces. A lack of bile salts can cause a buildup of toxins in our bodies.

People who don't produce enough bile salts, possibly because they've had their gallbladders removed, can experience, Diarrhea, trapped gas, bad-smelling gas, stomach cramps, erratic bowel movements, weight loss, pale-colored stools.

MICELLE

Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions. In aqueous solution, molecules having both polar or charged groups and non polar regions (amphiphilic molecules) form aggregates called micelles. In a micelle, polar or ionic heads form an outer shell in contact with water, while non polar tails are sequestered in the interior. Hence, the core of a micelle, being formed of long non polar tails, resembles an oil or gasoline drop. The length of the non polar tail, the nature and size of the polar or ionic head, the acidity of the solution, the temperature, and the presence of added salts are the most important factors determining the kind of the obtained aggregate. If those parameters are changed, it is possible to change shape and size of the micelles. The number of amphiphilic molecules forming the aggregate is called aggregation number; it is a way to describe the size of the micelle.



micelle

reverse micelle

Micelles are widely used in industrial and biological fields for their ability to dissolve and move non polar substances through an aqueous medium, or to carry drugs which are, often, scarcely soluble in water. The carrying ability of micelles can be altered if parameters determining their size and shape are changed.

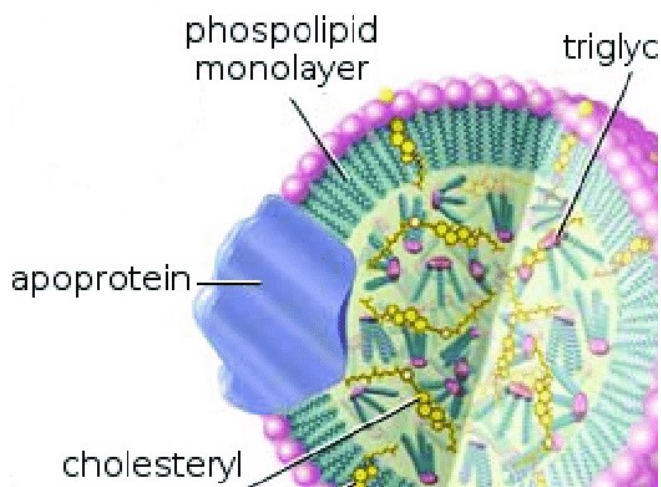
Micellization depends on the balance of two main effects: the tendency of the non polar tails to avoid contacts with water and the repulsion among the polar or charged heads, a destabilizing effect on the aggregation process. Hydrocarbon tails avoid contacts with the solvent molecules pointing toward the aggregate interior, which lacks of water. Instead, the repulsion among the charged heads on the surface of the micelle is attenuated by the presence of oppositely charged ions (counter-ions). The favourable association among the non polar tails in the interior of the micelle occurs through the hydrophobic interaction, which is the prevailing effect in the formation process of these aggregates.

Amphiphilic molecules can form micelles not only in water, but also in non polar organic solvents. In such cases, micelle aggregates are called inverse micelles because the situation is inverted as respect to water. In fact, hydrocarbon tails are exposed to the solvent, while the polar heads point toward the interior of the aggregate to escape the contacts with the solvent.

Reverse micelles are able to hold relatively large amounts of water in their interior. In that way, a "pocket" is formed which is particularly suited for the dissolution and transportation of polar solutes through a non polar solvent.

LIPOPROTEIN

Lipoprotein, any member of a group of substances containing both lipid (fat) and protein. Lipoproteins are basically a core full of fat and cholesterol, along with a lipid membrane that contains proteins called apolipoproteins. They occur in both soluble complexes—as in egg yolk and mammalian blood plasma—and insoluble ones, as in cell membranes. Lipoproteins in blood plasma have been intensively studied because they are the mode of transport for cholesterol through the bloodstream and lymphatic fluid.



Cholesterol is insoluble in the blood, and so it must be bound to lipoproteins in order to be transported. Two types of lipoprotein are involved in this function: low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). LDLs transport cholesterol from its site of synthesis in the liver to the body's cells, where the cholesterol is separated from the LDL and is then used by the cells for various purposes. HDLs probably transport excess or unused cholesterol from the body's tissues back to the liver, where the cholesterol is broken down to bile acids and is then excreted. About 70 percent of all cholesterol in the blood is carried by LDL particles, and most of the remainder is carried by HDLs. LDL-bound cholesterol is primarily responsible for the atherosclerotic buildup of fatty deposits on the blood vessel walls, while HDL particles may actually reduce or retard such atherosclerotic buildups and are thus beneficial to health.

Body cells extract cholesterol from the blood by means of tiny coated pits (receptors) on their surfaces; these receptors bind with the LDL particles (and their

attached cholesterol) and draw them from the blood into the cell. There are limits to how much cholesterol a body cell can take in, however, and a cell's capture of LDL particles inhibits the making of more LDL receptors on that cell's surface, thus lowering its future intake of cholesterol. Fewer receptors on the body cells means that less cholesterol is ingested by the cells and that more remains in the bloodstream, thus increasing the risk of cholesterol accumulating in the interior walls of blood vessels.

Several hereditary genetic disorders, called hyperlipoproteinemias, involve excessive concentrations of lipoproteins in the blood. Other such diseases, called hypolipoproteinemias, involve abnormally reduced lipoprotein levels in the blood.

BIMOLECULAR LEAFLET MODEL

Harvey and Coley (1931) and Danielli and Harvey (1935) studied surface tension of cell membrane and on the basis of their observation they pointed out the existence of protein molecules adsorbed on the surface of lipid droplets which reduce the surface tension of droplets.

This conclusion led **James Danielli and Hugh Davson** in 1935 to suggest bimolecular leaflet model of cell membrane. Danielli and Davson model was the first attempt to describe membrane structure in terms of molecules and to relate the structure to biological and chemical properties.

According to bimolecular model of Danielli and Davson, plasma membrane consists of two layers of phospholipid molecules (a bimolecular leaflet) in which phospholipid molecules are arranged in such a way that hydrophilic heads of the phospholipid molecules face outside and hydrophobic non-polar lipid chains are associated in the inner region of leaflet.

The hypothesis also suggested that the polar ends of lipid molecules are associated with monomolecular layer of globular proteins. The plasma membrane would thus consist of a double layer of phospholipid molecules sandwiched between two essentially continuous layers of protein (Fig. 2.2).

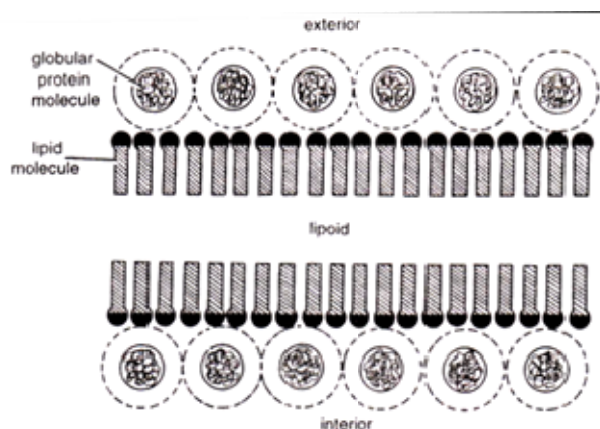


Fig. 2.2 Sandwich model of Danielli and Davson.

This basic model has been modified from time to time. Danielli (1938) suggested the presence of two types of proteins; tangentially arranged in contact with the lipid and globular proteins on the outer surface. Again Davson and Danielli (1943) and Danielli (1954) considered proteins to be in the form of a folded P-chain.

Perhaps, these units form micelles of membranes indicated in recent electron micrographs. Membrane models are usually postulated to contain protein lined polar pores of about 7 Å diameter which probably permit the passage of small ions and water molecules across the membrane.

In still other variations the proteins are thought to be in coiled or globular form on both sides of lipid layers or they are thought to be asymmetrical, with a folded P-chain on one side and globular proteins on the other.

NUCLEOTIDES

DNA and RNA are made up of monomers known as nucleotides. The nucleotides combine with each other to form a polynucleotide: DNA or RNA. Each nucleotide is made up of three components:

1. a nitrogenous base
2. a pentose (five-carbon) sugar
3. a phosphate group

Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to one or more phosphate groups.

NITROGENOUS BASE

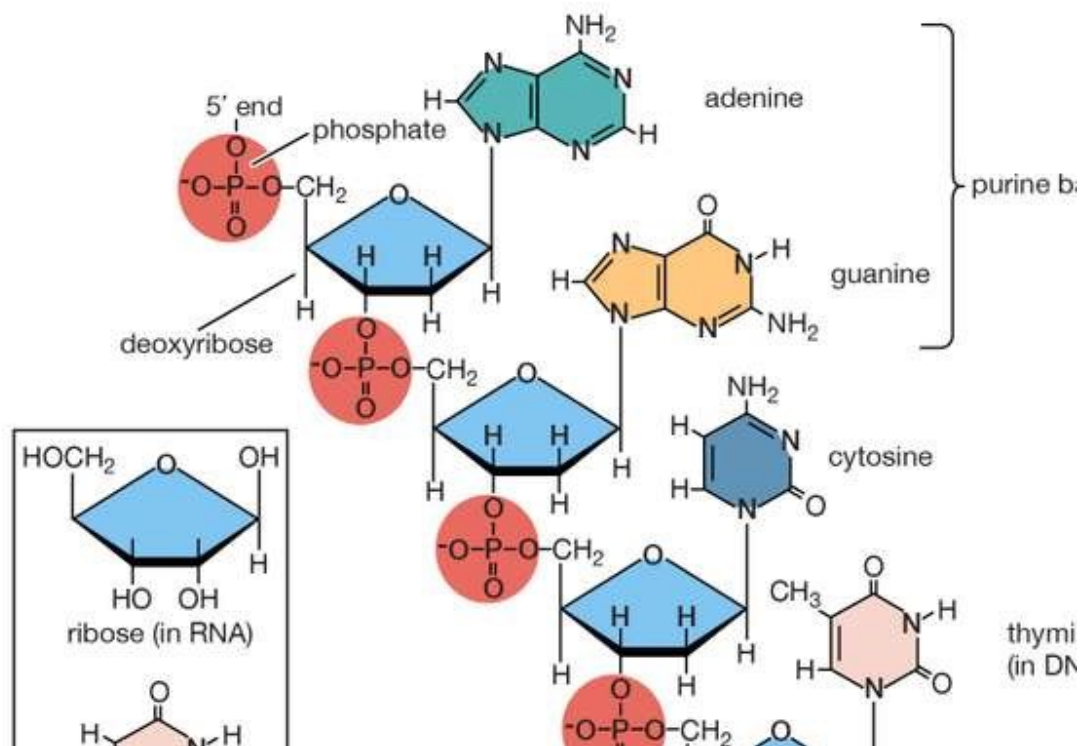
The nitrogenous bases are organic molecules and are so named because they contain carbon and nitrogen. They are bases because they contain an amino group that has the potential of binding an extra hydrogen, and thus, decreasing the hydrogen ion concentration in its environment, making it more basic. Each nucleotide in DNA contains one of four possible nitrogenous bases: adenine (A), guanine (G) cytosine (C), and thymine (T).

Adenine and guanine are classified as purines. The primary structure of a purine consists of two carbon-nitrogen rings. Cytosine, thymine, and uracil are classified as pyrimidines which have a single carbon-nitrogen ring as their primary structure. Each of these basic carbon-nitrogen rings has different functional groups attached to it. In molecular biology shorthand, the nitrogenous bases are simply known by their symbols A, T, G, C, and U. DNA contains A, T, G, and C whereas RNA contains A, U, G, and C.

DNA

DNA, abbreviation of deoxyribonucleic acid, organic chemical of complex molecular structure that is found in all prokaryotic and eukaryotic cells and in many viruses. DNA codes genetic information for the transmission of inherited traits.

The chemical DNA was first discovered in 1869, but its role in genetic inheritance was not demonstrated until 1943. In 1953 James Watson and Francis Crick, aided by the work of biophysicists Rosalind Franklin and Maurice Wilkins, determined that the structure of DNA is a double-helix polymer, a spiral consisting of two DNA strands wound around each other. The breakthrough led to significant advances in scientists' understanding of DNA replication and hereditary control of cellular activities.



Each strand of a DNA molecule is composed of a long chain of monomer nucleotides. The nucleotides of DNA consist of a deoxyribose sugar molecule to which is attached a phosphate group and one of four nitrogenous bases: two purines (adenine and guanine) and two pyrimidines (cytosine and thymine). The nucleotides are joined together by covalent bonds between the phosphate of one nucleotide and the sugar of the next, forming a phosphate-sugar backbone from which the nitrogenous bases protrude. One strand is held to another by hydrogen bonds between the bases; the sequencing of this bonding is specific—i.e., adenine bonds only with thymine, and cytosine only with guanine.

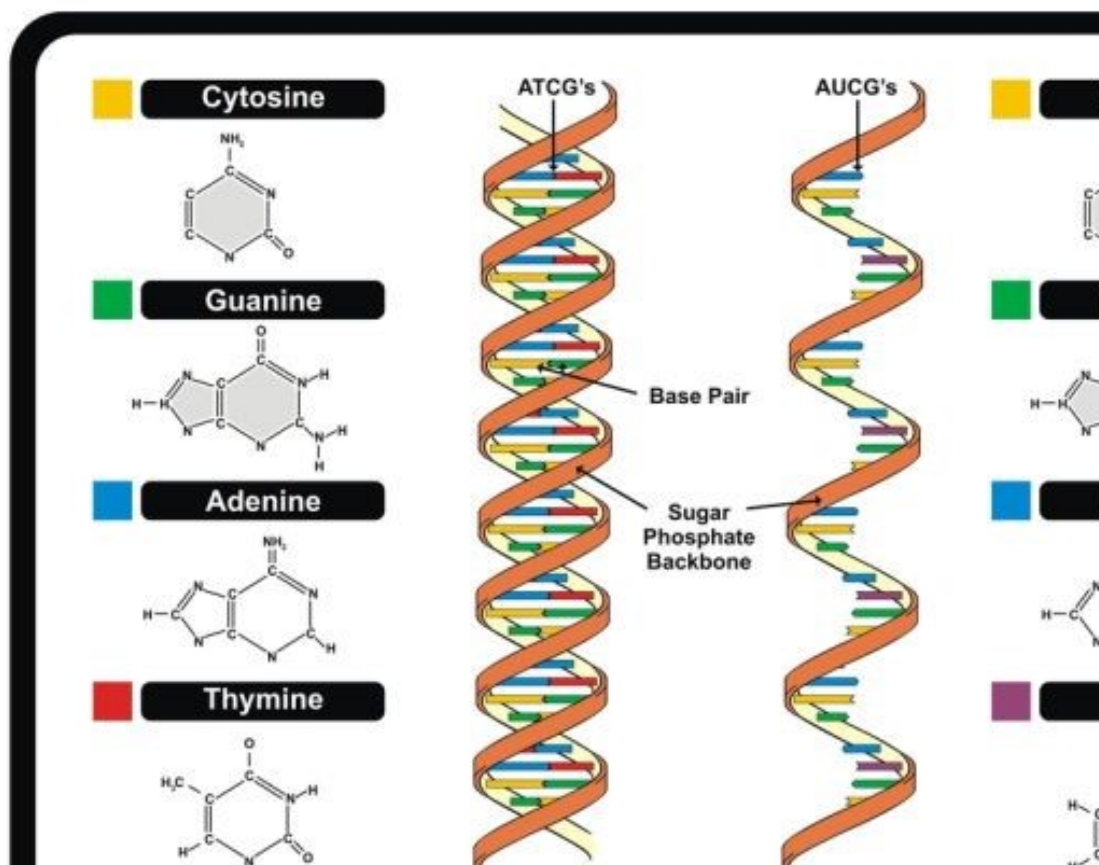
Function

- DNA replicates and stores genetic information.
- It is a blueprint for all genetic information contained within an organism.
- Replication
- Encoding Information
- Mutation and Recombination
- Gene Expression

RNA

RNA is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group. The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions. The RNA-specific pyrimidine uracil forms a complementary base pair with adenine and is used instead of the thymine used in DNA.

RNA is a ribonucleic acid that helps in the synthesis of proteins in our body. This nucleic acid is responsible for the production of new cells in the human body. It is usually obtained from the DNA molecule. RNA resembles the same as that of DNA, the only difference being that it has a single strand unlike the DNA which has two strands and it consists of an only single ribose sugar molecule in it. Hence is the name Ribonucleic acid. RNA is also referred to as an enzyme as it helps in the process of chemical reactions in the body.



TYPES OF RNA

Messenger RNA (mRNA) is an intermediate between a protein-coding gene and its protein product. If a cell needs to make a particular protein, the gene encoding the protein will be turned “on,” meaning an RNA-polymerizing enzyme will come and make an RNA copy, or transcript, of the gene’s DNA sequence. The transcript carries the same information as the DNA sequence of its gene. However, in the RNA molecule, the base T is replaced with U. For instance, if a DNA coding strand has the sequence 5’-AATTGCGC-3’, the sequence of the corresponding RNA will be 5’-AAUUGCGC-3’.

Once an mRNA has been produced, it will associate with a ribosome, a molecular machine that specializes in assembling proteins out of amino acids. The ribosome uses the information in the mRNA to make a protein of a specific sequence, “reading out” the mRNA’s nucleotides in groups of three (called codons) and adding a particular amino acid for each codon.

Ribosomal RNA (rRNA) is a major component of ribosomes, where it helps mRNA bind in the right spot so its sequence information can be read out. Some rRNAs also act as enzymes, meaning that they help accelerate (catalyze) chemical reactions – in this case, the formation of bonds that link amino acids to form a protein. RNAs that act as enzymes are known as ribozymes.

Transfer RNAs (tRNAs) are also involved in protein synthesis, but their job is to act as carriers – to bring amino acids to the ribosome, ensuring that the amino acid added to the chain is the one specified by the mRNA. Transfer RNAs consist of a single strand of RNA, but this strand has complementary segments that stick together to make double-stranded regions. This base-pairing creates a complex 3D structure important to the function of the molecule.

Function

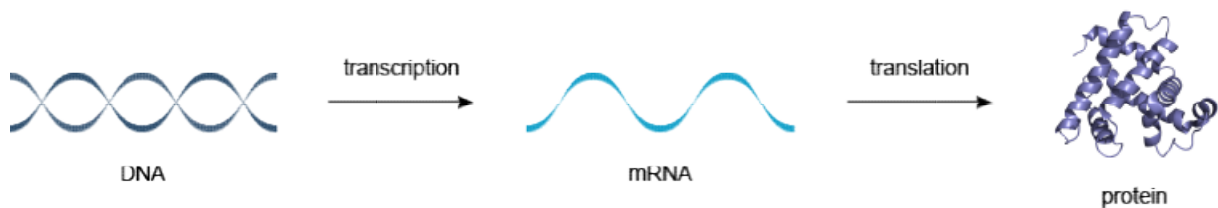
- Facilitate the translation of DNA into proteins
- Functions as an adapter molecule in protein synthesis
- Serves as a messenger between the DNA and the ribosomes.
- They are the carrier of genetic information in all living cells
- Promotes the ribosomes to choose the right amino acid which is required in building up of new proteins in the body.

DNA, RNA AND PROTEIN SYNTHESIS

The genetic material is stored in the form of DNA in most organisms. In humans, the nucleus of each cell contains 3×10^9 base pairs of DNA distributed over 23 pairs of chromosomes, and each cell has two copies of the genetic material. This is known collectively as the human genome. The human genome contains around 30 000 genes, each of which codes for one protein.

Large stretches of DNA in the human genome are transcribed but do not code for proteins. These regions are called introns and make up around 95% of the genome. The nucleotide sequence of the human genome is now known to a reasonable degree of accuracy but we do not yet understand why so much of it is non-coding. Some of this non-coding DNA controls gene expression but the purpose of much of it is not yet understood. This is a fascinating subject that is certain to advance rapidly over the next few years.

The Central Dogma of Molecular Biology states that DNA makes RNA makes proteins (Figure below).



The process by which DNA is copied to RNA is called transcription, and that by which RNA is used to produce proteins is called translation.

DNA REPLICATION

Each time a cell divides, each of its double strands of DNA splits into two single strands. Each of these single strands acts as a template for a new strand of complementary DNA. As a result, each new cell has its own complete genome. This process is known as DNA replication. Replication is controlled by the Watson-Crick pairing of the bases in the template strand with incoming deoxynucleoside triphosphates, and is directed by DNA polymerase enzymes. It is a complex process, particularly in eukaryotes, involving an array of enzymes. A simplified version of bacterial DNA replication is described in Figure 2.

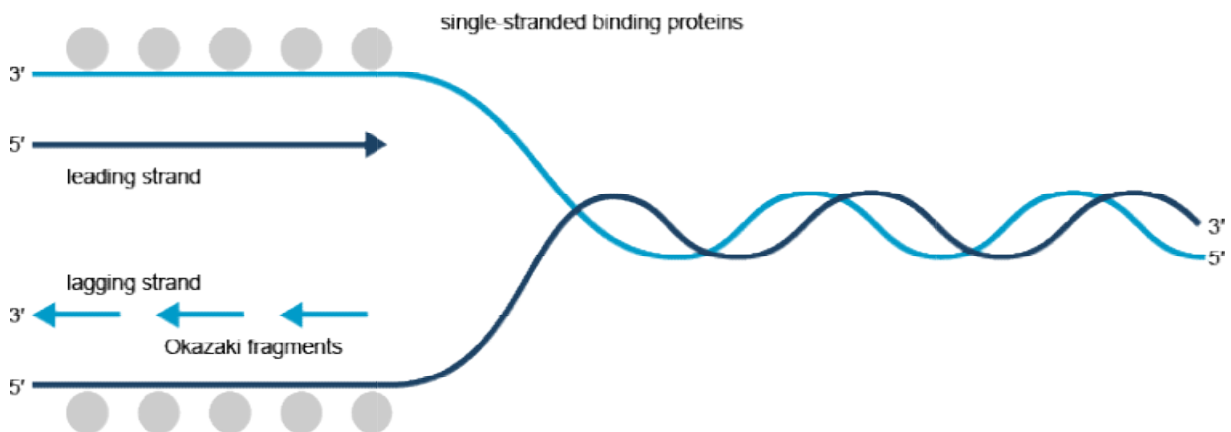


Figure 2 | DNA replication in bacteria - Simplified representation of DNA replication in bacteria.

DNA biosynthesis proceeds in the 5'- to 3'-direction. This makes it impossible for DNA polymerases to synthesize both strands simultaneously. A portion of the double helix must first unwind, and this is mediated by helicase enzymes.

The leading strand is synthesized continuously but the opposite strand is copied in short bursts of about 1000 bases, as the lagging strand template becomes available. The resulting short strands are called Okazaki fragments (after their discoverers, Reiji and Tsuneko Okazaki). Bacteria have at least three distinct DNA polymerases: Pol I, Pol II and Pol III; it is Pol III that is largely involved in chain elongation. Strangely, DNA polymerases cannot initiate DNA synthesis de novo, but require a short primer with a free 3'-hydroxyl group. This is produced in the lagging strand by an RNA polymerase (called DNA primase) that is able to use the DNA template and synthesize a short piece of RNA around 20 bases in length. Pol III can then take over, but it eventually encounters one of the previously synthesized short RNA fragments in its path. At this point Pol I takes over, using its 5'- to 3'-exonuclease activity to digest the RNA and fill the gap with DNA until it reaches a continuous stretch of DNA. This leaves a gap between the 3'-end of the newly synthesized DNA and the 5'-end of the DNA previously synthesized by Pol III. The gap is filled by DNA ligase, an enzyme that makes a covalent bond between a 5'-phosphate and a 3'-hydroxyl group (Figure 3). The initiation of DNA replication at the leading strand is more complex and is discussed in detail in more specialized texts.

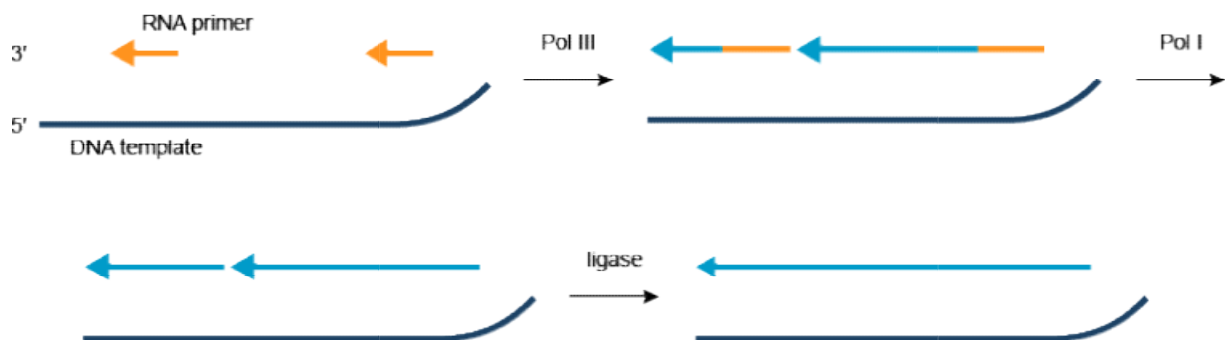


Figure 3 | DNA polymerases in DNA replication - Simplified representation of the action of DNA polymerases in DNA replication in bacteria.

TRANSCRIPTION

Transcription is the process by which DNA is copied (transcribed) to mRNA, which carries the information needed for protein synthesis. Transcription takes place in two broad steps. First, pre-messenger RNA is formed, with the involvement of RNA polymerase enzymes. The process relies on Watson-Crick base pairing, and the resultant single strand of RNA is the reverse-complement of the original DNA sequence. The pre-messenger RNA is then "edited" to produce the desired mRNA molecule in a process called RNA splicing.

Formation of pre-messenger RNA:

The mechanism of transcription has parallels in that of DNA replication. As with DNA replication, partial unwinding of the double helix must occur before transcription can take place, and it is the RNA polymerase enzymes that catalyze this process.

Unlike DNA replication, in which both strands are copied, only one strand is transcribed. The strand that contains the gene is called the sense strand, while the complementary strand is the antisense strand. The mRNA produced in transcription is a copy of the sense strand, but it is the antisense strand that is transcribed.

Ribonucleoside triphosphates (NTPs) align along the antisense DNA strand, with Watson-Crick base pairing (A pairs with U). RNA polymerase joins the ribonucleotides together to form a pre-messenger RNA molecule that is complementary to a region of the antisense DNA strand. Transcription ends when the RNA polymerase enzyme reaches a triplet of bases that is read as a "stop" signal. The DNA molecule re-winds to re-form the double helix.

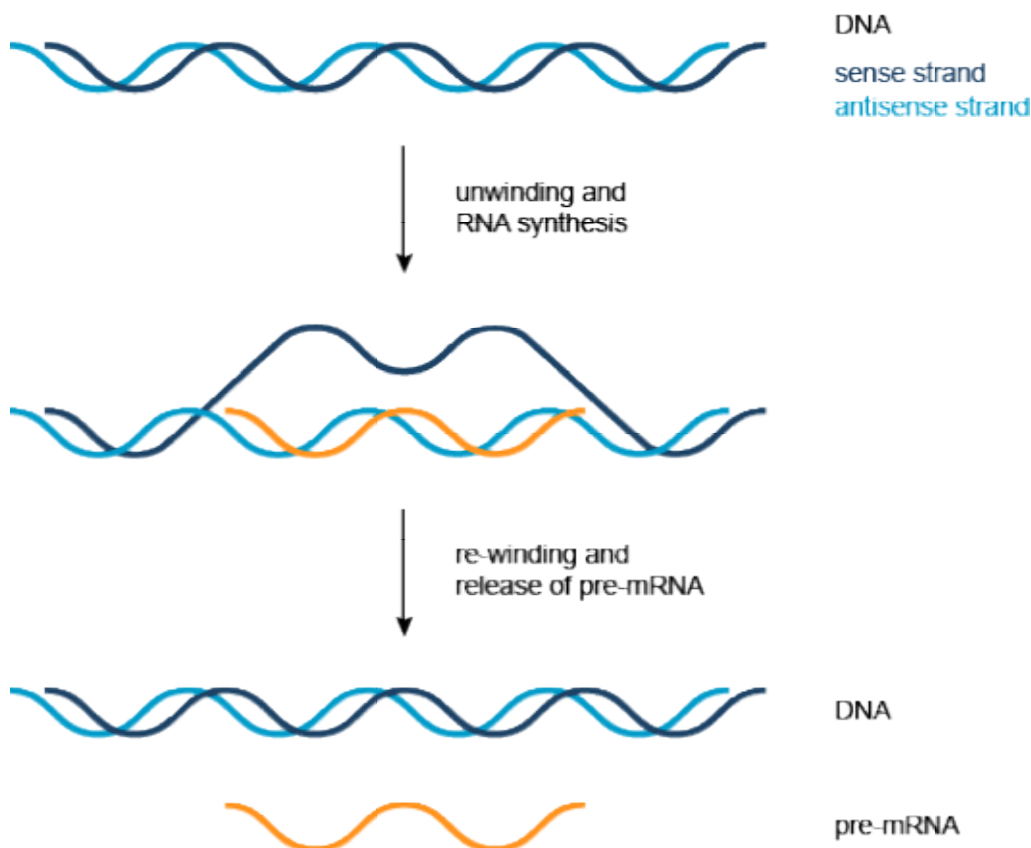


Figure 4 | Transcription - Simplified representation of the formation of pre-messenger RNA (orange) from double-stranded DNA (blue) in transcription.

REVERSE TRANSCRIPTION

In reverse transcription, RNA is "reverse transcribed" into DNA. This process, catalyzed by reverse transcriptase enzymes, allows retroviruses, including the human immunodeficiency virus (HIV), to use RNA as their genetic material. Reverse transcriptase enzymes have also found applications in biotechnology, allowing scientists to convert RNA to DNA for techniques such as PCR.

TRANSLATION

The mRNA formed in transcription is transported out of the nucleus, into the cytoplasm, to the ribosome (the cell's protein synthesis factory). Here, it directs protein synthesis. Messenger RNA is not directly involved in protein synthesis – transfer RNA (tRNA) is required for this. The process by which mRNA directs protein synthesis with the assistance of tRNA is called translation.

The ribosome is a very large complex of RNA and protein molecules. Each three-base stretch of mRNA (triplet) is known as a codon, and one codon contains the information for a specific amino acid. As the mRNA passes through the ribosome, each codon interacts with the anticodon of a specific transfer RNA (tRNA) molecule by Watson-Crick base pairing. This tRNA molecule carries an amino acid at its 3'-terminus, which is incorporated into the growing protein chain. The tRNA is then expelled from the ribosome. Figure 7 shows the steps involved in protein synthesis.

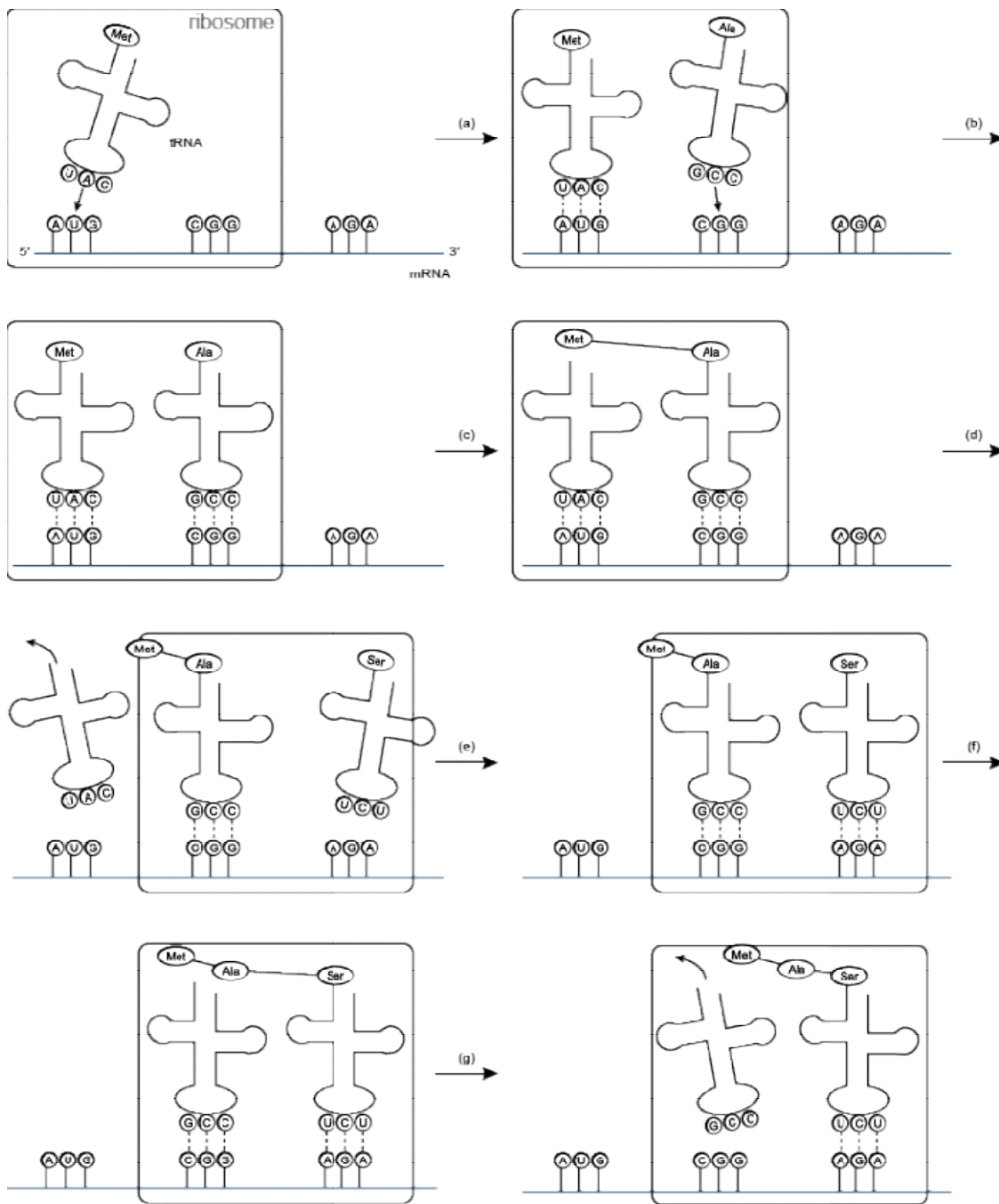


Figure 7 | Translation (a) and (b) tRNA molecules bind to the two binding sites of the ribosome, and by hydrogen bonding to the mRNA; (c) a peptide bond forms between the two amino acids to make a dipeptide, while the tRNA molecule is left uncharged; (d) the uncharged tRNA molecule leaves the ribosome, while the ribosome moves one codon to the right (the dipeptide is translocated from one binding site to the other); (e) another tRNA molecule binds; (f) a peptide bond forms between the two amino acids to make a tripeptide; (g) the uncharged tRNA molecule leaves the ribosome.

10

Serology Concepts

10.1 Serological Reagents

10.1.1 Immunogens and Antigens

A foreign substance that is capable of eliciting antibody formation when introduced into a host is called an *immunogen*. Natural immunogens are usually macromolecules such as proteins and polysaccharides. Other molecular structures can also act as immunogens, for example, glycolipids (such as A, B, and O blood group antigens) and glycoproteins (such as Rh and Lewis antigens). However, they must be foreign to their hosts. The molecular structure of an immunogen, usually a small portion recognized by an antibody, is called an *epitope* or determinant site. An immunogen usually consists of multiple epitopes and is thus considered *multivalent* (Figure 10.1). Each epitope can elicit the production of its own corresponding antibody.

An *antigen* is a foreign substance that is capable of reacting with an antibody. All immunogens can be considered antigens, but not all antigens can elicit antibody formation. *Hapten* is one example of a substance that is antigenic but not immunogenic. Haptens are chemical compounds that are too small to elicit antibody production when they are introduced to a host animal. However, a hapten can be coupled to a *carrier*, usually a macromolecule, to produce antibodies. A hapten-conjugated carrier can become immunogenic to elicit the formation of an antibody specific to the hapten. The resulting antibody can bind to free haptens. Certain controlled substances such as cocaine and amphetamines are haptens and can be detected through corresponding antibodies for forensic toxicological analysis.

10.1.2 Antibodies

Antibodies, also known as *immunoglobulins*, are capable of binding specifically to antigens and are designated with an Ig prefix. The five major classes of immunoglobulins are designated IgG, IgA, IgM, IgD, and IgE. Additionally, IgG immunoglobulins can be further divided into four subclasses (IgG1–IgG4) and IgA immunoglobulins can be further divided into two subclasses (IgA1 and IgA2). Thus, there are a total of nine immunoglobulin isotypes in humans. *Isotypes* are the immunoglobulins that differ based on the molecular variations in the constant domains of the heavy and light chains (Figure 10.2). IgD, IgE, and IgG are usually monomers. IgM can be a membrane-bound monomer or a cross-linked pentamer (secreted form). IgA can be a monomer, dimer, or trimer. In an immune response, an initial exposure to an immunogen elicits a primary response, producing IgM immunoglobulins. Further exposure to the immunogen can elicit a secondary response, producing IgG, IgA, IgE, and IgD immunoglobulins. IgG is the most abundant immunoglobulin in serum. A majority of serology tests are based on the IgG immunoglobulins.

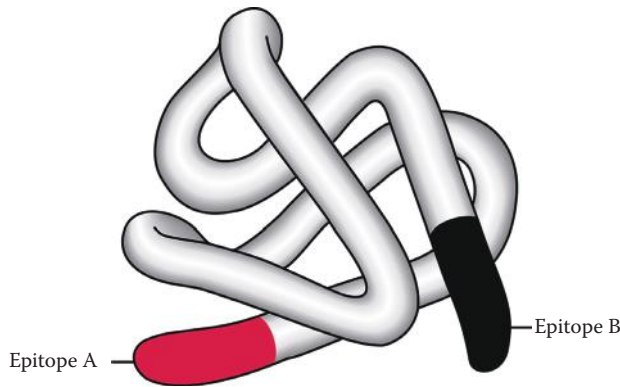


Figure 10.1 Multivalent immunogen. A protein with two different epitopes is shown. (© Richard C. Li)

Immunoglobulins have many similarities in their molecular structures. Figure 10.2 illustrates a diagram of the IgG molecule. The structures of immunoglobulins were first revealed by Gerald Edelman and Rodney Porter who shared a Nobel Prize in 1972. Immunoglobulins are composed of four polypeptide chains: two heavy (H) chains and two light (L) chains. The polypeptide chains are linked by disulfide bonds into a Y-shaped complex. The H chain can be divided into fragment antigen-binding (*Fab*) and fragment crystallizable (*Fc*) fragments. The L chain consists of a *Fab* fragment only. A typical antibody has two identical antigen-binding sites and is thus considered *bivalent*. The antigen-binding activity is located within the *Fab* fragments. In particular, the N-terminal ends of the L and H chains together form antigen-binding sites. At the amino acid sequence level, both H and L chains have *variable* and *constant* domains (Figure 10.2). The variable domains are located at the N-terminal ends of the immunoglobulins. Additionally, three small *hypervariable regions* are located within the variable domain of each chain.

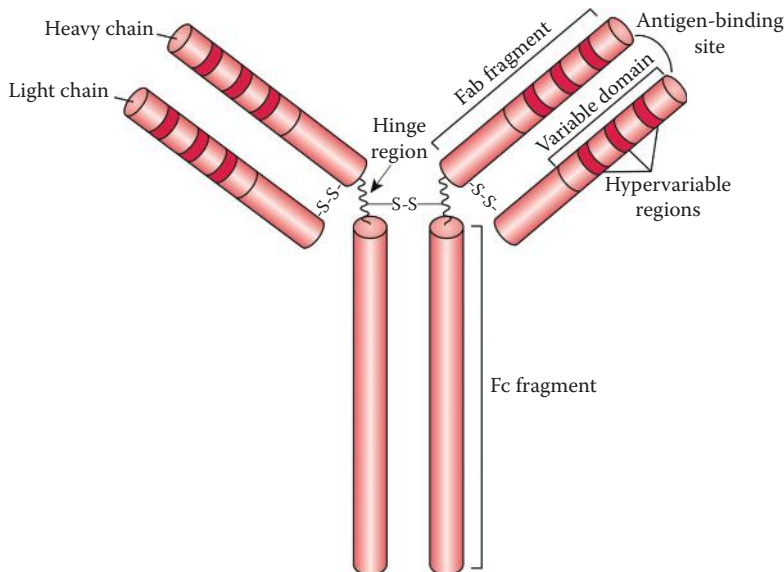


Figure 10.2 Immunoglobulin IgG structure. Immunoglobulin IgG is composed of light and heavy chains that contain variable domains. The remaining portions of chains form the constant domains. The variable domains of both light and heavy chains contain three hypervariable regions that form the antigen-binding site of the immunoglobulin. (© Richard C. Li.)

The diversity in the amino acid sequences of these hypervariable regions determines the specificity of the antigen-binding sites. The hinge regions provide flexibility to the antibody molecule and are important for the efficiency of the binding and cross-linking reactions. The basal portion of the H chain consists of *Fc* fragment.

The binding affinity and specificity of antibodies make them useful reagents for serological testing. Two types of antibodies are commonly used: polyclonal and monoclonal antibodies.

10.1.2.1 Polyclonal Antibodies

To produce an antibody, an immunogen is usually introduced into a host animal. A multivalent immunogen is capable of eliciting a mixture of antibodies with diverse specificities for the immunogen. As a result, a polyclonal antibody is produced by different B lymphocyte clones in response to the different epitopes of the immunogen. Antibodies can be circulating (in blood or other bodily fluids) or tissue bound (in cell surface antibodies). Circulating immunoglobulins are referred to as humoral antibodies.

The blood from an immune host is drawn and allowed to clot, resulting in the formation of a solid consisting largely of blood cells and a liquid portion known as *serum* containing antibodies (Figure 10.3). Such a preparation of humoral antibodies is also called a *polyclonal antiserum*. Depending on the type of animal used, the antibodies produced are classified as avian (B), rabbit (R), or horse (H) type. The characteristics of polyclonal antibodies may vary if they are produced from different individual host animals of the same species. Variations in reactions among different sources of antibodies should be monitored by quality-control procedures.

10.1.2.2 Monoclonal Antibodies

To produce a monoclonal antibody, spleen cells are harvested from a host animal, such as a mouse, inoculated with an immunogen (Figure 10.4). Next, the plasma cells of the spleen, which produce antibodies, are fused with myeloma cells. Since only a small population of cells fuse, a selection step is needed to allow only fused cells to grow (Figure 10.4). The fused cells, called *hybridoma* cells, are immortal (proliferate indefinitely) in cell cultures. Pools of hybridoma cells are diluted into single clones and are allowed to proliferate. The clones are then screened for the specific antibody of interest.

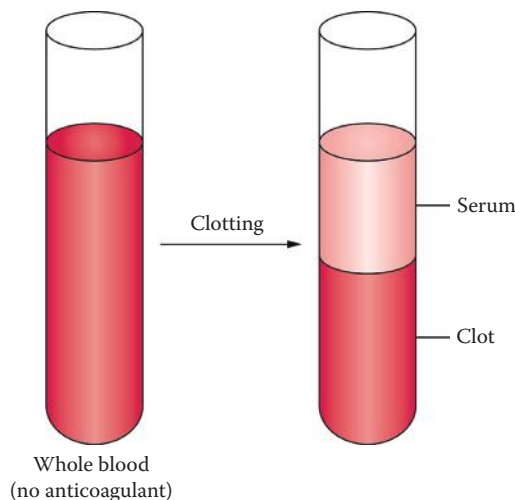


Figure 10.3 Serum component of blood. The blood of an immunized animal is collected in the absence of an anticoagulant and is allowed to clot. The resulting liquid portion of the blood is serum. (© Richard C. Li.)

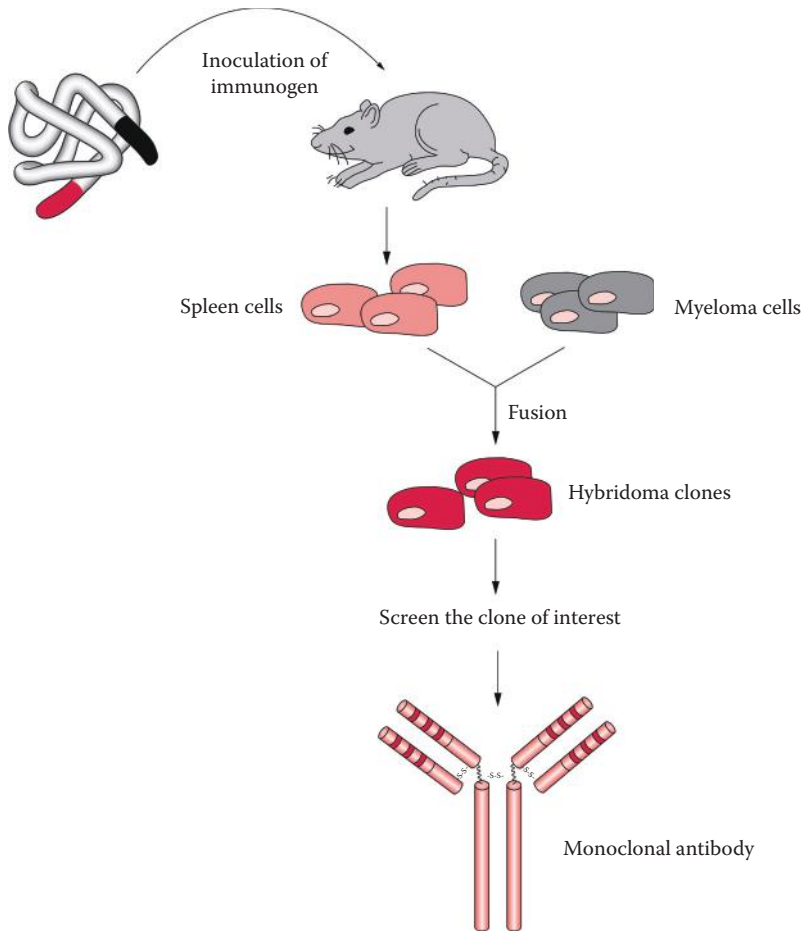


Figure 10.4 Preparation of a monoclonal antibody. A mouse is immunized with an immunogen, and its spleen cells are fused with myeloma cells to generate hybridoma cells. The clone that synthesizes and secretes the monoclonal antibody of interest can then be identified. (© Richard C. Li.)

The desired hybridoma clone can be maintained indefinitely, and it produces a monoclonal antibody that reacts with a single epitope. The hybridoma-derived monoclonal antibodies are specific and homogenous, and can be obtained in unlimited quantities. Monoclonal antibodies have been utilized in many serological assays, as discussed in Chapter 11. However, they have certain limitations in serology assays. For instance, monoclonal antibodies react with only a single epitope of a multivalent antigen and, therefore, cannot form cross-linked networks in precipitation assays (see Section 10.3.2.1).

10.1.2.3 Antiglobulins

Immunoglobulins are proteins that can also function as immunogens. If a purified foreign immunoglobulin or a fragment of a foreign immunoglobulin is introduced into a host, the antibodies produced are known as *antiglobulins*. Antiglobulins that are specific to a particular isotype can be produced in laboratories. In addition to specific antiglobulins, it is possible to produce nonspecific antiglobulins, which recognize an epitope that is common to all isotypes of an immunoglobulin class, such as the Fc portion of the heavy chain of all subclasses of human IgG. Antiglobulins are important reagents in many serological tests.

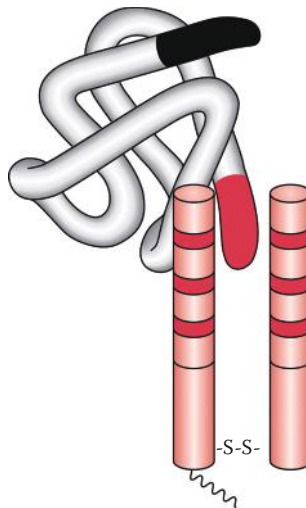


Figure 10.5 Affinity is a measure of the interaction between a single epitope on an antigen and a single binding site on an antibody. (© Richard C. Li.)

10.2 Strength of Antigen–Antibody Binding

The binding of an antigen to its specific antibody is mediated by the interaction between the epitope of an antigen and the binding site of its antibody. Noncovalent bonds can be formed during antigen–antibody binding. Various forces act cooperatively during antigen–antibody binding. These include hydrogen bonding, hydrophobic interactions that exclude water molecules from the area of contact, and van der Waals forces arising from the asymmetric distribution of the charges of electrons. The binding process occurs rapidly and the formation of the antigen–antibody complex is reversible. Such binding occurs at short distances when the antigen and antibody are in close proximity. Additionally, the strongest binding occurs only if the shape of the epitope fits the binding site of the antibody. The strength of the interaction between the antigen and the antibody depends on two characteristics, designated affinity and avidity.

Affinity is the energy of the interaction of a single epitope on an antigen and a single binding site on a corresponding antibody (Figure 10.5). The strength of the interaction depends on the specificity of the antibody for the antigen. Nevertheless, antibodies can bind with lower strength to antigens that are structurally similar to the immunogen. Such binding is known as *cross-reaction*.

Avidity is the overall strength of the binding of an antibody and an antigen (Figure 10.6). Since an antigen is usually considered multivalent and an antibody is bivalent, the avidity reflects the combined synergistic strength of the binding of all the binding sites of antigens and antibodies rather than the sum of individual affinities. It also reflects the overall stability of an antigen–antibody complex that is essential for many serological assays.

10.3 Antigen–Antibody Binding Reactions

The binding of an antigen to an antibody is an equilibrium reaction consisting of three types of reactions. The primary and secondary reactions form the basis for many forensic serological assays and will be discussed in the following subsections. The third type is called the tertiary reaction. It is used to measure *in vivo* immune responses such as inflammation and phagocytosis. Because most forensic serology tests are *in vitro* assays, the tertiary reaction is not commonly utilized in forensic serology testing and will not be discussed here.

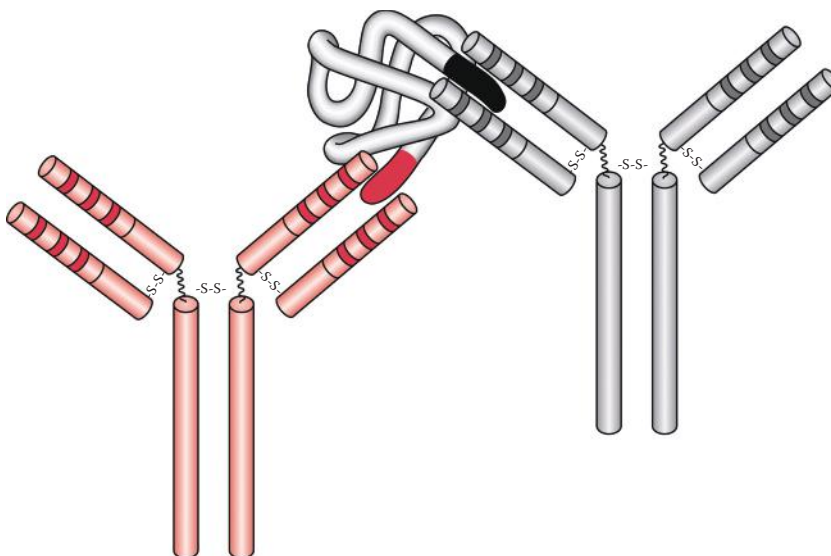
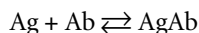


Figure 10.6 Avidity is a measure of the overall strength of the binding between antigens and antibodies. (© Richard C. Li.)

10.3.1 Primary Reactions

A primary reaction is the initial binding of a single epitope of an antigen (Ag) and a single binding site of an antibody (Ab) to form an antigen–antibody complex (Figure 10.7). This rapid and reversible binding reaction can be expressed as



At equilibrium, the strength of the interaction can be expressed as the affinity constant (K_a) that reflects the affinity of binding, where:

$$K_a = \frac{[\text{AgAb}]}{[\text{Ag}][\text{Ab}]}$$

The square brackets indicate the concentration of each component at equilibrium. K_a is the reciprocal of the concentration of free epitopes when half the antibody-binding sites are occupied. Thus, a higher K_a corresponds to a stronger binding interaction.

Techniques such as enzyme immunoassays, immunofluorescence assays, radioimmunoassays, and dye-labeled immunochromatography can measure the concentrations of antigen–antibody complexes formed by primary reactions (Chapter 11). These techniques are the most sensitive for detecting the presence of an antigen and an antibody in a sample. Additionally, many forensic serology assays are based on the detection of primary reactions and will be discussed in Chapters 12 through 17.

10.3.2 Secondary Reactions

The primary reaction between an antigen and an antibody is often followed by a secondary reaction. The three types of secondary reactions are precipitation, agglutination, and complement fixation. The techniques that detect secondary reactions are usually less sensitive but easier to perform than primary reaction assays. The precipitation and agglutination reactions form the basis for many serologic assays performed in forensic laboratories. These reactions will be discussed in the following subsections in detail. The third type of reaction is called *complement fixation*. If an antigen is located on a cell surface, the binding of the antigen and the antibody

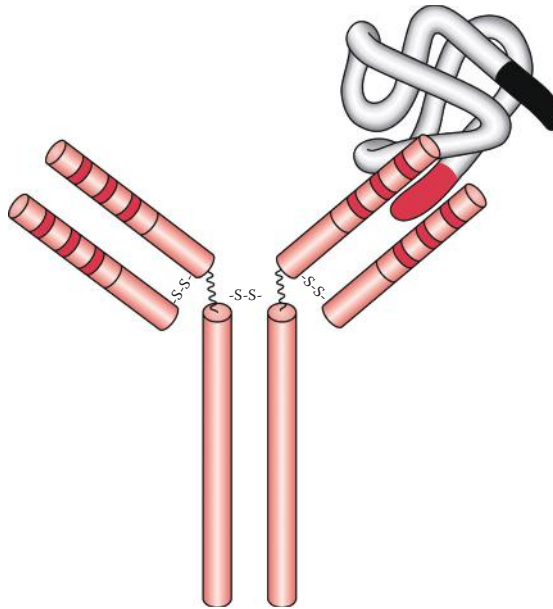


Figure 10.7 Primary reaction. Initial binding forms an antigen–antibody complex. (© Richard C. Li.)

may activate the classical complement pathway and lead to cell lysis, also known as a complement fixation reaction. The detection of this type of reaction is not commonly used in forensic serology.

10.3.2.1 Precipitation

If a soluble antigen is mixed and incubated with its antibody, the antigen–antibody complexes can form cross-linked complexes at the optimal ratio of antigen-to-antibody concentration. The cross-linked complex is insoluble and eventually forms a precipitate that settles to the bottom of a test tube. Antibodies that produce such precipitation are also called *precipitins*.

This precipitation reaction can be characterized by examining the effect of varying the relative ratio of antigen and antibody. If an increasing amount of soluble antigen is mixed with a constant amount of antibody, the amount of precipitate formed can be plotted. A precipitin curve (Figure 10.8)

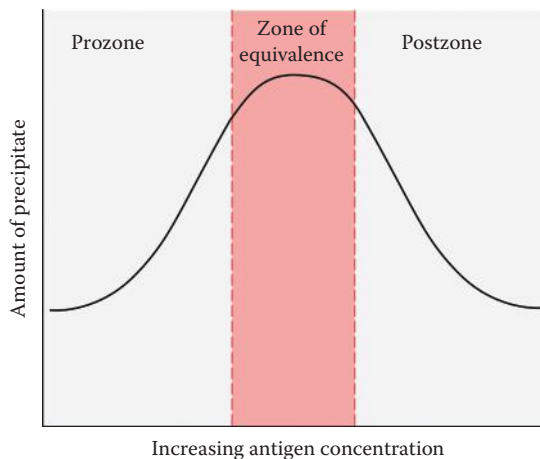


Figure 10.8 Precipitin curve. (© Richard C. Li.)

illustrates the results observed when antigens and antibodies are mixed in various concentration ratios. The curve can be divided into three zones known as the prozone, the zone of equivalence, and the postzone.

10.3.2.1.1 Prozone

At this zone, the ratio of antigen–antibody concentration is low. In other words, the antibody is in excess. Each antigen molecule is rapidly saturated with antibody, thus preventing cross-linking (Figure 10.9a). No precipitate is formed at the prozone stage.

10.3.2.1.2 Zone of Equivalence

As the concentrations of antigen increase, the amount of precipitate increases until it reaches a maximum. The amount of precipitation depends on the relative proportions of antigens and antibodies present. The maximum precipitation occurs in what is called the zone of equivalence. In the zone of equivalence, the ratio of antibody to antigen concentration is optimal and

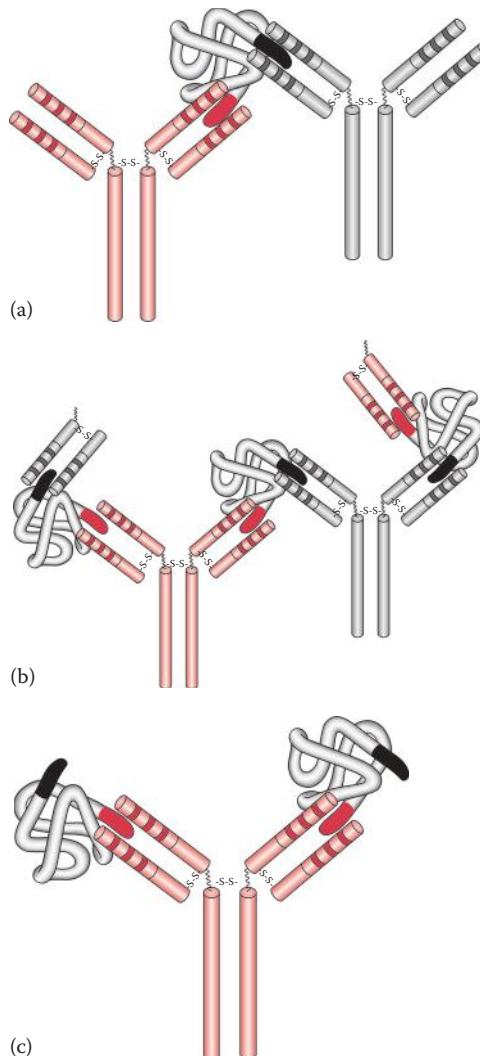


Figure 10.9 Antigen–antibody binding in (a) prozone, (b) zone of equivalence, and (c) postzone. (© Richard C. Li.)

precipitation occurs as a result of forming cross-linked networks (Figure 10.9b). Precipitation assays are usually carried out under the condition of the zone of equivalence, forming a sufficient quantity of precipitation to be detected.

10.3.2.1.3 Postzone

With the addition of more antigens, the ratio of antigen–antibody concentrations is high. In other words, the antigen is in excess. The amount of precipitate decreases and eventually diminishes. Each antibody molecule is saturated with antigen molecules (Figure 10.9c). Cross-linkage cannot form, and precipitation does not occur.

10.3.2.2 Agglutination

As discussed in the previous subsection, precipitation reactions involve soluble antigens. If the antigens are located on the surfaces of cells or carriers (carrier cells such as sheep erythrocytes,

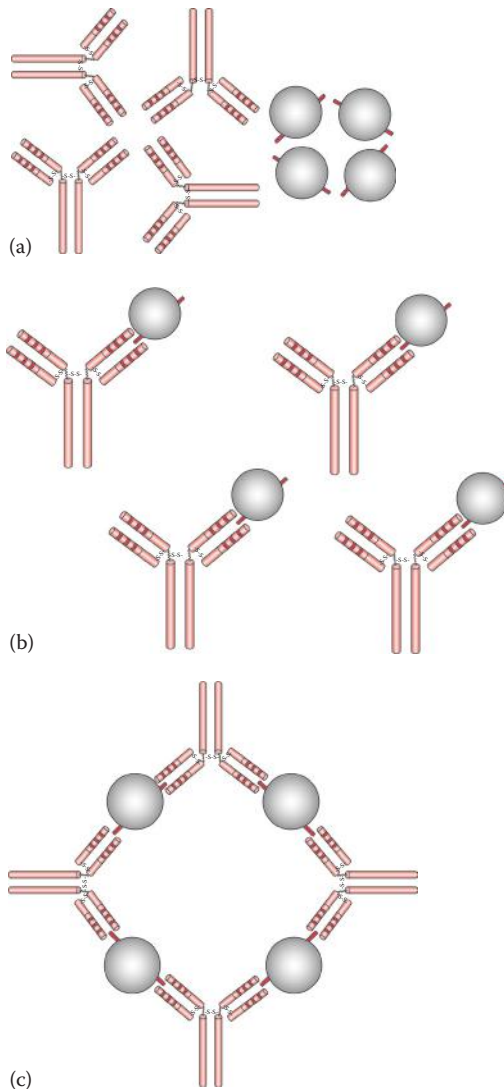


Figure 10.10 Agglutination reaction: (a) antigens are mixed with antibodies, (b) an antigen–antibody complex is formed during initial binding, and (c) the lattice is formed. (© Richard C. Li.)

bacteria, or latex particles), antibodies can bind to the surface antigens and can form cross-links among cells or carriers, causing them to aggregate. This aggregation is referred to as *agglutination*. In agglutination, a visible clumping can be observed as an indicator of the reaction of the antigen and the antibody. If the antigen is located on an erythrocyte, the agglutination reaction is designated *hemagglutination*. Agglutination is a two-step process that includes initial binding and lattice formation (Figure 10.10a).

10.3.2.2.1 Initial Binding

The first step of the reaction involves antigen–antibody binding at a single epitope on the cell surface (Figure 10.10b). This initial binding is rapid and reversible.

10.3.2.2.2 Lattice Formation

The second step involves the formation of a cross-linked network resulting in visible aggregates that constitute a lattice (Figure 10.10c). This involves an antibody binding to multiple epitopes because each antibody has two binding sites and antigens are multivalent. Lattice formation is a much slower process than the initial binding step. The cross-linking of cells requires physical contact. Additionally, an antibody must bind to epitopes on two different cells. The ability to cross-link cells depends on the nature of the antibody. Antibodies that produce such reactions are often called *agglutinins*.

Additionally, a *complete antibody* is capable of carrying out both primary and secondary interactions that result in agglutination. An antibody that can carry out initial binding but fails to form agglutination is called an *incomplete antibody*. This type of antibody is believed to have only one active antigen-binding site and is thus not capable of agglutination. It is potentially caused by the presence of steric obstruction due to the conformation of the incomplete antibody molecule, preventing the binding of antigens at the second binding site. However, other incomplete antibodies have two active sites but cannot bridge the distance between cells, thus failing to form lattices. Certain antibodies such as IgG are small and lack flexibility at the hinge region, and this may prevent agglutination. In contrast, the large IgM antibodies produce agglutination much more easily than IgG. Agglutination reactions have a wide variety of applications in the detection of antigens and antibodies. Such assays have high degrees of sensitivity and have been used for many years in forensic serology.

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