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Chapter 1
Plasma Membrane

1.0. Introduction

One of the basic concepts of biology is that all living organisms are composed of cells. Some organisms consist of a single cell, while others have multiple cells organized into tissues and tissues organized into organs. In many living organisms, organs function together as an organ system. However, even in these complex organisms, the basic biology revolves around the activities of the cell.

Robert Hooke. in the 1600s examined a thin slice of cork through the microscope and name the compartments as cells.. Later Anton Van Leeuwenhoek, made further observations of plant, animal, and microorganism cells. In 1838, Matthias Schleiden proposed that all plants are composed of cells. In 1839, Theodore Schwann, concluded that all animals are also composed of cells as well. In 1858, Rudolf Virchow proposed that all living things are made of cells and that all cells arise from preexisting cells. These premises have come down to us as the *cell theory.*

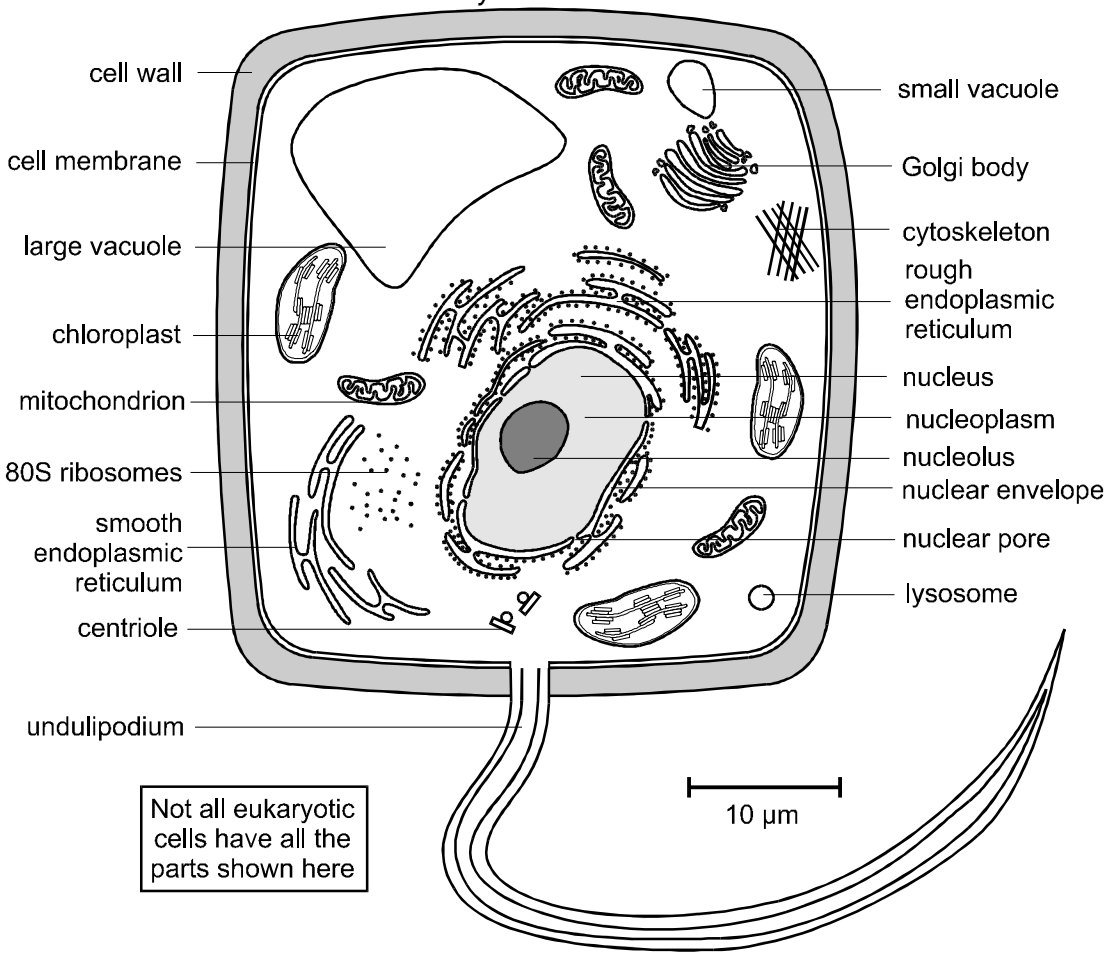


Fig.1.1. A Generalized Eukaryotic Cell

1.1. Prokaryote and Eukaryote Cell Structure

During the 1950s, scientists developed the concept that all organisms may be classified as **prokaryotes** or **eukaryotes.** The cells of all prokaryotes and eukaryotes possess two basic features: a plasma membrane and cytoplasm. However, the cells of prokaryotes are simpler than those of eukaryotes. For example,

prokaryotic cells lack a nucleus, while eukaryotic cells have a nucleus. Prokaryotic cells lack internal cellular bodies (organelles), while eukaryotic cells possess them. Examples of prokaryotes are bacteria and cyanobacteria (formerly known as **blue-green algae**). Examples of eukaryotes are protozoa, fungi, plants, and animals.

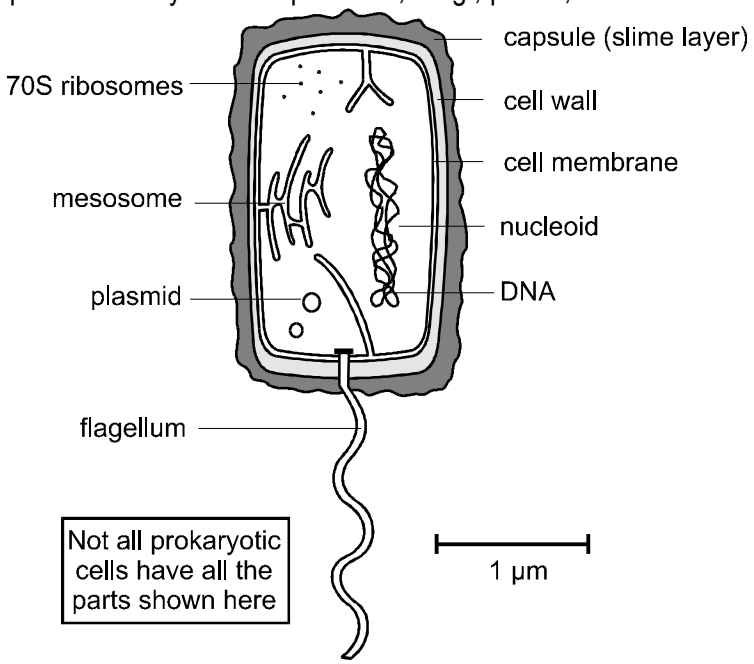


Fig.1.2. A generalized Diagram of a Prokaryote cell

1.2. Structure of Plasma Membrane

The **plasma membrane** separates internal metabolic events from the external environment and controls the movement of materials into and out of the cell.

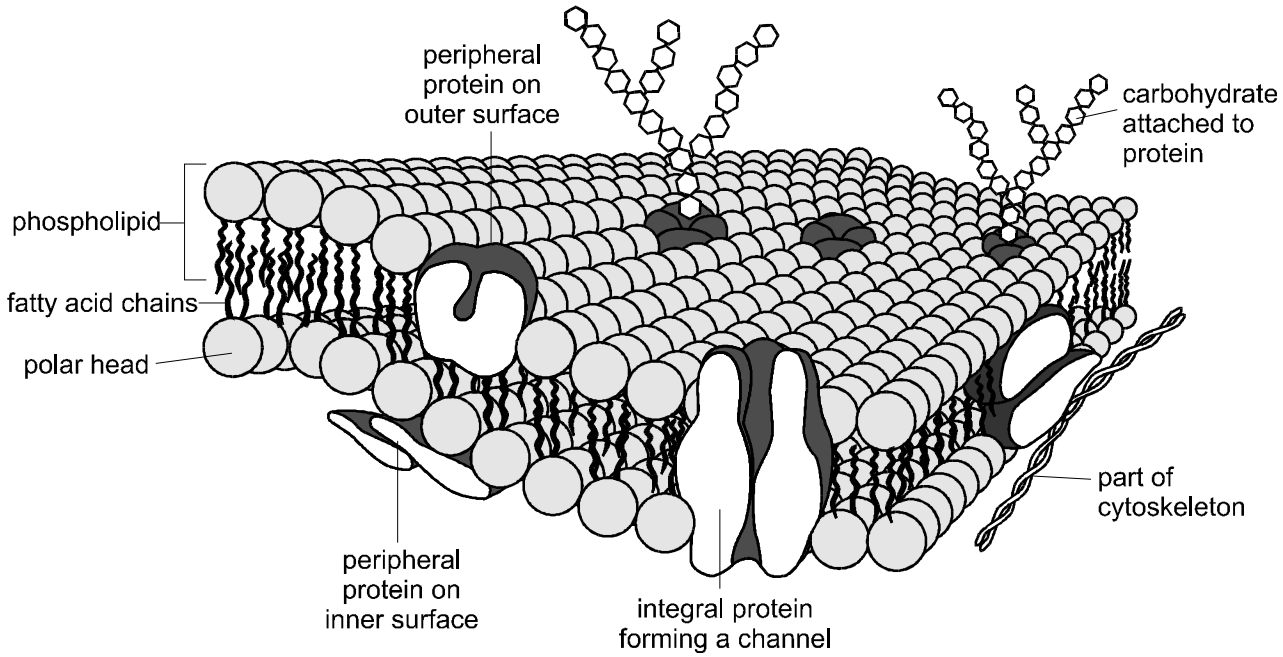


Fig. 1.3. Structure of the cell membrane (Fluid Mosaic Model)

It is a double phospholipid membrane (lipid bilayer) with the nonpolar hydrophobic tails pointing toward the inside of the membrane and the polar hydrophilic heads forming the inner and outer faces of the Proteins and cholesterol molecules are scattered throughout the flexible phospholipid membrane. Proteins may attach loosely to the inner or outer surface of the plasma membrane (peripheral proteins), or they may lie across the membrane, extending from inside to outside (integral proteins). The mosaic nature of scattered proteins within a flexible matrix of phospholipid molecules describes the fluid mosaic model of the cell membrane.

1.3. Functions of the Components of Plasma Membrane

Physiological features of the plasma membrane include.

- The phospholipid bilayer is selectively permeable. Only small, uncharged, polar molecules, such as H_2O and CO_2, and hydrophobic molecules—nonpolar molecules like O_2 and lipid soluble molecules such as hydrocarbons—can freely cross the membrane.
- **Channel proteins** provide passageways through the membrane for certain hydrophilic (water-soluble) substances such as polar and charged molecules.
- Transport proteins spend energy (ATP) to transfer materials across the membrane. When energy is used to provide passageway for materials, the process is called active transport.
- Recognition proteins distinguish the identity of neighboring cells. These proteins have oligosaccharide (short polysaccharide) chains extending out from their cell surface.
- Adhesion proteins attach cells to neighboring cells or provide anchors for the internal filaments and tubules that give stability to the cell.
- **Receptor proteins** initiate specific cell responses once hormones or other trigger molecules bind to them.
- **Electron transfer proteins** are involved in moving electrons from one molecule to another during chemical reactions.

1,4. Movement through the Plasma Membrane

In order for the cell cytoplasm to communicate with the external environment, materials must be able to move through the plasma membrane. This movement occurs through several mechanisms.

(A) Diffusion

Diffusion is the movement of molecules from a region of higher concentration to one of lower concentration. This movement occurs because the molecules are constantly colliding with one another. The net movement of the molecules is away from the region of high concentration to the region of low concentration.
Diffusion is a random movement of molecules down the pathway called the **concentration gradient.** Molecules are said to move down the concentration gradient because they move from a region of higher concentration to a region of lower concentration. A drop of dye placed in a beaker of water illustrates diffusion as the dye molecules spread out and color the water.

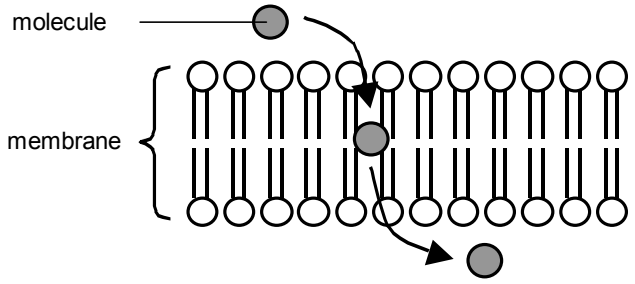


Fig.1.4. Substance movement in a lipid bilayer of the cell membrane by Diffusion

(B) Osmosis

Another method of movement across the membrane is osmosis. **Osmosis** is the movement of water from a region of higher concentration to one of lower concentration. Osmosis often occurs across a membrane that is semipermeable. A semipermeable membrane lets only certain molecules pass through while keeping other molecules out. Osmosis is really a type of diffusion involving only water molecules.

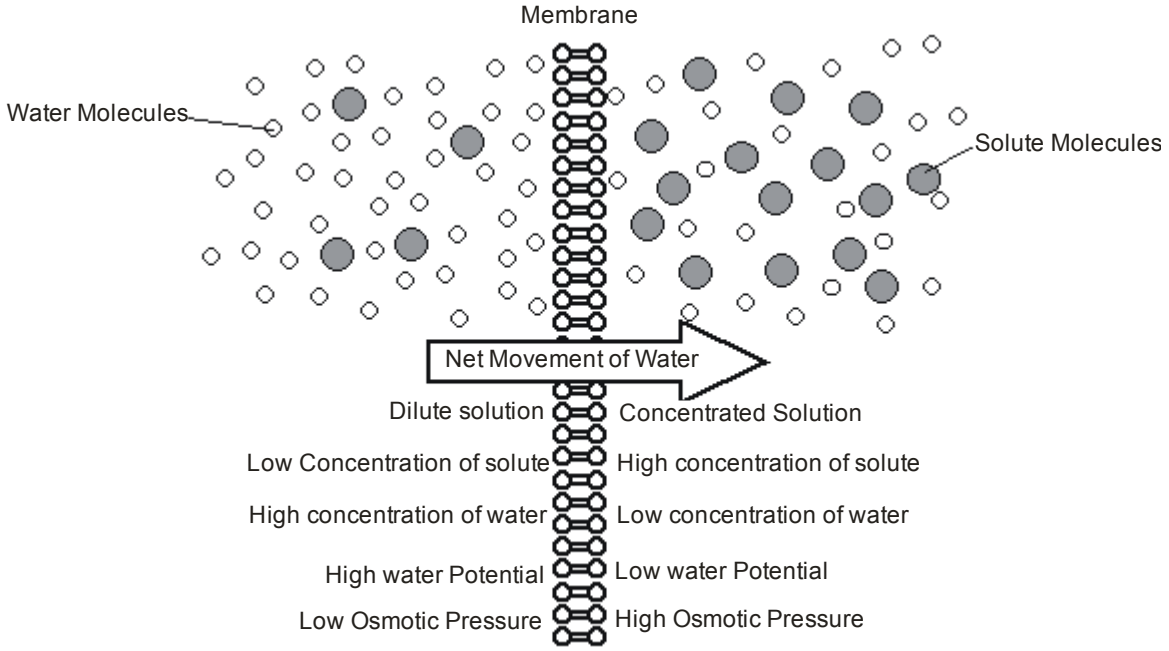


Fig.1.5.. Process of Osmosis

The concentration (or Osmotic pressure-OP) of the solution that surrounds a cell will affect the state of the cell, due to osmosis. There are three possible concentrations of solution to consider:

Isotonic solution which is a solution of equal OP (or concentration) to a cell
Hypertonic solution is a solution of higher OP (or concentration) than a cell
Hypotonic solution is a solution of lower OP (or concentration) than a cell

The effects of these solutions on cells are shown in this diagram:

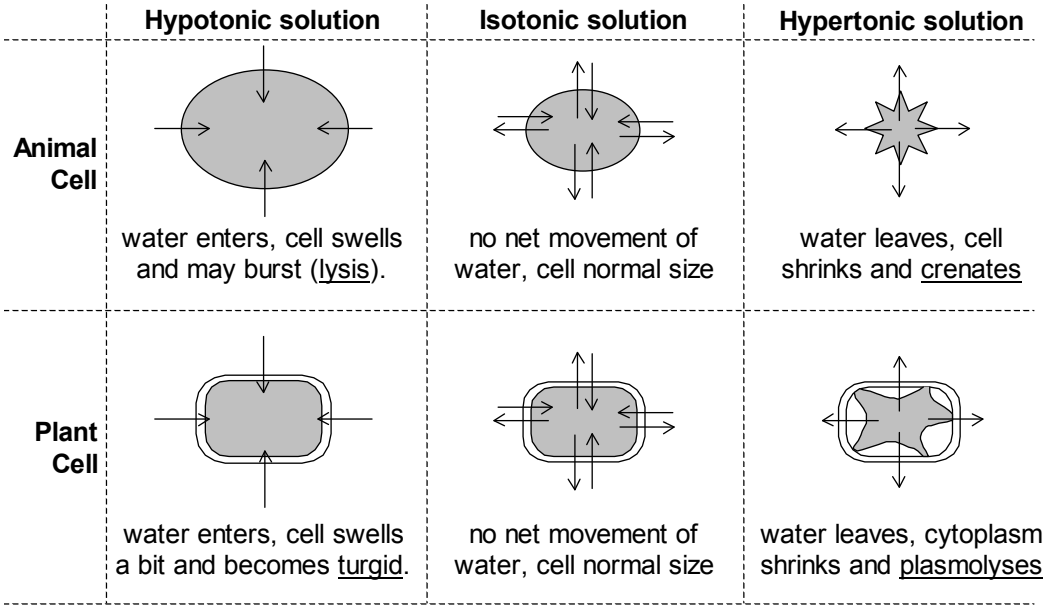


Fig.1.6. Osmosis in animal and plant cells

These are problems that living cells face all the time. For example: Simple animal cells (protozoans) in fresh water habitats are surrounded by a **hypotonic solution** and constantly need to expel water using **contractile vacuoles** to prevent swelling and lysis. Cells in marine environments are surrounded by a **hypertonic solution**, and must actively pump ions into their cells to reduce their water potential and so reduce water loss by osmosis. Young non-woody plants rely on cell **turgor** for their support, and without enough water they wilt. Plants take up water through their root hair cells by osmosis, and must actively pump ions into their cells to keep them **hypertonic** compared to the soil. This is particularly difficult for plants rooted in salt water.

(C) Facilitated Diffusion

Certain proteins in the membrane assist facilitated diffusion by permitting only certain molecules to pass across the membrane. The proteins encourage movement in the direction that diffusion would normally take place, from a region with a higher concentration of molecules to a region of lower concentration. This is referred to as passive transport. Passive transport is the transport of substances across a membrane by a **trans-membrane protein** molecule. The transport proteins tend to be specific for one molecule (a bit like enzymes), so substances can only cross a membrane if it contains the appropriate protein. As the name suggests, this is a passive diffusion process, so no energy is involved and substances can only move down their concentration gradient. There are two kinds of transport protein:

- **Channel Proteins** form a water-filled pore or channel in the membrane. This allows charged substances (usually ions) to diffuse across membranes. Most channels can be **gated** (opened or closed), allowing the cell to control the entry and exit of ions.
- **Carrier Proteins** have a binding site for a specific solute and constantly flip between two states so that the site is alternately open to opposite sides of the membrane. The substance will bind on the side where it at a **high concentration** and be released where it is at a **low concentration**.

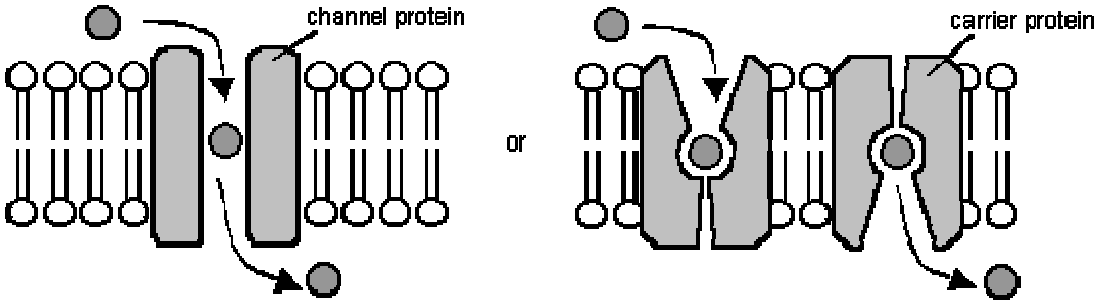


Fig.1.7..Process of Facilitated Diffusion (Passive transport)

(D) Active Transport

Active transport is the pumping of substances across a membrane by a trans-membrane **protein pump** molecule. The protein binds a molecule of the substance to be transported on one side of the membrane, changes shape, and releases it on the other side. The proteins are highly specific, so there is a different protein pump for each molecule to be transported. The protein pumps are also **ATPase enzymes**, since they catalyse the splitting of **ATP to ADP + phosphate (Pi),** and use the energy released to change shape and pump the molecule. Pumping is therefore an **active process,** and is the only transport mechanism that can transport substances **up** their concentration gradient.

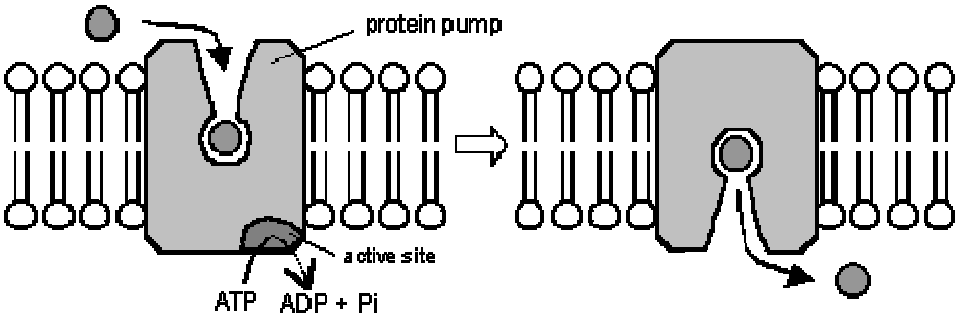


Fig.1.8.. Process of Active Transport

ATP. An example of active transport occurs in human nerve cells. Here, sodium ions are constantly transported out of the cell into the external fluid bathing the cell, a region of high concentration of sodium by an ATPase called **Na^+K^+ Pump.** This transport protein is present in the cell membranes of all animal cells and is the most abundant and important of all membrane pumps.

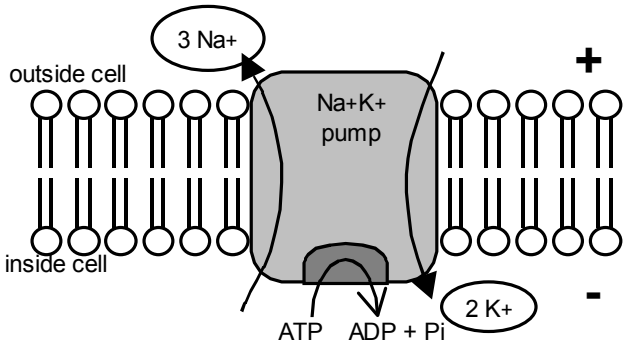


Fig.1.9.. Process of Na^+K^+ Pump

The Na^+K^+ pump is a complex pump, simultaneously pumping **three sodium ions** out of the cell and **two potassium ions** into the cell for each molecule of ATP split. This means that, apart from moving ions around, it also generates a potential difference across the cell membrane. This is called the **membrane potential**, and all animal cells have it. It varies from 20 to 200 mV, but and is always negative inside the cell. In most cells the Na^+K^+ pump runs continuously and uses 30% of all the cell's energy (70% in nerve cells).

(E) Transport of Vesicles

Large molecules (such as proteins, polysaccharides and nucleotides) and even whole cells are moved in and out of cells by using membrane vesicles by the process of **endocytosis** and **exocytosis.**

(i) Endocytosis

Endocytosis is a process in which a small patch of plasma membrane encloses particles or tiny volumes of fluid that are at or near the cell surface. The membrane enclosure then sinks into the cytoplasm and pinches off from the membrane, forming a vesicle that moves into the cytoplasm.

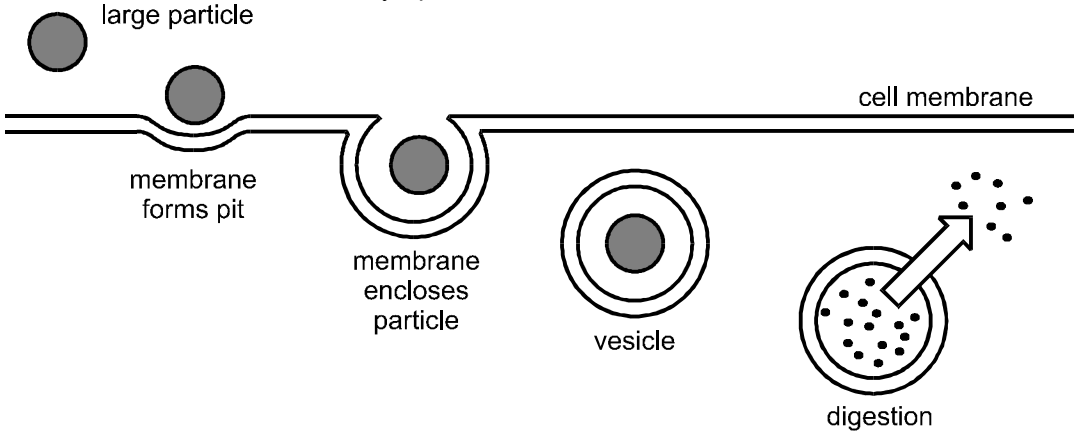


Fig.1.10. Vesicular membrane transport

When the vesicle contains particulate matter, the process is called **phagocytosis.** When the vesicle contains droplets of fluid, the process is called **pinocytosis.**

Receptor-mediated endocytosis occurs when specific molecules in the fluid surrounding the cell bind to specialized receptors in the plasma membrane. The plasma membrane folds inward and the formation of a vesicle follows. Certain hormones are able to target specific cells by receptor-mediated endocytosis.

(ii) Exocytosis

Exocytosis is the transport of materials out of a cell. It is the exact reverse of endocytosis. Materials to be exported must first be enclosed in a membrane vesicle, usually from the RER and Golgi Body. **Hormones** and **digestive enzymes** are secreted by exocytosis from the **secretory cells** of the intestine and endocrine glands. Sometimes materials can pass straight through cells without ever making contact with the cytoplasm by being taken in by endocytosis at one end of a cell and passing out by exocytosis at the other end

Along with the other mechanisms for transport across the plasma membrane, endocytosis ensures that the internal cellular environment will be able to exchange materials with the external environment and that the cell will continue to thrive and function.

1.5. Cell Junctions

The plasma membranes of adjacent cells are usually separated by extracellular fluids that allow transport of nutrients and wastes to and from the bloodstream. In certain tissues, however, the membranes of adjacent cells may join and form a junction. Three kinds of cell junctions are recognized thus desmosomes, tight junctions and gap junctions. :

(A) Desmosomes

Desmosomes are protein attachments between adjacent cells. Inside the plasma membrane, a desmosome bears a disk-shaped structure from which protein fibers extend into the cytoplasm. Desmosomes act like spot welds to hold together tissues that undergo considerable stress (such as skin or heart muscle).

(B) Tight Junctions

Tight junctions are tightly stitched seams between cells. The junction completely encircles each cell, preventing the movement of material between the cell. Tight junctions are characteristic of cells lining the digestive tract, where materials are required to pass through cells (rather than intercellular spaces) to penetrate the bloodstream.

(C) Gap junctions

Gap junctions are narrow tunnels between cells that consist of proteins called connexons. The proteins allow only the passage of ions and small molecules. In this manner, gap junctions allow communication between cells through the exchange of materials or the transmission of electrical impulses.

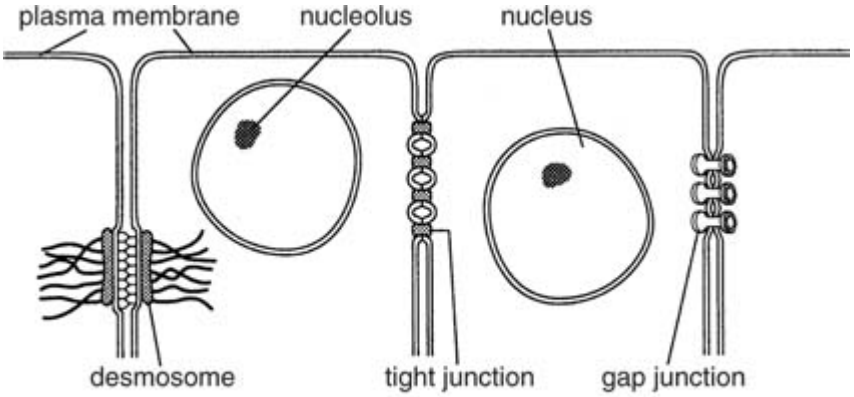


Fig.1.11. The three types of Cell Junctions

1.6. Microvilli

These are small finger-like extensions of the cell membrane found in certain cells such as in the **epithelial cells** of the **intestine** and **kidney**, where they **increase the surface area for absorption** of materials. They are just visible under the light microscope as a **brush border**.

1.7. Cilia and Flagella

Cilia and flagella are organelles of motility. They are consist of a cylindrical array of 9 filaments consisting of microtubules and the motor protein dynein and a central pair constituting a 9+2 pattern assembled in an extension of plasma membrane..

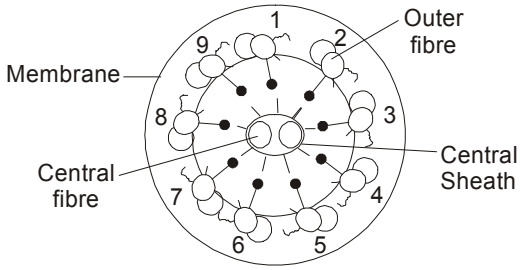


Fig.1.12. Structure of cilium and flagellum

Chapter 2
Cytoplasm and Endomembrane System

All prokaryote and eukaryote cells also have **cytoplasm** (or **cytosol**), a semiliquid substance that composes the foundation of a cell. Essentially, cytoplasm is the gel-like material enclosed by the plasma membrane. Within the cytoplasm of eukaryote cells are a number of membrane-bound bodies called **organelles** that provide a specialized function within the cell.

2.1. Cytoplasm

The cytoplasm refers to the entire area of the cell outside of the nucleus. The cytoplasm has two parts, the organelles and the **cytosol**, a grayish gel-like liquid that fills the interior of the cell. The cytosol provides a home for the nucleus and organelles as well as a location for protein synthesis and other fundamental chemical reactions.

2.2. Cytoskeleton

The cytoskeleton is a protein structure that maintains cell shape and helps move organelles around the cell. There are two types of cytoskeleton proteins: **microtubules** and **microfilaments**. Microtubules are thick, hollow rods that provide a strong scaffold for the cell. The smaller microfilaments are thin rods made of a protein called actin; they are entangled around the perimeter of the cell to help it withstand strain.

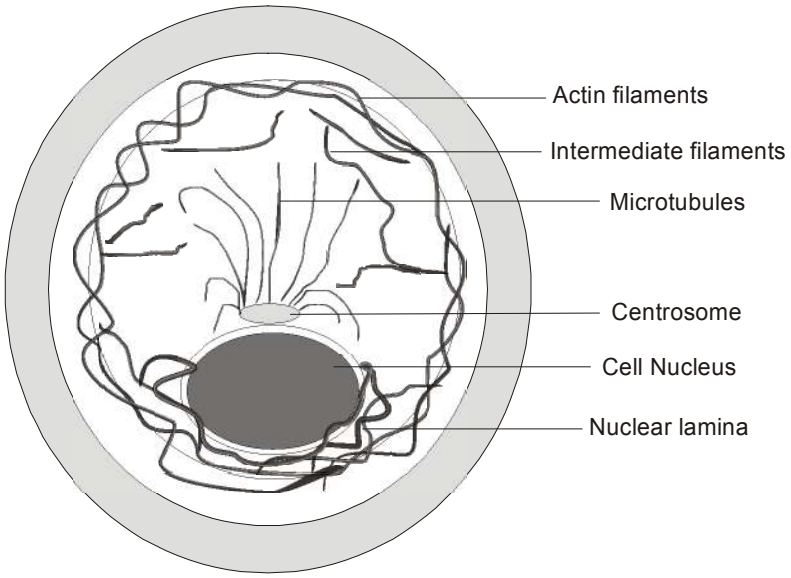


Fig.2.1. Cytoskeleton

In some organisms, the microtubules power limbs called cilia and flagella, creating movement. Contraction of the microfilaments powers muscle movement in animals and facilitates the creeping motion of creatures like amoebas. The microtubules also form protein tracks on which organelles can slide around the cell.

2.3. The Organelles

Floating in the cytoplasm are the many membrane-bound organelles, each with a distinct structure and an important function in the processes of the cell.

(A) Nucleus

The nucleus stores the cell's genetic material in strands of **DNA** and choreographs life functions by sending detailed messages to the rest of the cell. The interior of the nucleus is separated from the cytosol by a membrane called the **nuclear envelope**, which lets only select molecules in and out. The DNA itself is wrapped around proteins known as **histones** in an entangled fibrous network called **chromatin**. When the nucleus is about to split in two, this amorphous mass coils more tightly, forming distinct structures called **chromosomes**. The nucleus also houses a small, dark structure called the **nucleolus**, the site of manufacturing of ribosomes.

(B) Endoplasmic Reticulum:

These are extensive network of flattened membrane sacs that manufactures proteins. These proteins are transferred to the Golgi apparatus, from which they will be exported from the cell. There are two types of endoplasmic reticulum: rough and smooth. **Rough endoplasmic reticulum** is studded by ribosomes covering its exterior. These ribosomes make the rough endoplasmic reticulum a prime location for protein synthesis. The **smooth endoplasmic reticulum** moves the proteins around the cell and then packages them into vesicles that travel to the Golgi apparatus. The smooth endoplasmic reticulum also functions in the synthesis of fats and lipids.

(C) Ribosomes

Ribosomes synthesize proteins. Some ribosomes are mounted on the surface of the endoplasmic reticulum, and others float freely in the cytoplasm. All ribosomes have two unequally sized subunits made of proteins and a substance called RNA. All living cells, prokaryotic and eukaryotic alike, have ribosomes.

(D) Golgi Aapparatus

It is a complex of membrane-bound sacs that package proteins for export from the cell. Proteins enter the Golgi complex from the endoplasmic reticulum and proceed through the stacks, where they are modified and stored before secretion. When proteins are ready for export, pieces of the Golgi membrane bud off, forming vesicles that send them to the cell membrane.

(E) Lysosomes

They are small membrane-bound packages of acidic enzymes that digest compounds and worn-out cellular components that the cell no longer needs.

(F) Peroxisomes

Peroxisomes are about the size of lysosomes and like them are bound by a single membrane. They also resemble lysosomes in being filled with enzymes. However, peroxisomes bud off from the endoplasmic reticulum, not the Golgi apparatus (that is the source of lysosomes). The enzymes and other proteins destined for peroxisomes are synthesized in the cytosol. Each contains a **p**eroxisomal **t**argeting **s**ignal (**PTS**) that binds to a receptor molecule that takes the protein into the peroxisome and then returns for another load

(G) Centriole

This is a pair of short microtubules involved in **cell division**. Before each division the centriole replicates itself and the two centrioles move to opposite ends of the cell, where they initiate the spindle that organises and separates the chromosomes.

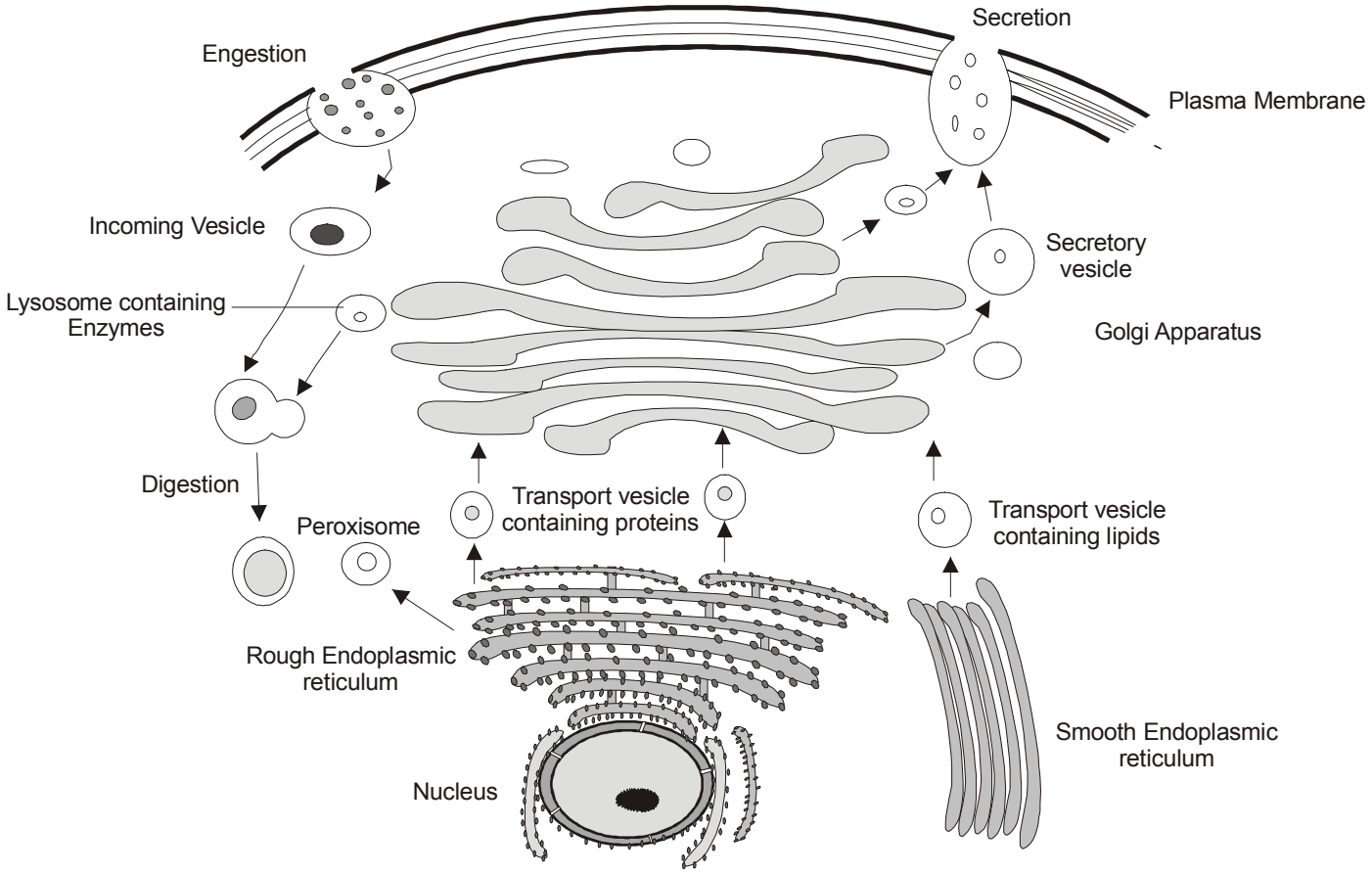


Fig.2.2. The functional aspect of endombrane system

(H) Mitochondria:

They produce energy for the cell through a process called cellular respiration. The mitochondria has two membranes; the inside membrane has many folds, called cristae. Many of the key cell-respiration enzymes are embedded in this second membrane. The chemical reactions of respiration take place in the compartment formed by the second membrane, a region called the **mitochondrial matrix**.

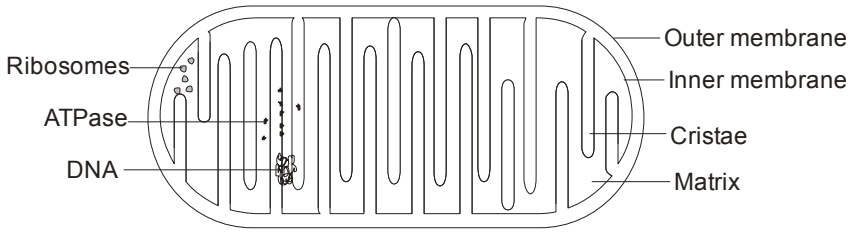


Fig.12.2.Structure of Mitochondrion

2.4. Plant Cell Organelles

The organelles described above are found in both animal and plant eukaryotic cells. But plants have additional organelles—chloroplasts, vacuoles, and cell walls—that support their unique life cycles.

(A) Chloroplasts:

Chloroplasts are the organelles in which **photosynthesis** takes place. They are large oval-shaped structures containing a green pigment called **chlorophyll** that absorbs sunlight. Chloroplasts, like mitochondria, are built from two membranes: an external membrane forming the boundary of the organelle and a stacked inner membrane within the organelle.

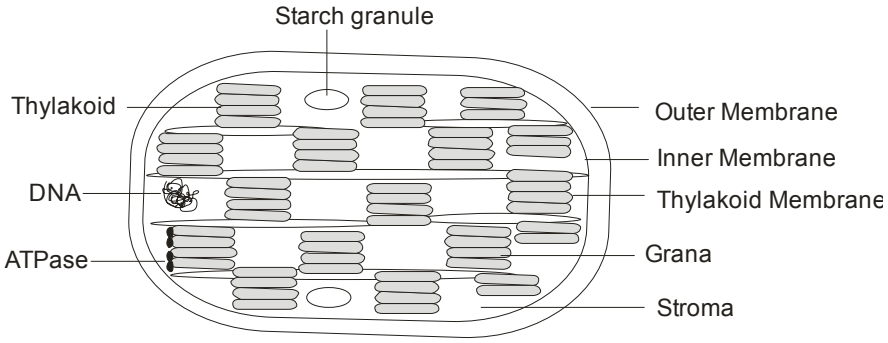


Fig.11.2. Structure of Chloroplasts

(B) Vacuoles:

large liquid-filled storage organelle found in plant cells.. Some animal cells in freshwater microorganisms have specialized contractile vacuoles that pump water out of the cell to prevent bursting.

(C) Cell wall:

Plant cells have a rigid cell wall surrounding their cell membrane. This wall is made of a compound called cellulose. The tough wall gives the plant cell added stability and protection from harm.

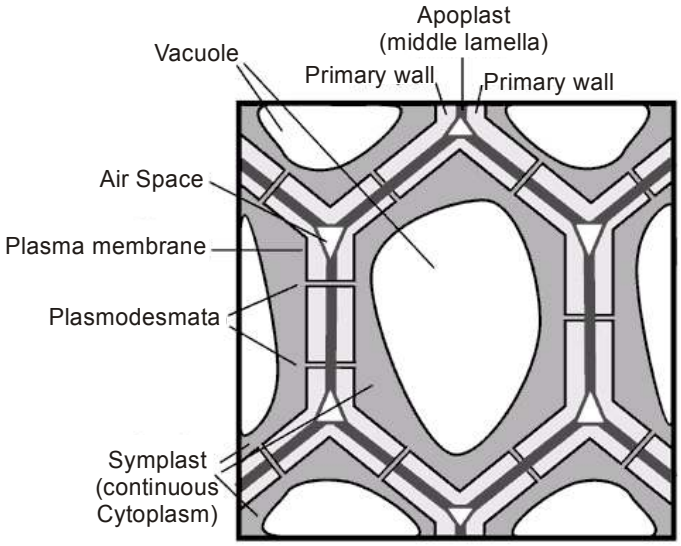


Fig.2.5. Structure of a plant cell

Chapter 3
Cellular Metabolism

Metabolism refers to all the chemical reactions taking place in a cell. There are thousands of these in a typical cell, and to make them easier to understand, biochemists arrange them into **metabolic pathways**. The intermediates in these metabolic pathways are called **metabolites**. Reactions that release energy (usually breakdown reactions) are called **catabolic reactions** (e.g. respiration). Reactions that use up energy (usually synthetic reactions) are called **anabolic reactions** (e.g. photosynthesis).

Enzymes

Enzymes are **biological catalysts**. There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction. They increase the rate of reactions by a factor of between 10^6 to 10^{12} times, allowing the chemical reactions that make life possible to take place at normal temperatures. They were discovered in fermenting yeast in 1900 by **Buchner**, and the name enzyme means "in yeast". As well as catalysing all the metabolic reactions of cells (such as **respiration, photosynthesis** and **digestion**), they also act as **motors**, **membrane pumps** and **receptors.**

3.1. Enzyme Structure

Enzymes are **proteins**, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the **active site**, while the rest of the protein acts as "scaffolding". This is shown in this diagram of a molecule of the enzyme **amylase,** with a short length of starch being digested in its active site. The amino acids around the active site attach to the **substrate molecule** and hold it in position while the reaction takes place. This makes the enzyme **specific** for one reaction only, as other molecules won't fit into the active site.

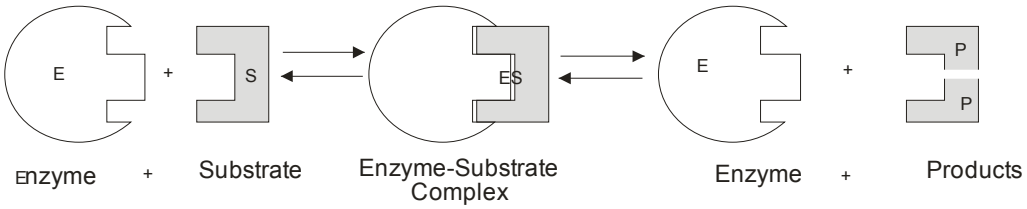
Many enzymes need **cofactors** (or **coenzymes**) to work properly. These can be metal ions (such as Fe^{2+}, Mg^{2+}, Cu^{2+}) or organic molecules (such as haem, biotin, FAD, NAD or coenzyme A). Many of these are derived from dietary vitamins, which is why they are so important. The complete active enzyme with its cofactor is called a **holoenzyme,** while just the protein part without its cofactor is called the **apoenzyme**.

3.2. Specificity and the Concept of Active Sites

There are three ways of thinking about enzyme catalysis. They all describe the same process, though in different ways.

(A) Reaction Mechanism

In any chemical reaction, a **substrate** (S) is converted into a **product** (P): (There may be more than one substrate and more than one product, but that doesn't matter here.) In an enzyme-catalysed reaction, the substrate first binds to the **active site** of the enzyme to form an **enzyme-substrate** (ES) **complex**, then the substrate is converted into product while attached to the enzyme, and finally the product is released. This mechanism can be shown as:



3.1. Reaction Mechanism in the mechanism of enzyme action

The enzyme is then free to start again. The end result is the same (S → P), but a different route is taken, so that the S → P reaction as such never takes place. In by-passing this step, the reaction can be made to happen much more quickly.

(B) Molecule Geometry

The **substrate molecules** fit into the **active** site of the enzyme molecule like a key fitting into a lock (in fact it is sometimes called a **lock and key** mechanism). Once there, the **enzyme changes shape** slightly, **distorting the molecules** in the active site, and making it more likely to **change into the product.** For example if a bond in the substrate is to be formed, the two substrate molecules will be compressed by the enzyme, making it more likely to bind together. Alternatively the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen.

It's a bit more complicated than that though. Although enzymes **can change the speed** of a chemical reaction, they **cannot change its direction**, otherwise they could make "impossible" reactions happen and break the **laws of thermodynamics**. So an enzyme can just as easily turn a product into a substrate as turn a substrate into a product, depending on which way the reaction would go anyway. In fact the active site doesn't really fit the substrate (or the product) at all, but instead fits a sort of half-way house, called the **transition state**. When a substrate (or product) molecule binds, the **active site changes shape** and fits itself around the molecule, distorting it into forming the **transition state**, and so speeding up the reaction. This is sometimes called the **induced fit mechanism.**

(C) Energy Changes

The way enzymes work can also be shown by considering the energy changes that take place during a chemical reaction. We shall consider a reaction where the product has a lower energy than the substrate, so the substrate naturally turns into product (in other words the equilibrium lies in the direction of the product). Before it can change into product, the substrate must overcome an "energy barrier" called the **activation energy** (E_A). The larger the activation energy, the slower the reaction will be because only a few substrate molecules will by chance have sufficient energy to overcome the activation energy barrier. Imagine pushing boulders over a hump before they can roll down hill and you have the idea. Most physiological reactions have large activation energies, so they simply don't happen on a useful time scale. Enzymes dramatically reduce the activation energy of a reaction, so that most molecules can easily get over the activation energy barrier and quickly turn into product.

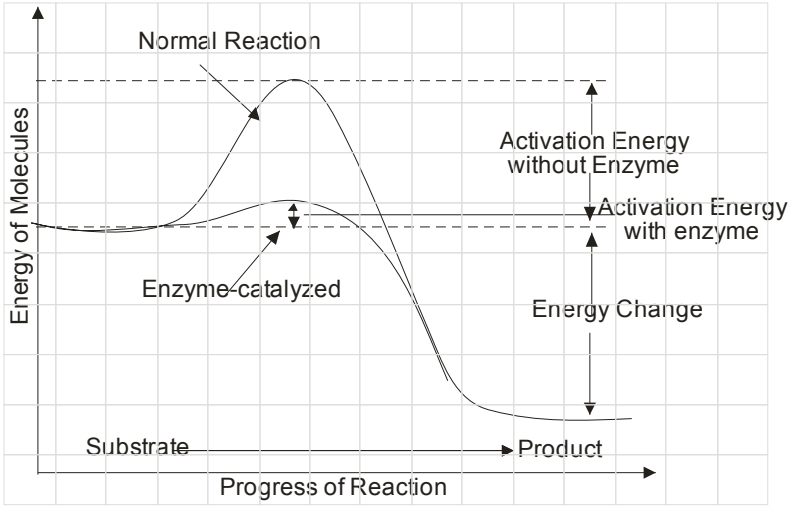


Fig.3.2. Energy changes during the enzyme catalysed reaction

For example for the **catalase** reaction ($2H_2O_2 \rightarrow 2H_2O + O_2$) the activation energy is 86 kJ mol^{-1} with no catalyst, 62 kJ mol^{-1} with an inorganic catalyst of iron filings, and just 1 kJ mol^{-1} in the presence of the enzyme catalase.

The activation energy is actually the energy required to form the **transition state**, so enzymes lower the activation energy by stabilising the transition state, and they do this by changing the conditions within the active site of the enzyme. So the three ideas above are really three ways of describing the same process.

3.3. Factors that Affect the Rate of Enzyme Reactions

(A) Temperature

Enzymes have an **optimum temperature** at which they work fastest. For mammalian enzymes this is about 40°C, but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at -10°C, and enzymes from thermophilic bacteria work at 90°C.

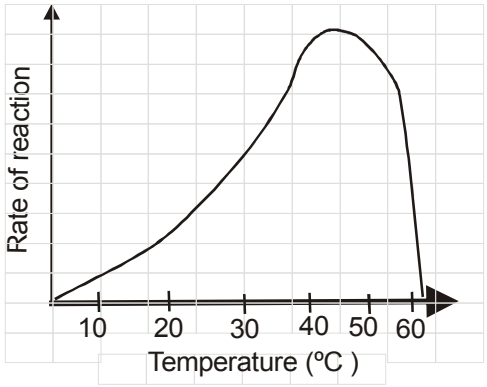


Fig.3.3. Effect of Temperature on Enzyme catalyzed reactions

Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more **kinetic energy** so collide more often, and also because more molecules have sufficient energy to overcome the (greatly reduced) **activation energy**. The increase in rate with temperature can be quantified as a $\mathbf{Q_{10}}$, which is the relative increase for a 10°C rise in temperature. Q_{10} is usually 2-3 for enzyme-catalysed reactions (i.e. the rate doubles every 10°C) and usually less than 2 for non-enzyme reactions.

$$Q_{10} = \frac{\text{Rate at Temperature } (t + 10)^{o}C}{\text{Rate at Temperature } (t\ ^{o}C)}$$

The rate is not zero at 0°C, so enzymes still work in the fridge (and food still goes bad), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

Above the optimum temperature the rate decreases as more and more of the enzyme molecules **denature**. The thermal energy breaks the hydrogen bonds holding the secondary and tertiary structure of the enzyme together, so the enzyme (and especially the active site) loses its shape to become a random coil. The substrate can no longer bind, and the reaction is no longer catalysed. At very high temperatures this is irreversible. Remember that only the weak hydrogen bonds are broken at these mild temperatures; to break strong covalent bonds you need to boil in concentrated acid for many hours.

(B) pH

Enzymes have an **optimum pH** at which they work fastest. For most enzymes this is about pH 7-8 (physiological pH of most cells), but a few enzymes can work at extreme pH, such as protease enzymes in animal stomachs, which have an optimum of pH 1. The pH affects the charge of the amino acids at the active site, so the properties of the active site change and the substrate can no longer bind. For example a carboxyl acid R groups will be uncharged at low pH (COOH), but charged at high pH (COO^-).

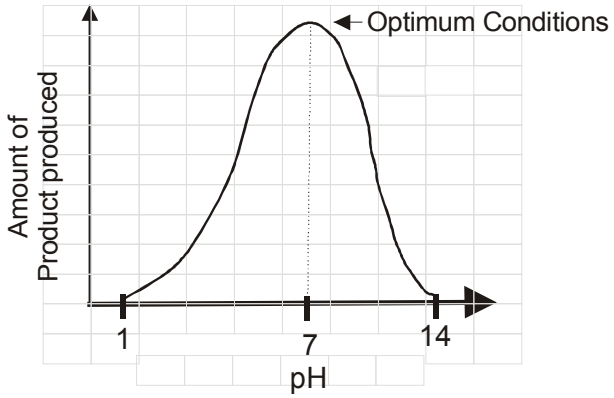


Fig.3.4. Effect of pH on Enzyme catalyzed reactions

(C) Enzyme concentration

As the enzyme concentration increases the rate of the reaction increases linearly, because there are more enzyme molecules available to catalyse the reaction. At very high enzyme concentration the substrate concentration may become **rate-limiting,** so the **rate stops increasing**. Normally enzymes are present in cells in rather low concentrations.

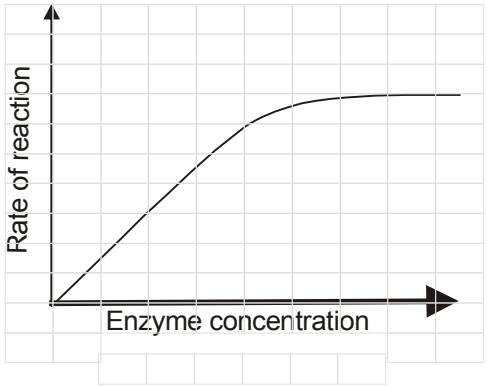


Fig.3,5. Effect of Enzyme concentration on Enzyme catalyzed reactions

(D) Substrate concentration

The rate of an enzyme-catalysed reaction shows a curved dependence on substrate concentration. As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place. At higher concentrations the enzyme molecules become **saturated** with substrate, so there are few free enzyme molecules, so adding more substrate doesn't make much difference (though it will increase the rate of E-S collisions).

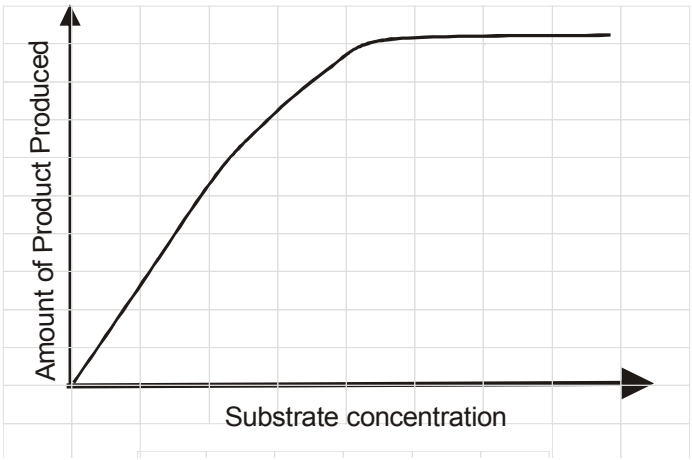


Fig.3.6. Effect of Substrate concentration on Enzyme catalyzed reactions

The maximum rate at infinite substrate concentration is called v_{max}, and the substrate concentration that give a rate of half v_{max} is called K_M. These quantities are useful for characterising an enzyme. A good enzyme has a high v_{max} and a low K_M.

(E) Covalent modification

The activity of some enzymes is controlled by other enzymes, which modify the protein chain by cutting it, or adding a phosphate or methyl group. This **modification** can turn an **inactive enzyme** into an **active enzyme** (or vice versa), and this is used to control many metabolic enzymes and to switch on enzymes in the gut e.g. hydrochloric acid in stomach→ activates pepsin→ activates rennin.

3.4. Inhibitors

Inhibitors inhibit the activity of enzymes, reducing the rate of their reactions. They are found naturally, but are also used artificially as drugs, pesticides and research tools. There are two kinds of inhibitors and allosteric effects.

(A) Competitive Inhibition

A **competitive inhibitor** molecule has a similar structure to the normal substrate molecule, and it can fit into the active site of the enzyme. It therefore **competes** with the substrate **for the active site**, so the reaction is slower. Competitive inhibitors increase K_M for the enzyme, but have no effect on v_{max}, so the rate can approach a normal rate if the substrate concentration is increased high enough. The **sulphonamide anti-bacterial drugs** are competitive inhibitors.

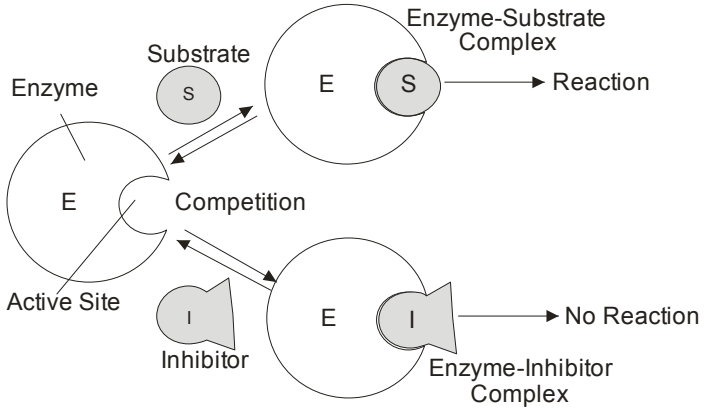


Fig.3.7. Competitive Inhibition

(B) Non-competitive Inhibition

A **non-competitive inhibitor** molecule is quite different in structure from the substrate molecule and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Non-competitive inhibitors therefore simply reduce the amount of active enzyme (just like decreasing the enzyme concentration), so they decrease v_{max}, but have no effect on K_M. Inhibitors that bind fairly weakly and can be washed out are sometimes called **reversible inhibitors**, while those that bind tightly and cannot be washed out are called **irreversible inhibitors**. Poisons like **cyanide**, heavy metal ions and some insecticides are all non-competitive inhibitors.

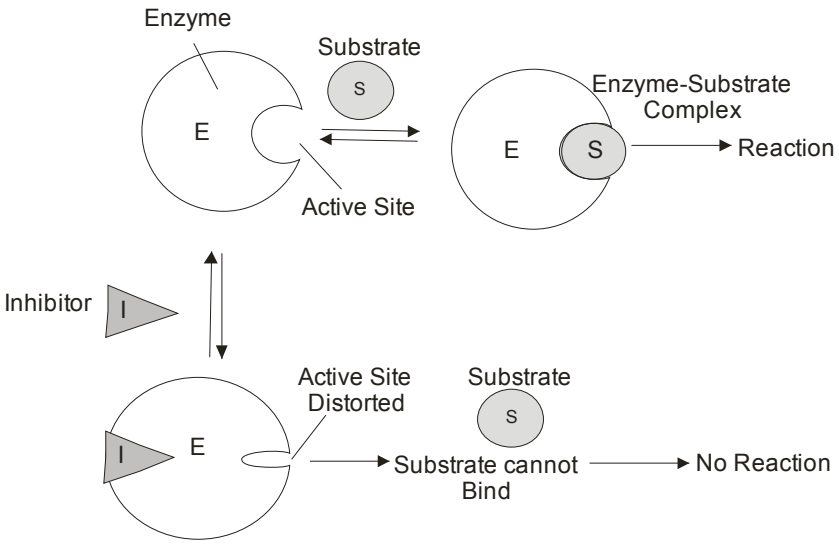


Fig.3.8. Non-Competitive Inhibition

(C) Allosteric Effectors

The activity of some enzymes is controlled by certain molecules binding to a **specific regulatory** (or **allosteric**) site on the enzyme, distinct from the active site. Different molecules can inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway. They are generally activated by the substrate of the pathway and inhibited by the product of the pathway, thus only turning the pathway on when it is needed.

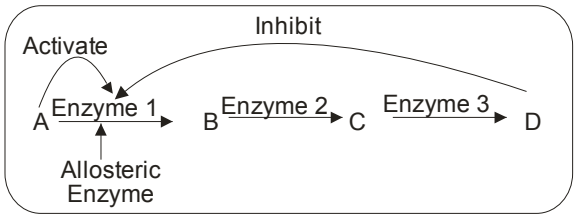


Fig.3.9. Allosteric Effect

In Allosteric Inhibition, an inhibitor I and substrate S each have binding sites on an enzyme E. Unlike non-competitive inhibition, the binding of I to E does not cause a conformational change in the resulting complex which prevents S from binding

3.5. How to Measure Enzyme Kinetics

This means measuring the rate of enzyme reactions.

Firstly you need a signal to measure that shows the progress of the reaction. The signal should change with either substrate or product concentration, and it should preferably be something that can be measured continuously. Typical signals include colour changes, pH changes, mass changes, gas production, volume changes or turbidity changes. If the reaction has none of these properties, it can sometimes be linked to a second reaction which does generate one of these changes.

If you mix your substrate with enzyme and measure your signal, you will obtain a **time-course**. If the signal is proportional to substrate concentration it will start high and decrease, while if the signal is proportional to product it will start low and increase. In both cases the time-course will be curved (actually an exponential curve).

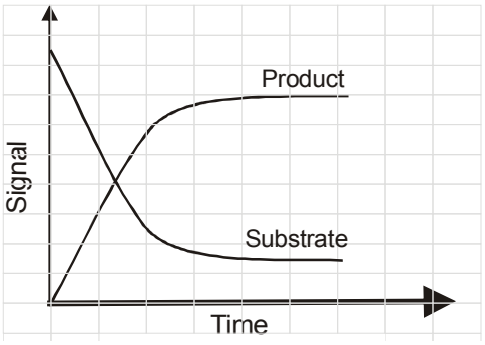


Fig.3,9.. Time-Coure Curve

How do you obtain a rate from this time-course? One thing that is not a good idea is to measure the time taken for the reaction, for as the time-course shows it is very difficult to say when the reaction ends: it just gradually approaches the end-point. A better method is to measure the **initial rate** - that is the initial slope of the time-course. This also means you don't need to record the whole time-course, but simply take one measurement a short time after mixing.

Repeat this initial rate measurement under different conditions (such as different substrate concentrations) and then plot a graph of rate *vs.* the factor. Each point on this second graph is taken from a separate initial rate measurement (or better still is an average of several initial rate measurements under the same conditions). Draw a smooth curve through the points.

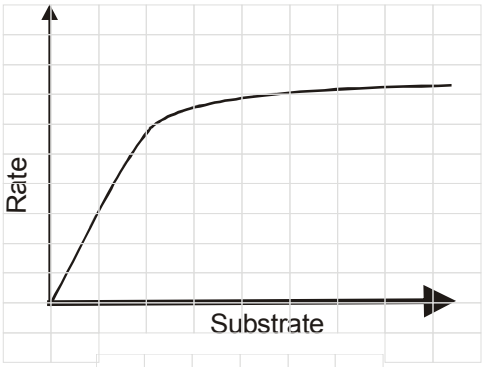
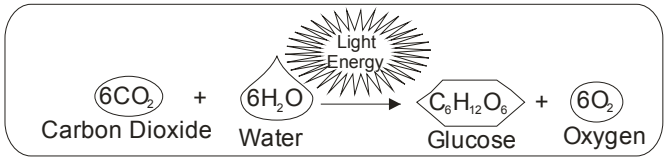


Fig.3.10.Substrate-Rate Curve

Photosynthesis

Photosynthesis as a process in which light energy is used in the synthesis of organic molecules simplified by the following equation



Equation 1. Summary of Photosynthesis

The process of photosynthesis is divided into two parts: the energy-fixing reaction (also called the light reaction) and the carbon-fixing reaction (also called the light-independent reaction, or the dark reaction).

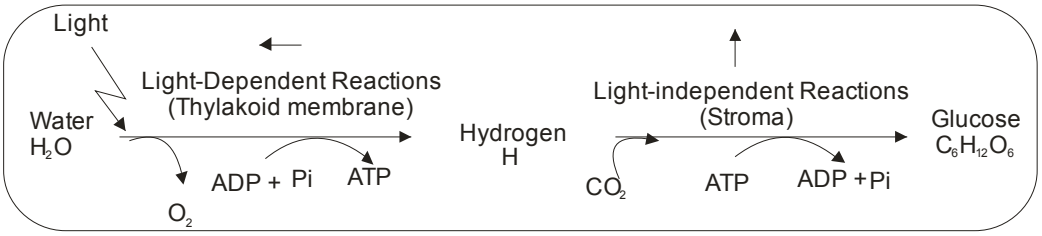


Fig.3.11.. Summary of Photosynthetic pathways

These two step ca be represented in the following diagram.

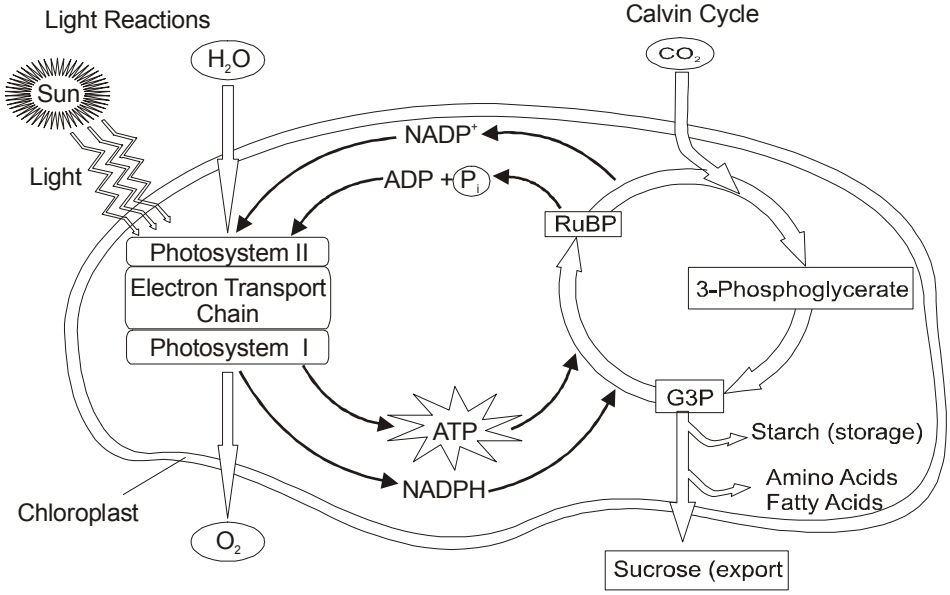


Fig.3.12.. Overview of the process of Photosynthesis

(A) Energy-Fxing Reaction

The energy-fixing reaction of photosynthesis begins when light is absorbed in photosystem II in the thylakoid membranes. The energy of the sunlight, captured in the P680 reaction center, activates electrons to jump out of the chlorophyll molecules in the reaction center. These electrons pass through a series of cytochromes in the nearby electron-transport system.

(i) Cyclic Phosporylation

After passing through the electron transport system, the energy-rich electrons eventually enter photosystem 1. Some of the energy of the electron is lost as the electron moves along the chain of acceptors, but a portion of the energy pumps protons across the thylakoid membrane, and this pumping sets up the potential for chemiosmosis.

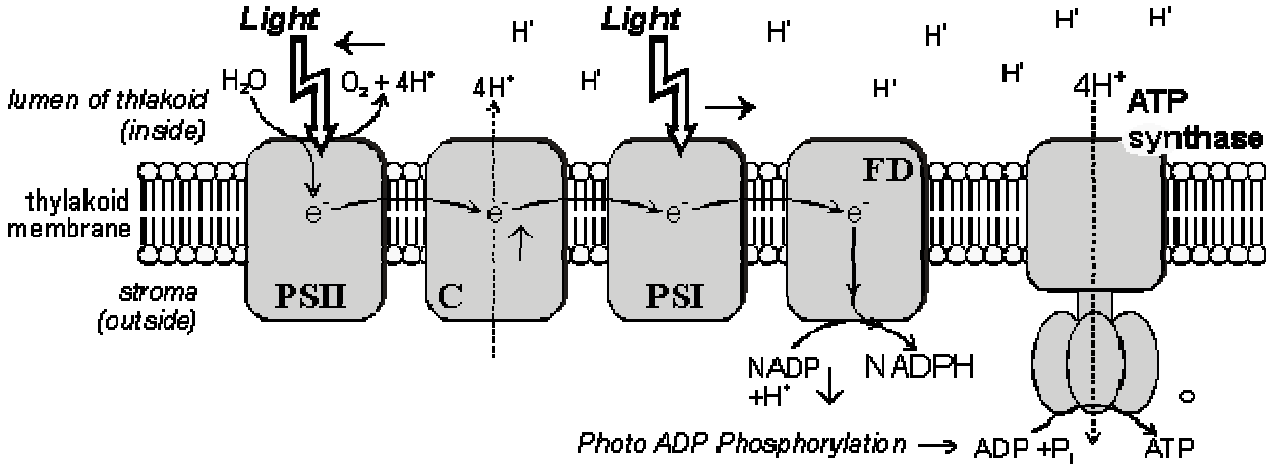


Fig.3.13. Light-dependent on the thylakoid membranes

The spent electrons from P680 enter the P700 reaction center in photosystem I. Sunlight now activates the electrons, which receive a second boost out of the chlorophyll molecules. There they reach a high energy level. Now the electrons progress through a second electron transport system, but this time there is no proton pumping. Rather, the energy reduces NADP. This reduction occurs as two electrons join NADP and energize the molecule. Because NADP acquires two negatively charged electrons, it attracts two positively charged protons to balance the charges. Consequently, the NADP molecule is reduced to NADPH, a molecule that contains much energy.

Because electrons have flowed out of the P680 reaction center, the chlorophyll molecules are left without a certain number of electrons. Electrons secured from water molecules replace these electrons. Each split water molecule releases two electrons that enter the chlorophyll molecules to replace those lost. The split water molecules also release two protons that enter the cytoplasm near the thylakoid and are available to increase the chemiosmotic gradient.

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e^-$$

Equation 2

The third product of the disrupted water molecules is oxygen. Two oxygen atoms combine with one another to form molecular oxygen, which is given off as the byproduct of photosynthesis; it fills the atmosphere and is used by all oxygen-breathing organisms, including plant and animal cells.

(ii) Non-Cyclic Phosporylation

Certain plants are also known to participate in *cyclic energy-fixing reactions*. These reactions involve only photosystem I and the P700 reaction center. Excited electrons leave the reaction center, pass through coenzymes of the electron transport system, and then follow a special pathway back to P700. Each electron powers the proton pump and encourages the transport of a proton across the thylakoid membrane. This process enriches the proton gradient and eventually leads to the generation of ATP.

ATP production in the energy-fixing reactions of photosynthesis occurs by the process of **chemiosmosis**. Essentially, this process consists of a rush of protons across a membrane (the thylakoid membrane, in this case), accompanied by the

synthesis of ATP molecules. Biochemists have calculated that the proton concentration on one side of the thylakoid is 10,000 times that on the opposite side of the membrane.

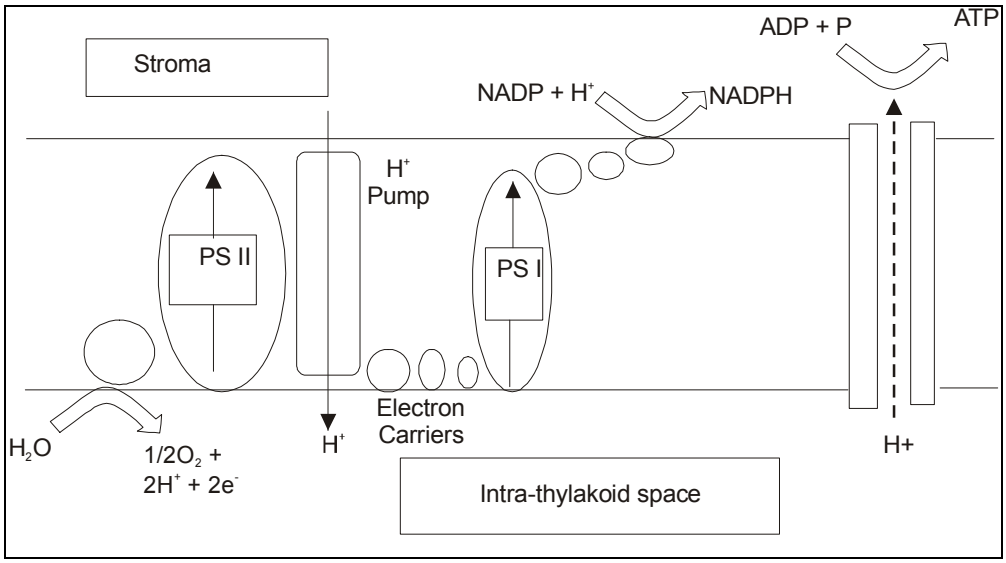


Fig.3.14. Non-Cyclic phosphorylation or Z-diagram

In photosynthesis, the protons pass back across the membranes through channels lying alongside sites where enzymes are located. As the protons pass through the channels, the energy of the protons is released to form high-energy ATP bonds. ATP is formed in the energy-fixing reactions along with the NADPH formed in the main reactions. Both ATP and NADPH provide the energy necessary for the synthesis of carbohydrates that occurs in the second major set of events in photosynthesis.

(B) Carbon-Fixing Reaction

Glucose and other carbohydrates are synthesized in the carbon-fixing reaction of photosynthesis, often called the *Calvin cycle* . This phase of photosynthesis occurs in the stroma of the plant cell.

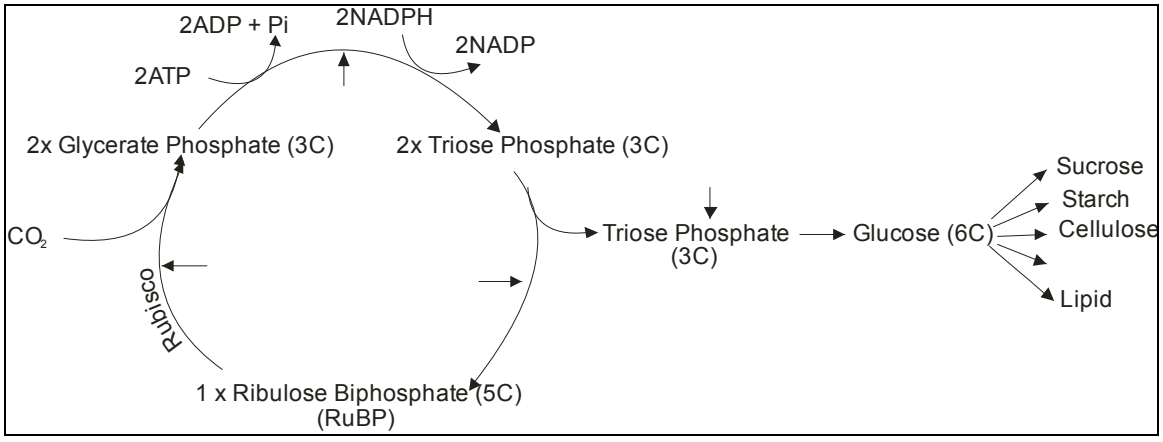


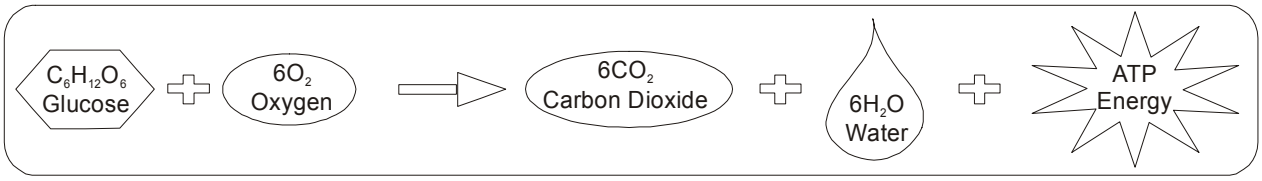
Fig.3.15. Light-dependent reactions (Calvin Cycle)

In the carbon-fixing reaction, an essential material is carbon dioxide, which is obtained from the atmosphere. The carbon dioxide is attached to a five-carbon compound called *ribulose diphosphate*. Ribulose diphosphate carboxylase catalyzes this reaction.

After carbon dioxide has been joined to ribulose diphosphate, a six-carbon product forms, which immediately breaks into two three-carbon molecules called *phosphoglycerate*. Each phosphoglycerate molecule converts to another organic compound, but only in the presence of ATP. The ATP used is the ATP synthesized in the energy-fixing reaction. The organic compound formed converts to still another organic compound using the energy present in NADPH. Again, the energy-fixing reaction provides the essential energy. The organic compounds that result each consist of three carbon atoms. Eventually, the compounds interact with one another and join to form a single molecule of six-carbon glucose. This process also generates additional molecules of ribulose diphosphate to participate in further carbon-fixing reactions.

Cellular Respiration

All living cells require energy, and this energy is provided by respiration. The overall summary of Respiration which is the reverse of photosynthesis is as follows:



Equation 3

The above equation is an over simplification of the process but in fact respiration is a complex metabolic pathway, comprising at least 30 separate steps.

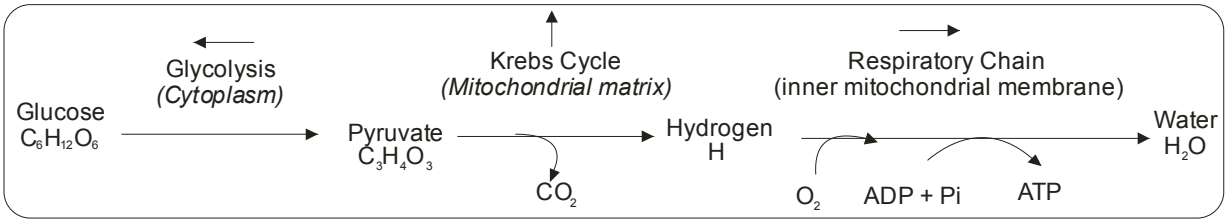


Fig.3.16. Highlight of respiration

The overall mechanism of cellular respiration involves four processes: glycolysis, in which glucose molecules are broken down to form pyruvic acid molecules; the Krebs cycle, in which pyruvic acid is further broken down and the energy in its molecule is used to form high-energy compounds, such as nicotinamide adenine dinucleotide (NADH); the electron transport system, in which electrons are transported along a series of coenzymes and cytochromes and the energy in the electrons is released; and chemiosmosis, in which the energy given off by electrons pumps protons across a membrane and provides the energy for ATP synthesis. The general pathways for cellular respiration are as follows:
Glucose is converted to acetyl-CoA in the cytoplasm, and then the Krebs cycle proceeds in the mitochondrion. Electron transport and chemiosmosis result in energy release and ATP synthesis

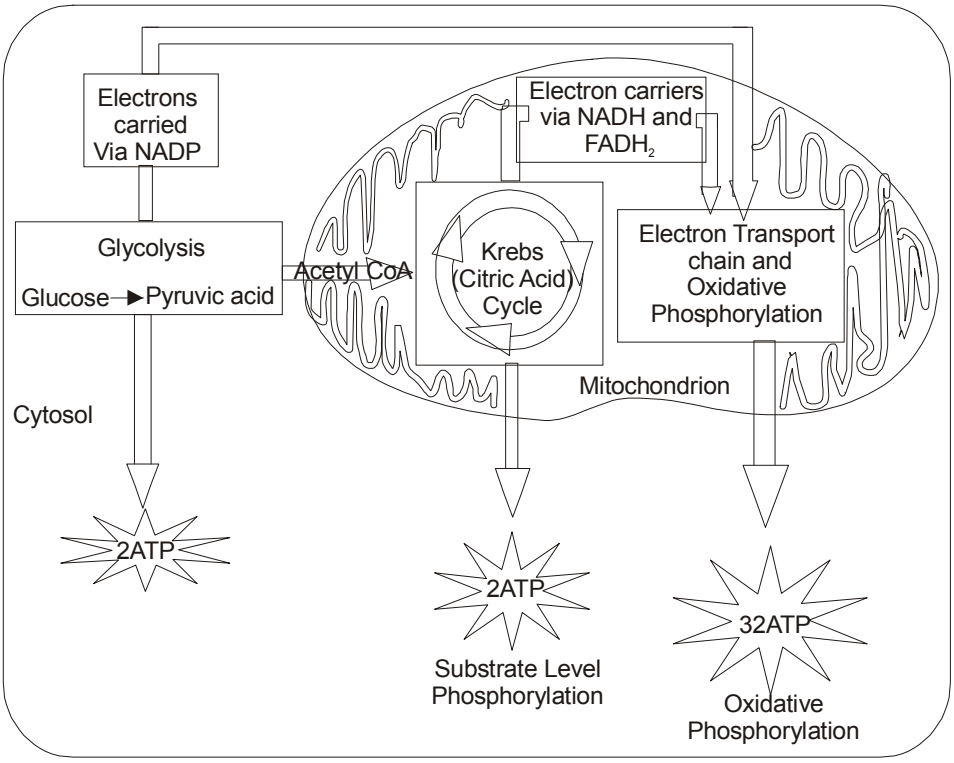


Fig.3.17. Overview of the respiratory pathways

(A) Glycolysis

Glycolysis is the process in which one glucose molecule is broken down to form two molecules of pyruvic acid. The glycolysis process is a multistep metabolic pathway that occurs in the cytoplasm of animal cells, plant cells, and the cells of microorganisms. At least six enzymes operate in the metabolic pathway. In the first and third steps of the pathway, ATP energizes the molecules. Thus, two ATP molecules must be expended in the process. Further along in the process, the six-carbon glucose molecule converts into intermediary compounds and then is split into two three-carbon compounds. The latter undergo additional conversions and eventually form pyruvic acid at the conclusion of the process.

During the latter stages of glycolysis, four ATP molecules are synthesized using the energy given off during the chemical reactions. Thus, four ATP molecules are synthesized and two ATP molecules are used during glycolysis, for a net gain of two ATP molecules.

Another reaction during glycolysis yields enough energy to convert NAD to NADH (plus a hydrogen ion). The reduced coenzyme (NADH) will later be used in the electron transport system, and its energy will be released. During glycolysis, two NADH molecules are produced.

Because glycolysis does not use any oxygen, the process is considered to be anaerobic. For certain anaerobic organisms, such as some bacteria and fermentation yeasts, glycolysis is the sole source of energy. Glycolysis is a somewhat inefficient process because much of the cellular energy remains in the two molecules of pyruvic acid that are created. Interestingly, this process is somewhat similar to a reversal of photosynthesis.

(B) Krebs Cycle

Following glycolysis, the mechanism of cellular respiration involves another multistep process—the Krebs cycle, which is also called the citric acid cycle or the tricarboxylic acid cycle. The **Krebs cycle** uses the two molecules of pyruvic acid formed in glycolysis and yields high-energy molecules of NADH and flavin adenine dinucleotide (FADH), as well as some ATP.

The Krebs cycle occurs in the mitochondrion of a cell. Located along the cristae are the important enzymes necessary for the proton pump and for ATP production. Prior to entering the Krebs cycle, the pyruvic acid molecules are altered. Each three-carbon pyruvic acid molecule undergoes conversion to a substance called acetyl-coenzyme A, or acetyl-CoA. During the process, the pyruvic acid molecule is broken down by an enzyme, one carbon atom is released in the form of carbon dioxide, and the remaining two carbon atoms are combined with a coenzyme called coenzyme A. This combination forms acetyl-CoA. In the process, electrons and a hydrogen ion are transferred to NAD to form high-energy NADH.

Acetyl-CoA now enters the Krebs cycle by combining with a four-carbon acid called oxaloacetic acid. The combination forms the six-carbon acid called citric acid. Citric acid undergoes a series of enzyme-catalyzed conversions. The conversions, which involve up to ten chemical reactions, are all brought about by enzymes. In many of the steps, high-energy electrons are released to NAD. The NAD molecule also acquires a hydrogen ion and becomes NADH. In one of the steps, FAD serves as the electron acceptor, and it acquires two hydrogen ions to become $FADH_2$. Also, in one of the reactions, enough energy is released to synthesize a molecule of ATP. Because for each glucose molecule there are two pyruvic acid molecules entering the system, two ATP molecules are formed.

Also during the Krebs cycle, the two carbon atoms of acetyl-CoA are released, and each forms a carbon dioxide molecule. Thus, for each acetyl-CoA entering the cycle, two carbon dioxide molecules are formed. Two acetyl-CoA molecules enter the cycle, and each has two carbon atoms, so four carbon dioxide molecules will form. Add these four molecules to the two carbon dioxide molecules formed in the conversion of pyruvic acid to acetyl-CoA, and it adds up to six carbon dioxide molecules. These six $C0_2$ molecules are given off as waste gas in the Krebs cycle. They represent the six carbons of glucose that originally entered the process of glycolysis.

At the end of the Krebs cycle, the final product is oxaloacetic acid. This is identical to the oxaloacetic acid that begins the cycle. Now the molecule is ready to accept another acetyl-CoA molecule to begin another turn of the cycle. The Krebs cycle forms (per two molecules of pyruvic acid) two ATP molecules, ten NADH molecules, and two $FADH_2$ molecules. The NADH and the $FADH_2$ will be used in the electron transport system.

(C) Electron Transport System

The electron transport system occurs in the cristae of the mitochondria, where a series of cytochromes (cell pigments) and coenzymes exist. These cytochromes and coenzymes act as carrier molecules and transfer molecules. They accept high-energy electrons and pass the electrons to the next molecule in the system. At key proton-pumping sites, the energy of the electrons transports protons across the membrane into the outer compartment of the mitochondrion.

Each NADH molecule is highly energetic, which accounts for the transfer of six protons into the outer compartment of the mitochondrion. Each $FADH_2$ molecule accounts for the transfer of four protons. The flow of electrons is similar to that taking place in photosynthesis. Electrons pass from NAD to FAD, to other cytochromes and coenzymes, and eventually they lose much of their energy. In cellular respiration, the final electron acceptor is an oxygen atom. In their energy-depleted condition, the electrons unite with an oxygen atom. The electron–oxygen combination then reacts with two hydrogen ions (protons) to form a water molecule (H_2O).

The role of oxygen in cellular respiration is substantial. As a final electron receptor, it is responsible for removing electrons from the system. If oxygen were not available, electrons could not be passed among the coenzymes, the energy in electrons could not be released, the proton pump could not be established, and ATP could not be produced. In humans, breathing is the essential process that brings oxygen into the body for delivery to the cells to participate in cellular respiration.

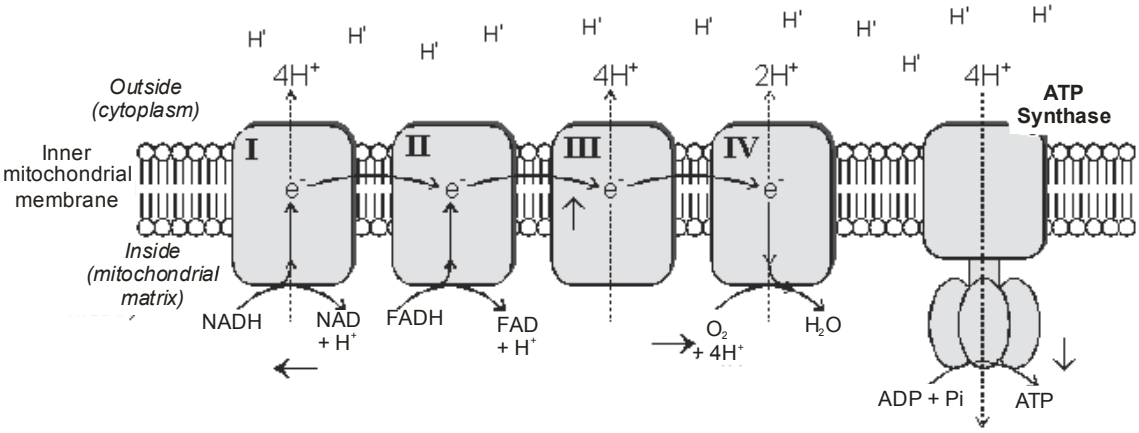


Fig.3.18. Oxidative Phosporylation

(D) Chemiosmosis

The actual production of ATP in cellular respiration takes place through the process of chemiosmosis. Chemiosmosis involves the pumping of protons through special channels in the membranes of mitochondria from the inner to the outer compartment. The pumping establishes a proton gradient. After the gradient is established, protons pass down the gradient through particles designated F1. In these particles, the energy of the protons generates ATP, using ADP and phosphate ions as the starting points.

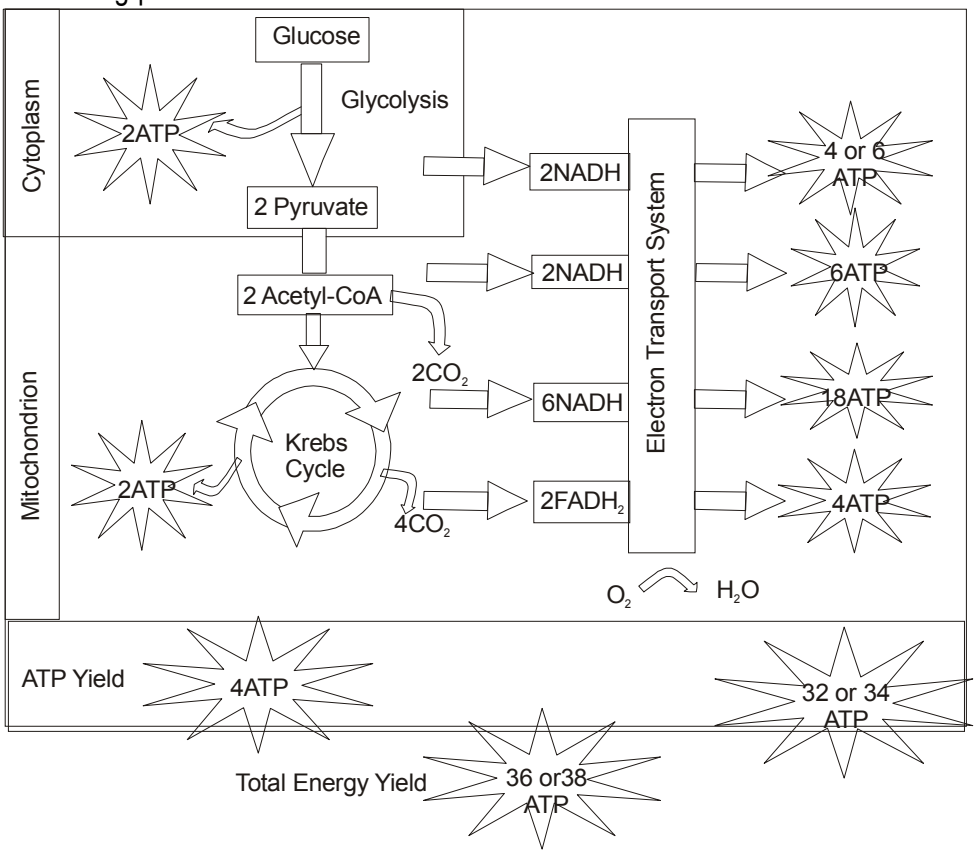


Fig.3.19. ATP Balance Sheet of Respiration

The energy production of cellular respiration is substantial. Most biochemists agree that 36 molecules of ATP can be produced for each glucose molecule during cellular respiration as a result of the Krebs cycle reactions, the electron transport system, and chemiosmosis. Also, two ATP molecules are produced through glycolysis, so the grand total is 38 molecules of ATP. These ATP

molecules may then be used in the cell for its needs. However, the ATP molecules cannot be stored for long periods of time, so cellular respiration must constantly continue in order to regenerate the ATP molecules as they are used. Each ATP molecule is capable of releasing 7.3 kilocalories of energy per mole.

(E) Fermentation

Fermentation is an anaerobic process in which energy can be released from glucose even though oxygen is not available. **Fermentation** occurs in yeast cells, and a form of fermentation takes place in bacteria and in the muscle cells of animals.

In yeast cells (the yeast used for baking and producing alcoholic beverages), glucose can be metabolized through cellular respiration as in other cells. When oxygen is lacking, however, glucose is still metabolized to pyruvic acid via glycolysis. The pyruvic acid is converted first to acetaldehyde and then to ethyl alcohol. The net gain of ATP to the yeast cell is two molecules—the two molecules of ATP normally produced in glycolysis.

Yeasts are able to participate in fermentation because they have the necessary enzyme to convert pyruvic acid to ethyl alcohol. This process is essential because it removes electrons and hydrogen ions from NADH during glycolysis. The effect is to free the NAD so it can participate in future reactions of glycolysis. The net gain to the yeast cell of two ATP molecules permits it to remain alive for some time. However, when the percentage of ethyl alcohol reaches approximately 15 percent, the alcohol kills the yeast cells.

Yeast is used both in bread and alcohol production. Alcohol fermentation is the process that yields beer, wine, and other spirits. The carbon dioxide given off during fermentation supplements the carbon dioxide given off during the Krebs cycle and causes bread to rise.

$$2 \text{ pyruvate} + 2 \text{ NADH} + 2 \text{ H}^+ \longrightarrow 2 \text{ Ethanol} + \text{CO}_2 + 2 \text{ NAD}^+$$

In muscle cells, another form of fermentation takes place. When muscle cells contract too frequently (as in strenuous exercise), they rapidly use up their oxygen supply. As a result, the electron transport system and Krebs cycle slow considerably, and ATP production is slowed. However, muscle cells have the ability to produce a small amount of ATP through glycolysis in the absence of oxygen. The muscle cells convert glucose to pyruvic acid. Then an enzyme in the muscle cells converts the pyruvic acid to lactic acid. As in the yeast, this reaction frees up the NAD while providing the cells with two ATP molecules from glycolysis. Eventually, however, the lactic acid buildup causes intense fatigue, and the muscle cell stops contracting.

$$2 \text{ pyruvate} + 2 \text{ NADH} + 2 \text{ H}^+ \longrightarrow 2 \text{ Lactate} + 2 \text{ NAD}^+$$

(F). Balancing Act

Photosynthesis and **respiration** are the reverse of each other, and you couldn't have one without the other. The net result of all the photosynthesis and respiration by living organisms is the **conversion of light energy to heat energy.**

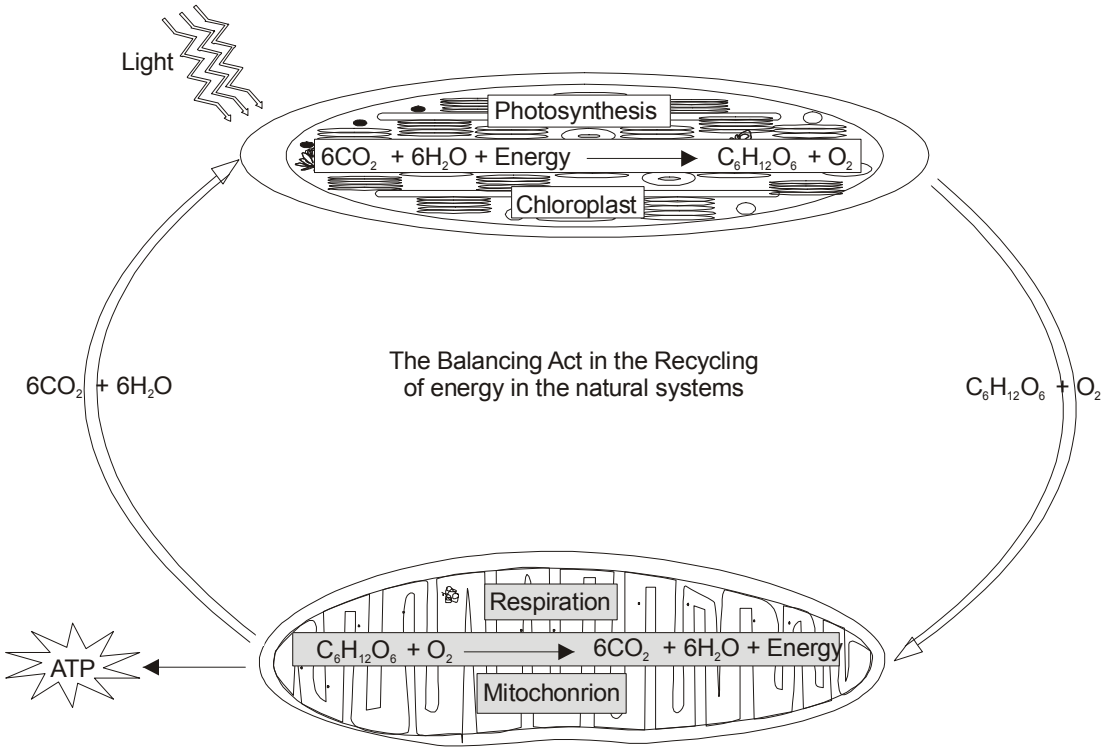


Fig.3.6. The Balancing act of metabolisms

Carbon-based compounds are the building blocks and energy stores of life. Plants assemble these compounds by **photosynthesis.** First they trap sunlight energy and convert it to chemical energy (in the form of certain bonds in ATP molecules).

Chapter Four
Cell Reproduction and Cytogenetics

A distinguishing feature of a living thing is that it reproduces independent of other living things. This reproduction occurs at the cellular level. In certain parts of the body, such as along the gastrointestinal tract, the cells reproduce often. In other parts of the body, such as in the nervous system, the cells reproduce less frequently. With the exception of only a few kinds of cells, such as red blood cells (which lack nuclei), all cells of the human body reproduce.
In eukaryotic cells, the structure and contents of the nucleus are of fundamental importance to an understanding of cell reproduction. The nucleus contains the hereditary material of the cell assembled into chromosomes. In addition, the nucleus usually contains one or more prominent **nucleoli** .

The nuclear material consists of deoxyribonucleic acid (DNA) organized into long strands. The strands of DNA are composed of nucleotides bonded to one another by covalent bonds. DNA molecules are extremely long relative to the cell; indeed, the length of a chromosome may be hundreds of times the diameter of its cell. However, in the chromosome, the **DNA** is condensed and packaged with protein into manageable bodies. The mass of DNA material and its associated protein is **chromatin.** To form chromatin, the DNA molecule is wound around globules of a protein called **histone.** The units formed in this way are *nucleosomes.* Millions of nucleosomes are connected by short stretches of histone protein much like beads on a string. The configuration of the nucleosomes in a coil causes additional coiling of the DNA and the eventual formation of the chromosome.

4.1. Cell Cycle

The **cell cycle** involves many repetitions of cellular growth and reproduction. With few exceptions (for example, red blood cells), all the cells of living things undergo a cell cycle.

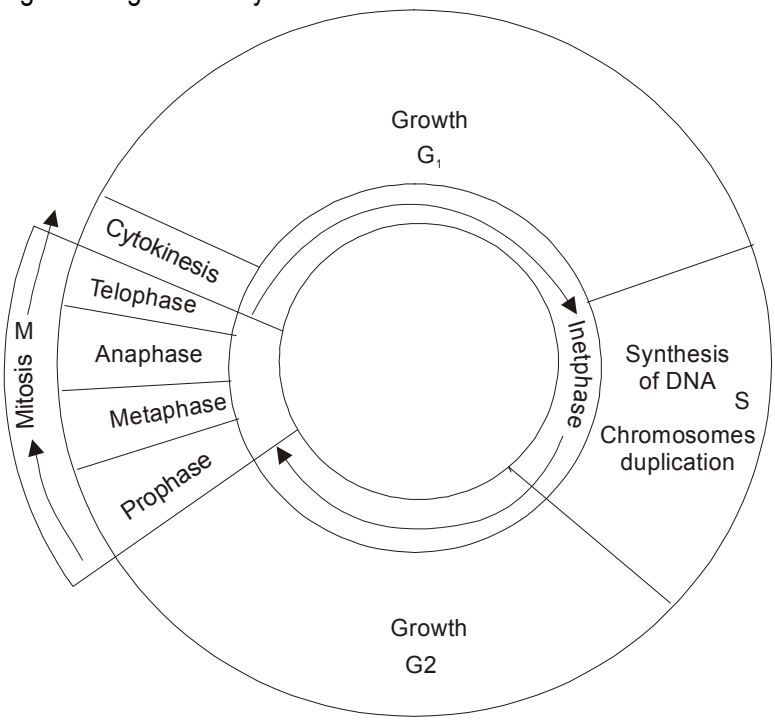


Fig.4.1.The Cell Cycle

The cell cycle is generally divided into two phases: **interphase** and **mitosis**. During **interphase,** the cell spends most of its time performing the functions that make it unique. *Mitosis* is the phase of the cell cycle during which the cell divides into two daughter cells.

(A) Interphase

The interphase stage of the cell cycle includes three distinctive parts: the **G_1 phase**, the **S phase,** and the **G_2 phase**. The **G1 phase** follows mitosis and is the period in which the cell is synthesizing its structural proteins and enzymes to perform its functions. For example, a pancreas cell in the G_1 phase will produce and secrete insulin, a muscle cell will undergo the contractions that permit movement, and a salivary gland cell will secrete salivary enzymes to assist digestion. During the G_1 phase, each chromosome consists of a single molecule of DNA and its associated histone protein. In human cells, there are 46 chromosomes per cell (except in sex cells with 23 chromosomes and red blood cells with no nucleus and hence no chromosomes).

During the **S phase** of the cell cycle, the DNA within the nucleus replicates. During this process, each chromosome is faithfully copied, so by the end of the S phase, two DNA molecules exist for each one formerly present in the G_1 phase. Human cells contain 92 chromosomes per cell in the S phase.

In the G_2 phase, the cell prepares for mitosis. Proteins organize themselves to form a series of fibers called the *spindle,* which is involved in chromosome movement during mitosis. The spindle is constructed from amino acids for each mitosis, and then taken apart at the conclusion of the process. Spindle fibers are composed of microtubules.

(B) Mitosis

During mitosis, the nuclear material becomes visible as threadlike chromosomes. The chromosomes organize in the center of the cell, and then they separate, and 46 chromosomes move into each new cell that forms. Mitosis is a continuous process, but for convenience in denoting which portion of the process is taking place, scientists divide mitosis into a series of phases: **prophase, metaphase, anaphase, telophase,** and **cytokinesis**.

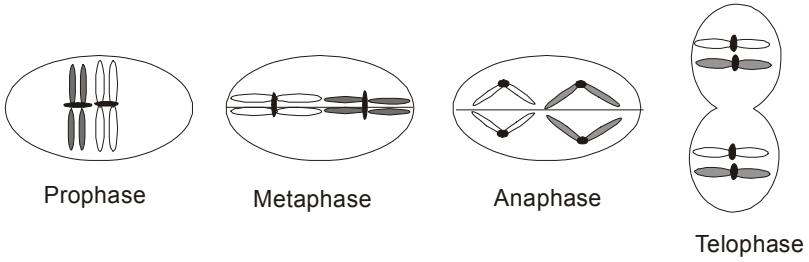


Fig.4.2. Mitosis

(i) Prophase

Mitosis begins with the condensation of the chromosomes to form visible threads in the phase called prophase. Two copies of each chromosome exist; each one is a **chromatid.** Two chromatids are joined to one another at a region called the **centromere.** As prophase unfolds, the chromatids become visible in pairs, the spindle fibers form, the nucleoli disappear, and the nuclear envelope dissolves.

In animal cells during prophase, microscopic bodies called the **centrioles** begin to migrate to opposite sides of the cell. When the centrioles reach the poles of the cell, they produce, and are then surrounded by, a series of radiating microtubules called an ***aster***. Centrioles and asters are not present in most plant or fungal cells.

As prophase continues, the chromatids attach to spindle fibers that extend out from opposite poles of the cell. The spindle fibers attach at the region of the centromere at a structure called the **kinetochore,** a region of DNA that has remained undivided. Eventually, all pairs of chromatids reach the center of the cell, a region called the ***equatorial plate***.

(ii) Metaphase

Metaphase is the stage of mitosis in which the pairs of chromatids line up on the equatorial plate. This region is also called the metaphase plate. In a human cell, 92 chromosomes in 46 pairs align at the equatorial plate. Each pair is connected at

centromere, where the spindle fiber is attached (more specifically at the kinetochore). At this point, the DNA at the kinetochore duplicates, and the two chromatids become completely separate from one another.

(iii) Anaphase
At the beginning of anaphase **,** the chromatids move apart from one another. The chromatids are **chromosomes** after the separation. Each chromosome is attached to a spindle fiber, and the members of each chromosome pair are drawn to opposite poles of the cell by the spindle fibers. They take on a rough V shape because of their midregion attachment to the spindle fibers. The movement toward the poles is accomplished by several mechanisms, such as an elongation of the spindle fibers, which results in pushing the poles apart. The result of anaphase is an equal separation and distribution of the chromosomes. In humans cells, a total of 46 chromosomes move to each pole as the process of mitosis continues.

(iv) Telophase
In telophase, the chromosomes finally arrive at the opposite poles of the cell. The distinct chromosomes begin to fade from sight as masses of chromatin are formed again. The events of telophase are essentially the reverse of those in prophase. The spindle is dismantled and its amino acids are recycled, the nucleoli reappear, and the nuclear envelope is reformed.

(v) Cytokinesis
Cytokinesis is the process in which the cytoplasm divides and two separate cells form. In animal cells, cytokinesis begins with the formation of a furrow in the center of the cell. With the formation of the furrow, the cell membrane begins to pinch into the cytoplasm, and the formation of two cells begins. This process is often referred to as *cell cleavage.* Microfilaments contract during cleavage and assist the division of the cell into two daughter cells.

In plant cells, cytokinesis occurs by a different process because a rigid cell wall is involved. Cleavage does not take place in plant cells. Rather, a new cell wall is assembled at the center of the cell, beginning with vesicles formed from the Golgi body. As the vesicles join, they form a double membrane called the *cell plate.* The cell plate forms in the middle of the cytoplasm and grows outward to fuse with the cell membrane. The cell plate separates the two daughter cells. As cell wall material is laid down, the two cells move apart from one another to yield two new daughter cells.

Mitosis serves several functions in living cells. In many simple organisms, it is the method for asexual reproduction (for example, in the cells of a fungus). In multicellular organisms, mitosis allows the entire organism to grow by forming new cells and replacing older cells. In certain species, mitosis is used to heal wounds or regenerate body parts. It is the universal process for cell division.

(C) Meiosis

Most plant and animal cells are diploid i.e. have two sets of chromosomes. In human cells, for example, 46 chromosomes are organized in 23 pairs. Hence, human cells are diploid in that they have two sets of 23 chromosomes per set.

During sexual reproduction, the sex cells of parent organisms unite with one another and form a fertilized egg cell. In this situation, each sex cell is a **gamete**. The gametes of human cells are **haploid**. This term implies that each gamete contains a single set of chromosomes—23 chromosomes in humans. When the human gametes unite with one another, the original diploid condition of 46 chromosomes is reestablished. Mitosis then brings about the development of the diploid cell into an organism.

The process by which the chromosome number is halved during gamete formation is **meiosis**. In meiosis, a cell containing the diploid number of chromosomes is converted into four cells, each having the haploid number of chromosomes. In human cells, for instance, a reproductive cell containing 46 chromosomes yields four cells, each with 23 chromosomes.

Meiosis occurs by a series of steps that resemble the steps of mitosis. Two major phases of meiosis occur: meiosis I and meiosis II. During meiosis I, a single cell divides into two. During meiosis II, those two cells each divide again. The same demarcating phases of mitosis take place in meiosis I and meiosis II.

First, the chromosomes of a cell duplicate and pass into two cells. The chromosomes of the two cells then separate and pass into four daughter cells. The parent cell has two sets of chromosomes and is diploid, while the daughter cells have a single set of chromosomes each and are haploid. Synapsis and crossing over occur in the Prophase I stage. The members of each chromosome pair within a cell are called *homologous chromosomes*. Homologous chromosomes are similar but not identical. They may carry different versions of the same genetic information. For instance, one homologous chromosome may carry the information for blond hair while the other homologous chromosome may carry the information for black hair. As a cell prepares to enter meiosis, each of its chromosomes has duplicated, as in mitosis. Each chromosome thus consists of two chromatids.

(i) Meiosis I

At the beginning of meiosis 1, a human cell contains 46 chromosomes, or 92 chromatids (the same number as during mitosis). Meiosis I proceeds through the following phases:

(a) Prophase I

Prophase I is similar in some ways to prophase in mitosis. The chromatids shorten and thicken and become visible under a microscope. An important difference, however, is that a process called synapsis occurs. A second process called crossing over also takes place during prophase 1.

During prophase 1, the two homologous chromosomes come near each other. Because each homologous chromosome consists of two chromatids, there are actually four chromatids aligned next to one another. This combination of four chromatids is called a *tetrad*, and the coming together is the process called *synapsis*.

After synapsis has taken place, the process of crossing over occurs. In this process, segments of DNA from one chromatid in the tetrad pass to another chromatid in the tetrad. They result in a genetically new chromatid. Crossing over is an important driving force of evolution. After crossing over has taken place, the four chromatids of the tetrad are genetically different from the original four chromatids.

(b) Metaphase I

In metaphase I of meiosis, the tetrads align on the equatorial plate (as in mitosis). The centromeres attach to spindle fibers, which extend from the poles of the cell. One centromere attaches per spindle fiber.

(c) Anaphase I

In anaphase 1, the homologous chromosomes separate. One homologous chromosome (consisting of two chromatids) moves to one side of the cell, while the other homologous chromosome (consisting of two chromatids) moves to the other side of the cell. The result is that 23 chromosomes (each consisting of two chromatids) move to one pole, and 23 chromosomes (each consisting of two chromatids) move to the other pole. Essentially, the chromosome number of the cell is halved. For this reason the process is a reduction-division.

(d) Telophase I

In telophase I of meiosis, the nucleus reorganizes, the chromosomes become chromatin, and a cytoplasmic division into two cells takes place. This process occurs differently in plant and animal cells, just as in mitosis. Each daughter cell (with 23 chromosomes each consisting of two chromatids) then enters interphase, during which there is no duplication of the DNA. The interphase period may be brief or very long, depending on the species of organism.

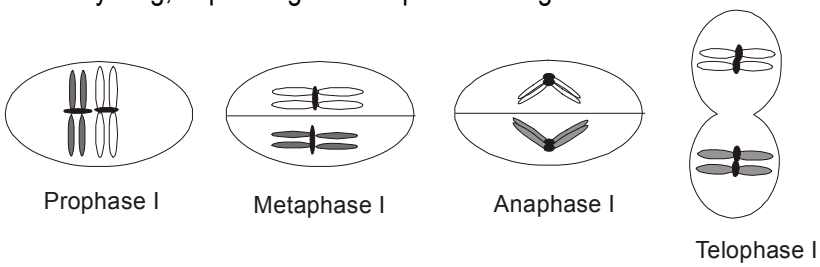


Fig.4.3. First Meiotic Cell Division

(ii) Meiosis II

Meiosis II is the second major subdivision of meiosis. It occurs in essentially the same way as mitosis. In meiosis II, a cell containing 46 chromatids undergoes division into two cells, each with 23 chromosomes. Meiosis II proceeds through the following phases:

(a) Prophase II

Prophase II is similar to the prophase of mitosis. The chromatin material condenses, and each chromosome contains two chromatids attached by the centromere. The 23 chromatid pairs, a total of 46 chromatids, then move to the equatorial plate.

(b) Metaphase II

In metaphase II of meiosis, the 23 chromatid pairs gather at the center of the cell prior to separation. This process is identical to metaphase in mitosis.

(c) Anaphase II

During anaphase II of meiosis, the centromeres divide, and the 46 chromatids become known as 46 chromosomes. Then the 46 chromosomes separate from one another. Spindle fibers move one chromosome from each pair to one pole of the cell and the other member of the pair to the other pole. In all, 23 chromosomes move to each pole. The forces and attachments that operate in mitosis also operate in anaphase 11.

(d) Telophase II

During telophase II, the chromosomes gather at the poles of the cells and become indistinct. Again, they form a mass of chromatin. The nuclear envelope develops, the nucleoli reappear, and the cells undergo cytokinesis as in mitosis.

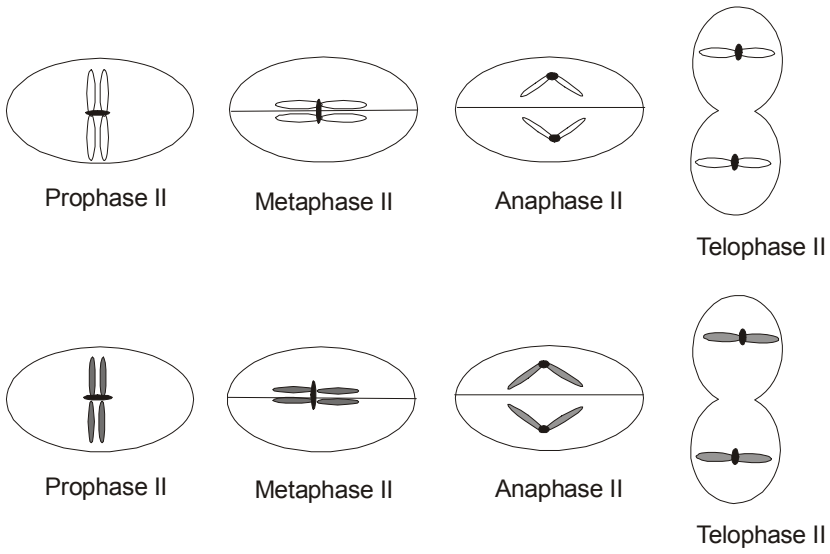


Fig.4.4. Second Meiotic Cell Division

During meiosis II, each cell containing 46 chromatids yields two cells, each with 23 chromosomes. Originally, there were two cells that underwent meiosis II; therefore, the result of meiosis II is four cells, each with 23 chromosomes. Each of the four cells is haploid; that is, each cell contains a single set of chromosomes. The 23 chromosomes in the four cells from meiosis are not identical because crossing over has taken place in prophase 1. The crossing over yields variation so that each of the four resulting cells from meiosis differs from the other three. Thus, meiosis provides a mechanism for producing variations in the chromosomes. Also, it accounts for the formation of four haploid cells from a single diploid cell.

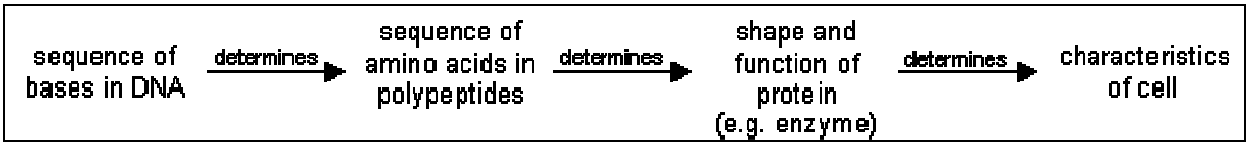
(III) Meiosis in Humans

In humans, meiosis is the process by which sperm cells and egg cells are produced. In the male, meiosis takes place after puberty. Diploid cells within the testes undergo meiosis to produce haploid **sperm** cells with 23 chromosomes. A

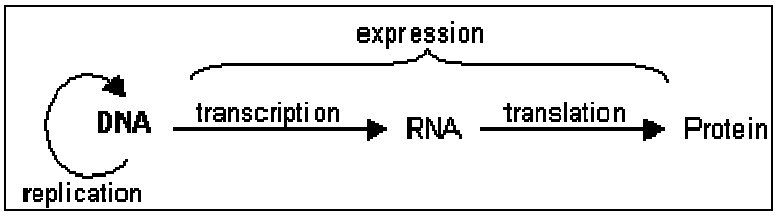
single diploid cell yields four haploid sperm cells through meiosis. In females, meiosis begins during the fetal stage when a series of diploid cells enter meiosis 1. At the conclusion of meiosis 1, the process comes to a halt, and the cells gather in the ovaries. At puberty, meiosis resumes. One cell at the end of meiosis I enters meiosis II each month. The result of meiosis II is a single egg cell per cycle (the other meiotic cells disintegrate). Each egg cell contains 23 chromosomes and is haploid.
The union of the egg cell and the sperm cell leads to the formation of a fertilized egg cell with 46 chromosomes, or 23 pairs. Fertilization restores the diploid number of chromosomes. The fertilized egg cell, a diploid, is a **zygote**. Further divisions of the zygote by mitosis eventually yield a complete human being.

4,2. Gene Expression

DNA is the genetic material, and genes are made of DNA. DNA therefore has two essential functions: replication and expression. Replication means that the DNA, with all its genes, must be copied every time a cell divides. Expression means that the genes on DNA must control characteristics. A gene was traditionally defined as a factor that controls a particular characteristic (such as flower colour), but a much more precise definition is that a gene is a section of DNA that codes for a particular protein. Characteristics are controlled by genes through the proteins they code for, like this:



Expression can be split into two parts: transcription (making RNA) and translation (making proteins). These two functions are summarised in this diagram (called the central dogma of genetics).



(A) DNA Replication

Before a cell enters the process of mitosis, its DNA replicates itself. Equal copies of the DNA pass into the daughter cells at the end of mitosis. In human cells, this means that 46 chromosomes (or molecules of DNA) replicate to form 92 chromosomes.

The process of DNA replication begins when specialized enzymes pull apart, or “unzip,” the DNA double helix (see Figure 1). As the two strands separate, the purine and pyrimidine bases on each strand are exposed. The exposed bases then attract their complementary bases. Deoxyribose molecules and phosphate groups are present in the nucleus. The enzyme **DNA polymerase** joins all the nucleotide components to one another, forming a long strand of nucleotides. Thus, the old strand of DNA directs the synthesis of a new strand of DNA through complementary base pairing. The old strand then unites with the new strand to reform a double helix. This process is called *semiconservative replication* because one of the old strands is conserved in the new DNA double helix.

DNA polymerase joins nucleotides in a 5'-3' direction on the leading strand, shown in Figure 1 . However, DNA polymerase does not elongate a DNA strand in a 3'-5' direction. Therefore, the 3'-5' strand, called the lagging strand, is synthesized in short segments in a 5'-3' direction. These short segments placed on the lagging strand are **Okazaki fragments** and are ultimately joined together by the enzyme DNA ligase to form a new DNA strand.

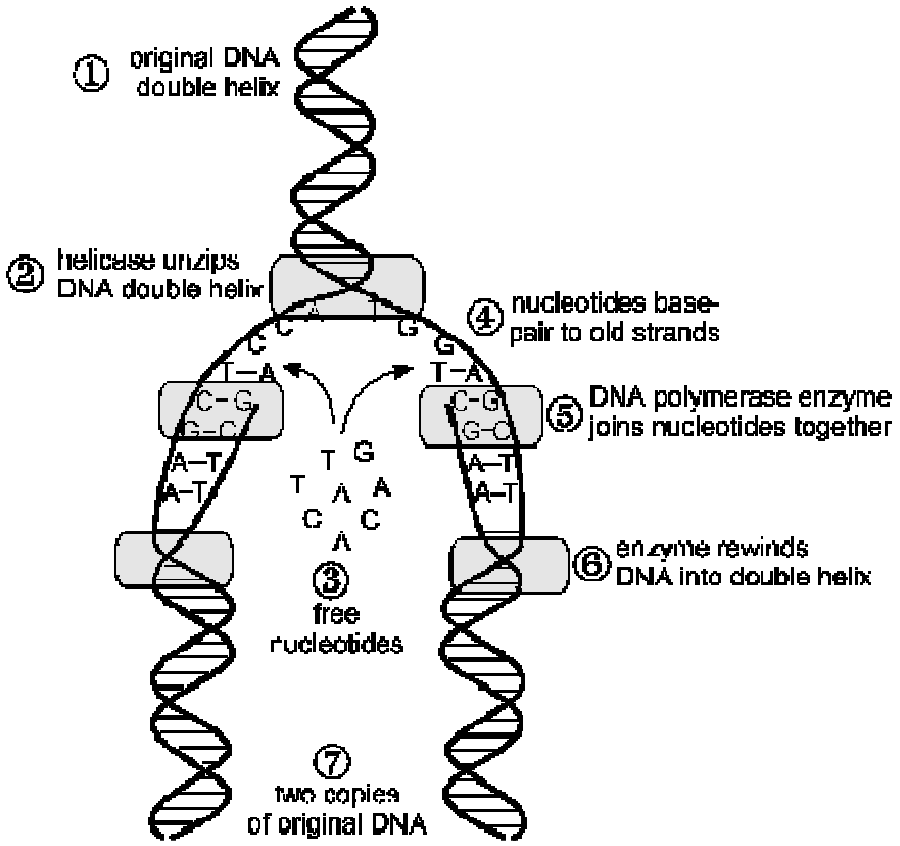


Fig.4,5. DNA Replication

DNA replication occurs during the S phase of the cell cycle. After replication has taken place, the chromosomal material shortens and thickens. The chromatids appear in the prophase of the next mitosis. The process then continues, and eventually two daughter cells form, each with the identical amount and kind of DNA as the parent cell. The process of DNA replication thus ensures that the molecular material passes to the offspring cells in equal amounts and types.

(B) Protein Synthesis

During the 1950s and 1960s, it became apparent that DNA is essential in the synthesis of proteins. **Proteins** are used in enzymes and as structural materials in cells. Many specialized proteins function in cellular activities. For example, in humans, the hormone insulin and the muscle cell filaments are composed of protein. The hair, skin, and nails of humans are composed of proteins, as are all the hundreds of thousands of enzymes in the body.

(i) Genetic Code

The key to a protein molecule is how the amino acids are linked. The sequence of amino acids in a protein is a type of code that specifies the protein and distinguishes one protein from another. A genetic code in the DNA determines this amino acid code. The genetic code consists of the sequence of nitrogenous bases in the DNA. How the nitrogenous base code is translated to an amino acid sequence in a protein is the basis for protein synthesis.

		SECOND BASE									
		U		C		A		G			
F	U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U	**T**
I		UUC		UCC		UAC		UGC		C	**H**
R		UUA	Leu	UCA	Ser	UAA	**Stop**	UGA	**Stop**	A	**I**
S		UUG		UCG		UAG		UGG	Trp	G	**R**

T B A S E (5'end)	C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U	**D** **B** **A** **S** **E** **(3'end)**
		CUC		CCC		CAC		CGC		C	
		CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A	
		CUG		CCG		CAG		CGG		G	
	A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U	
		AUC		ACC		AAC		AGC		C	
		AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A	
		AUG	**Met**	ACG		AAG		AGG		G	
	G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U	
		GUC		GCC		GAC		GGC		C	
		GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A	
		GUG		GCG		GAG		GGG		G	

**** Note that this table represents bases in mRNA. There are some tables that may only show the DNA code*

For protein synthesis to occur, several essential materials must be present, such as a supply of the 20 amino acids, which comprise most proteins. Another essential element is a series of enzymes that will function in the process. DNA and another form of nucleic acid called**ribonucleic acid (RNA)** are essential.

(ii) RNA

RNA is the nucleic acid that carries instructions from the nuclear DNA into the cytoplasm, where protein is synthesized. RNA is similar to DNA, with two exceptions. First, the carbohydrate in RNA is ribose rather than deoxyribose, and second, RNA nucleotides contain the pyrimidine uracil rather than thymine.

(iii) Types of RNA

In the synthesis of protein, three types of RNA function. The first type is called *ribosomal RNA (rRNA)*. This form of RNA is used to manufacture ribosomes. **Ribosomes** are ultramicroscopic particles of rRNA and protein. They are the places (the chemical "workbenches") where amino acids are linked to one another to synthesize proteins. Ribosomes may exist along the membranes of the endoplasmic reticulum or in the cytoplasm of the cell.

A second important type of RNA is *transfer RNA (tRNA)*. Transfer RNA exists in the cell cytoplasm and carries amino acids to the ribosomes for protein synthesis. When protein synthesis is taking place, enzymes link tRNA molecules to amino acids in a highly specific manner. For example, tRNA molecule X will link only to amino acid X; tRNA Y will link only to amino acid Y.

The third form of RNA is *messenger RNA (mRNA)*. In the nucleus, messenger RNA receives the genetic code in the DNA and carries the code into the cytoplasm where protein synthesis takes place. Messenger RNA is synthesized in the nucleus at the DNA molecules. During the synthesis, the genetic information is transferred from the DNA molecule to the mRNA molecule. In this way, a genetic code can be used to synthesize a protein in a distant location. **RNA polymerase**, an enzyme, accomplishes mRNA, tRNA, and rRNA synthesis.

(iv) Transcription

Transcription is one of the first processes in the mechanism of protein synthesis. In transcription, a complementary strand of mRNA is synthesized according to the nitrogenous base code of DNA. To begin, the enzyme RNA polymerase binds to an area of one of the DNA molecules in the double helix. (During transcription, only one DNA strand serves as a template for RNA synthesis. The other DNA strand remains dormant.) The enzyme moves along the DNA strand and "reads" the nucleotides one by one. Similar to the process of DNA replication, the new nucleic acid strand elongates in a 5'-3' direction. . The enzyme selects complementary bases from available nucleotides and positions them in an mRNA molecule according to the principle of complementary base pairing. The chain of mRNA lengthens until a "stop" message is received.

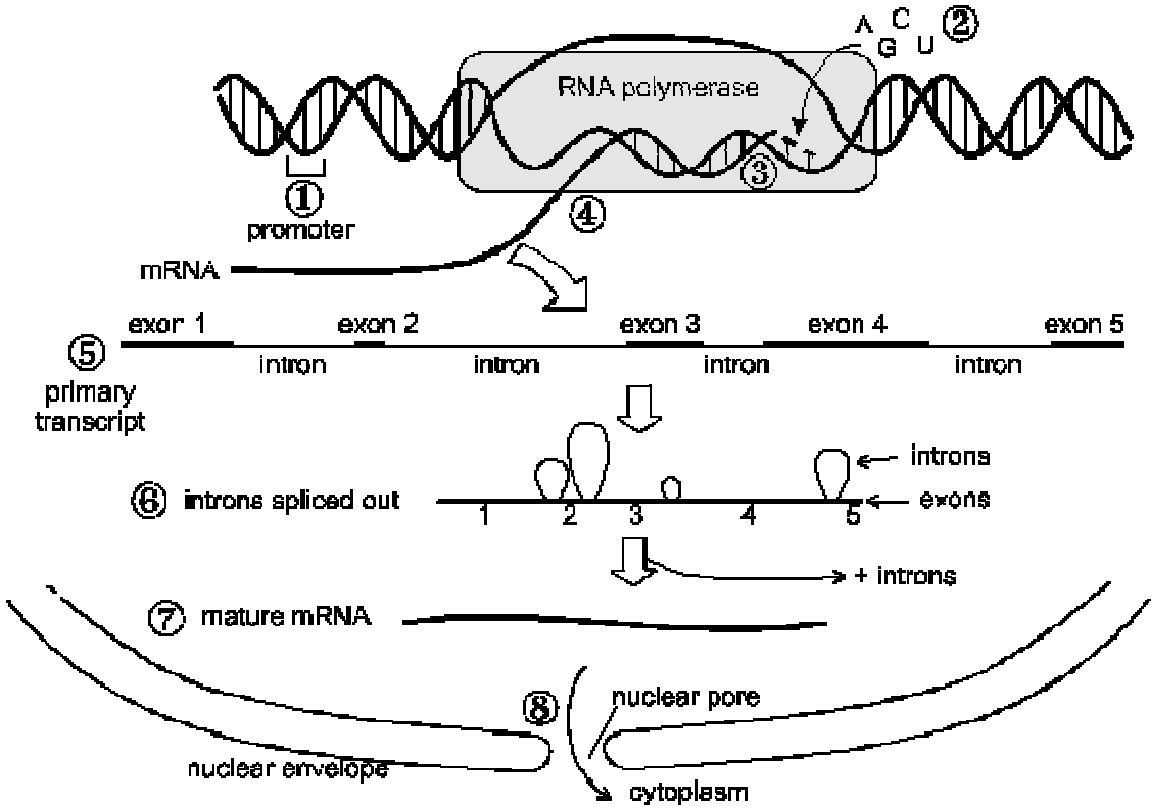


Fig.4,6, Transcription Process

The nucleotides of the DNA strands are read in groups of three. Each group is a *codon*. Thus, a codon may be CGA, or TTA, or GCT, or any other combination of the four bases, depending on their sequence in the DNA strand. Each codon will later serve as a "code word" for an amino acid. First, however, the codons are transcribed to the mRNA molecule. Thus, the mRNA molecule consists of nothing more than a series of codons received from the genetic message in the DNA.
After the "stop" codon is reached, the synthesis of the mRNA comes to an end. The mRNA molecule leaves the DNA molecule, and the DNA molecule rewinds to form a double helix. Meanwhile, the mRNA molecule passes through a pore in the nucleus and proceeds into the cellular cytoplasm where it moves toward the ribosomes.

(v) Translation

The genetic code is transferred to an amino acid sequence in a protein through the **translation** process, which begins with the arrival of the mRNA molecule at the ribosome. While the mRNA was being synthesized, tRNA molecules were uniting with their specific amino acids according to the activity of specific enzymes. The tRNA molecules then began transporting their amino acids to the ribosomes to meet the mRNA molecule. After it arrives at the ribosomes, the mRNA molecule exposes its bases in sets of three, the codons. Each codon has a complementary codon called an **anticodon** on a tRNA molecule. When the codon of the mRNA molecule complements the anticodon on the tRNA molecule, the latter places the particular amino acid in that position. Then the next codon of the mRNA is exposed, and the complementary anticodon of a tRNA molecule matches with it. The amino acid carried by the second tRNA molecule is positioned next to the first amino acid, and the two are linked. At this point, the tRNA molecules release their amino acids and return to the cytoplasm to link up with new molecules of amino acid.

When it's time for the next amino acid to be positioned in the growing protein, a new codon on the mRNA molecule is exposed, and the complementary three-base anticodon of a tRNA molecule positions itself opposite the codon. This brings another amino acid into position, and that amino acid links to the previous amino acids. The ribosome moves further down the mRNA molecule and exposes another codon, which attracts another tRNA molecule with its anticodon.

One by one, amino acids are added to the growing chain until the ribosome has moved down to the end of the mRNA molecule. Because of the specificity of tRNA molecules for their individual amino acids, and because of the base pairing between codons and anticodons, the sequence of codons on the mRNA molecule determines the sequence of amino acids in the protein being constructed. And because the codon sequence of the mRNA complements the codon sequence of the DNA, the DNA molecule ultimately directs the amino acid sequencing in proteins. The primary "start" codon on an mRNA molecule is AUG, which codes for the amino acid methionine. Therefore, each mRNA transcript begins with the AUG codon, and the resulting peptide begins with methionine.

The mRNA molecule proceeds to the ribosome where it meets tRNA molecules carrying amino acids. The tRNA molecule has a base code that complements the mRNA code and thereby brings a specific amino acid into position. The amino acids join together in peptide bonds and the tRNA molecules are released to pick up additional amino acid molecules.

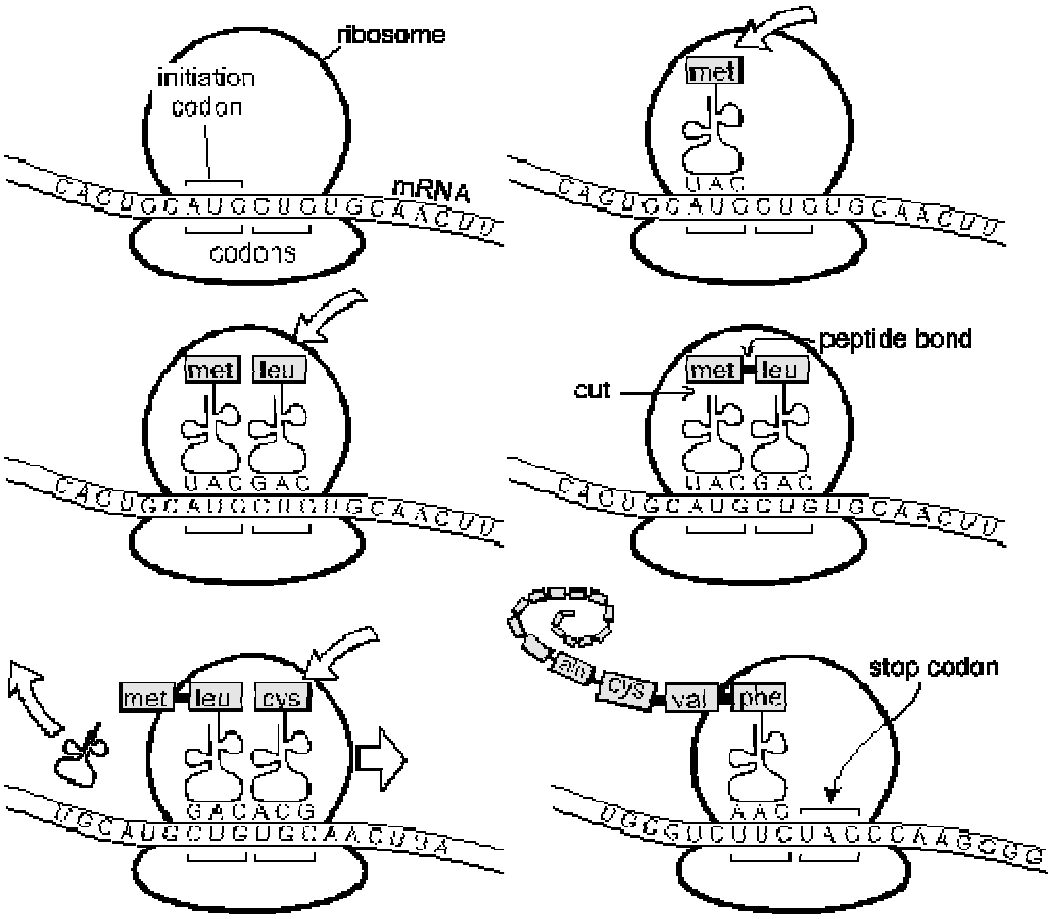


Fig.4.7. Translation

After the protein has been synthesized completely, it is removed from the ribosome for further processing and to perform its function. For example, the protein may be stored in the Golgi body before being released by the cell, or it may be stored in the lysosome as a digestive enzyme. Also, a protein may be used in the cell as a structural component, or it may be released as a hormone, such as insulin. After synthesis, the mRNA molecule breaks up and the nucleotides return to the nucleus. The tRNA molecules return to the cytoplasm to unite with fresh molecules of amino acids, and the ribosome awaits the arrival of a new mRNA molecule.

(vi) Gene Control

The process of protein synthesis does not occur constantly in the cell. Rather, it occurs at intervals followed by periods of genetic "silence." Thus the cell regulates and controls the gene expression process. The control of gene expression may

occur at several levels in the cell. For example, genes rarely operate during mitosis, when the DNA fibers shorten and thicken to form chromatids. The inactive chromatin is compacted and tightly coiled, and this coiling regulates access to the genes. Other levels of gene control can occur during and after transcription. In transcription, certain segments of DNA can increase and accelerate the activity of nearby genes. After transcription has taken place, the mRNA molecule can be altered to regulate gene activity. For example, researchers have found that an mRNA molecule contains many useless bits of RNA that are removed in the production of the final mRNA molecule. These useless bits of nucleic acid are called *introns*. The remaining pieces of mRNA, called *exons*, are then spliced to form the final mRNA molecule. Thus, through removal of introns and the retention of exons, the cell can alter the message received from the DNA and control gene expression.

In Bacteria, genes have been identified as structural genes, regulator genes, and control genes. The three units form a functional unit called the *operon.* Certain carbohydrates can induce the presence of the enzymes needed to digest those carbohydrates. When lactose is present, bacteria synthesize the enzyme needed to break down the lactose. Lactose acts as the inducer molecule in the following way: In the absence of lactose, a regulator gene produces a repressor, and the repressor binds to a control region called the operator. This binding prevents the structural genes from encoding the enzyme for lactose digestion. When lactose is present, however, it binds to the repressor and thereby removes the repressor at the operator site. With the operator site free, the structural genes are free to produce their lactose-digesting enzyme.

The operon system in bacteria shows how gene expression can occur in relatively simple cells. The gene is inactive until it is needed and is active when it becomes necessary to produce an enzyme.

(C) Recombinant DNA

Biotechnology is an industrial process that uses the scientific research on DNA for practical means. Biotechnology is synonymous with genetic engineering because the genes of an organism are changed during the process. Because the genes are changed, the DNA of the organism is said to be *recombined*. The result of the process is **recombinant DNA**. Recombinant DNA and biotechnology can be used to form proteins not normally produced in a cell, to produce drugs or vaccines, or to promote human health. In addition, bacteria that carry recombinant DNA can be released into the environment under carefully controlled conditions to increase the fertility of the soil, serve as an insecticide, or relieve pollution.

Biotechnology and recombinant DNA can also be used in forensic medicine to “fingerprint” individuals and identify DNA at a crime scene. In addition, transgenic plants and animals are being created. Humans can also have the genes in their cells modified to produce proteins that relieve health-related deficiencies. Finally, at the time of publication, scientists at Celera Genomics and the National Human Genome Research Institute have sequenced a substantial portion of the human genome in an effort to identify genes linked to human disease. The sequenced fragments of DNA are currently not organized into contiguous reading frames. Although a substantial portion of the genome has been sequenced, it may require several more years for all the pieces to come together to form a complete and accurate genetic map of the human genome.

…….. **End**

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