মানুষের জ্ঞান ও ভাবকে বইয়ের মধ্যে সঞ্চিত করিবার যে একটা প্রচুর সুবিধা আছে, সে কথা কেহই অস্বীকার করিতে পারে না। কিন্তু সেই সুবিধার দ্বারা মনের স্বাভাবিক শক্তিকে একেবারে আচ্ছন্ন করিয়া ফেলিলে বুদ্ধিকে বাবু করিয়া তোলা হয়।

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Any system of education which ignores Indian conditions, requirements, history and sociology is too unscientific to commend itself to any rational support.

— Subhas Chandra Bose

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NETAJI SUBHAS OPEN UNIVERSITY

Choice Based Credit System (CBCS)

SELF LEARNING MATERIAL

HZO

ZOOLOGY

Cell and Molecular Biology

CC-ZO-07

Under Graduate Degree Programme

PREFACE

In a bid to standardise higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core, discipline specific, generic elective, ability* and *skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility to choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry acquired credits. I am happy to note that the University has been accredited by NAAC with grade 'A'.

UGC (Open and Distance Learning Programmes and Online Learning Programmes) Regulations, 2020 have mandated compliance with CBCS for U.G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Under Graduate Degree Programme level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the *six* semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English / Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

Professor (Dr.) Subha Sankar Sarkar

Vice-Chancellor

D/Barun-2021/Netaji Subhas Open University/CC-VII BDP ZOOLOGY/Title New 2021/1/19.05.2022

Netaji Subhas Open University

Undergraduate Degree Programme

Choice Based Credit System (CBCS)

Subject : Honours in Zoology (HZO)

Course : Cell & Molecular Biology

Course Code : CC - ZO - 07

First Print : May, 2022

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Netaji Subhas Open University

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UG : Zoology (HZO)

Course : Cell & Molecular Biology
Course Code : CC - ZO - 07

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Unit-1 □ Plasma Membrane

Structure

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1.1 Objectives

This section of the unit of the module of Cell and Molecular Biology gives a clear picutre of the idea of plasma membrane. When the reader finishes this particular unit one will be able to understand the following :

- The basic idea of the structure of plasma membrane
- The variety of functions like
  - Cell-cell adhesion
  - Membrane transport
  - Neurotransmission
- How cellular junctions play important role in cell adhesion
- And finally about the structure of desmosome

1.2 Introduction

Every cell has a plasma membrane whether it is unicellular amoeba or a multi cellular- the familiar plants, fungi, and animals like cockroach or even human that represent only a small fraction of life on earth. Generally, a plasma membrane protects a cell by acting as a barrier between its own living content and outside the adjacent environment and proper performance is essential for maintaining our good health today. The regulation by the membrane what goes into and out of the cell makes a cell unique for the living being.

The membrane was called 'cell membrane' by C. Nageli and C. Cramer in 1855 and 'plasmalemma' by J.Q. Plower in 1931. The term 'plasma membrane' and 'plasmalemma' are preferred because the term 'cell membrane' may be confused with 'cell wall' in plants. In all cells, plasma membrane contains proteins, which help in cell identification, transport of materials to and from or in sending signals received from outside. Some of these proteins also help in maintaining ion gradients across the membrane to make possible for ATP synthesis or movement of solutes / ions through the membrane and tansmission of signals in all kinds of cells including nerve cells and muscles cells.

1.3 Membrane model

Each and every cell membrane consists of a phospholipid bilary with embedded proteins. Investigators noted that lipid soluble molecules entered cells very speedily

than water soluble molecules and suggested that one of the components of the plasma membrane is lipid. But later it has been found after chemical analysis that plasma membrane contains phospholipids. Gorter and Grended (1925) measured the quantity of the phospholipid from red blood cells and confirmed that there is enough of this amount to form a bilayer around the cells. They also suggested that the polar head which is hydrophilic are directed outward while non polar head as hydrophobic tails are directed inward. (Fig.1)

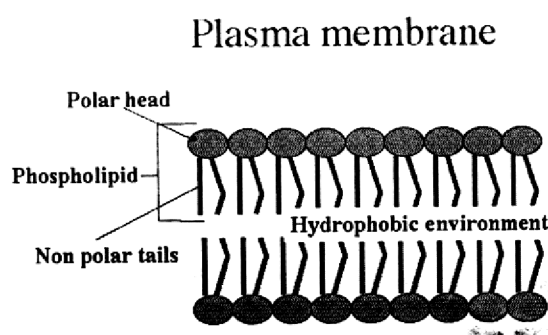


Fig.1 : Showing arrangements of phopholipid in membrane

In 1940 Daniellli and Davson proposed a sandwich morel in which the phospholipid bilayer is filled up by two continuous layers of proteins confirming proteins are a part of the membrane. (Fig.2)

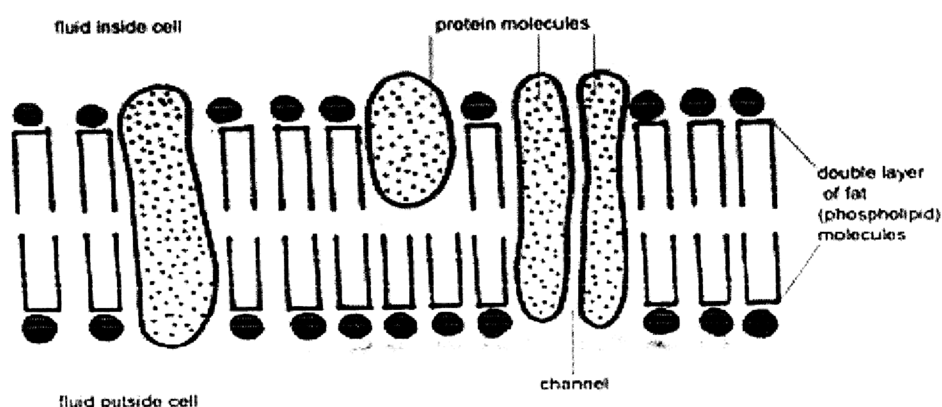


Fig.2 : Showing arrangements of phospholipid and protein in membrane

Further Roberson (1959) modified the sandwitch model and suggested that all membranes have basically the same composition of outer having proteins plus the hydrophilic heads of the phodspholipids and the interior to be the hydrophobic heads of the phospholipids. Simply, it is the alteration of sandwich model. So it faces the same shortcomings as of sandwich hypothesis.

1.3.1 Fluid-mosaic model

In 1972 Singer and Nicolson proposed finally, the most acceptable model as fluid-mosaic model of biomembrane structure. According to their proposition this model membrane does not have a uniform nature of proteins and lipids. Also, the membrane is not solid in structure rather a quasifluid.

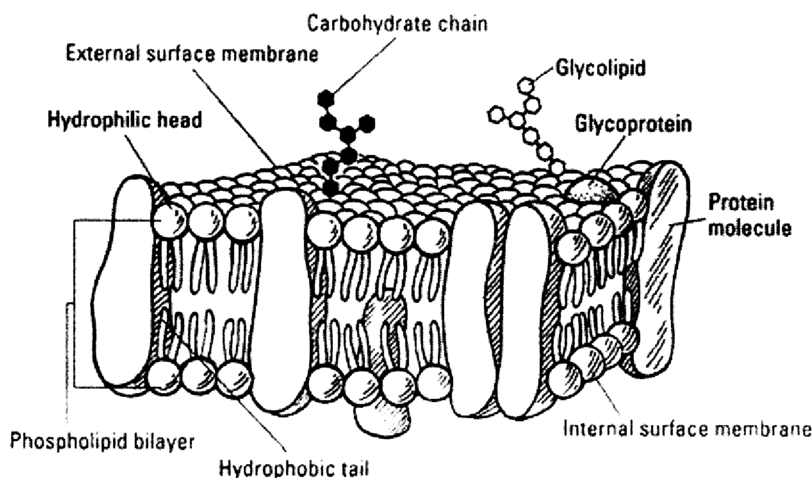


Fig.3 : Fluid-mosaic model of plasma membrane

Fluid-mosaic model hypothesizes that the lipid molecules are present in a viscous bilayer as in lipid layer. Protein molecules occur at place both inside and on the outer side of lipid bilayer. It is just like **Proteins icebergs in a sea of lipids.** It is mosaic in the sense that membrane structure consists of a fluid phospholipids bilayer where proteins are embedded to form a mosaic pattern. The proteins those are internal called as **intrinsic** or integral proteins. The external one likewise is known as **extrinsic** or **peripheral** protein. Out of the total membrane protein, 70% makes up for intrinsic protein and some of these proteins run throughout the lipid bilayer. Hence they are called tunnel proteins. Similarly, the extrinsic proteins are present apparently on the two surfaces of the membrane. Mostly the membrane proteins

behave as an enzyme. Somemay act as permeases. On the other hand some of the lipids present on the outer side contain small carbohydrate molecules to form **glycolipids.**

The main structure of the membrane is composed of phospholipid molecules that are amphiphilic or dual-loving. The hydrophilic or water-loving areas of these molecules are in contact with the aqueous fluid both inside and outside the cell. Hydrophobic, or water-hating molecules, tend to be non-polar. A phospholipid molecule consists of a three-carbon glycerol backbone with two fatty acid molecules attached to carbons 1 and 2, and a phosphate-containing group attached to the third carbon. (Fig. 4)

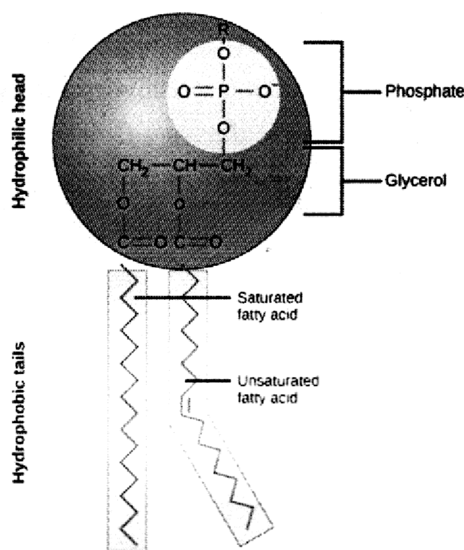


Fig.4 : The structure of a phospholipid molecule

This arrangement gives the overall moelcule an area described as its head (the phosphate-containing group), which has a polar character or negative charge, and an area called the tail (the fatty acids), which has no charge. They interact with other non-polar molecules in chemical reactions, but generally do not interact with polar molecules. When placed in water, hydrophobic molecules tend to form a ball or cluster. The hydrophilic regions of the phospholipids tend to form hydrogen bonds with water and other polar molecules on both the exterior and interior of the cell. Thus, the membrane surfaces that face the interior and exterior of the cell are hydrophillic. In contrast, the middle of the cell membrane is hydrophobic and will

not interact with water. Therefore, phospholipids form an excellent lipid bilayer cell membrane that separates fluid within the cell from the fluid outside of the cell.

Proteins make up the second major component of plasma membranes. Integral proteins (some specialized types are called intergrins) are, as their name suggests, integrated completely into the membrane structure, and their hydrophobic membrane spanning regions interact with the hydrophobic region of the phospholipids bilayer. Single-pass integral membrane proteins usually have a hydrophobic transmembrane segment that consists of 20-25 amino acids. Some span only part of the membrane— associating with a single layer— while others stretch from one side of the membrane to the other, and the exposed on either side. Some complex proteins are composed of up to 12 segments of a single protein, which are extensively folded and embedded in the membrane. This type of protein has a hydrophilic region or regions, and one or several mildly hydrophobic regions. This arrangement of regions of the protein tends to orient the protein alongside the phospholipids, with the hydrophobic region of the protein adjacent to the tails of the phospholipids and the hydrophilic region or regions of the protein protruding from the membrane and in contact with the cytosol or extracellular fluid.

Carbohydrates are the third major component of plasma membranes. They are always found on the exterior surface of cells and are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids). These carbohydrate chains may consist of 2-60 monosaccharide units and can be either strainght or branched. Along with peripheral proteins, carbohydrates form specialized sites on the cell surface that allow cells to recognize each other. This recognition function is very importrant to cells, as it allows the immune system to differentiate between body cells (called "self") and foreign cells or tissues (called "non-self"). Similar types of glycoproteins and glycolipids are found on the surface of viruses and may change frequently, preventing immune cells from recognizing and attacking them. Thee carbohydrates on the exterior surface of the cell— the carbohydrate components of both glycoproteins and glycolipids— are collectively referred to as the glycocalyx (meaning "sugar coating"). The glycocalyx is highly hydrophilic and atracts large amounts of water to the surface of the cell. This aids in the interaction of the cell with its watery environment and in the cell's ability to obtain substances dissolved in the water.

1.3.1.1 Supportive evidences for fluid mosaic model

1. The cell membrane both on the surface as well as on the interior contains protein particles.

2. Presence of different types of permeability by the membrane is clarified by this model.
3. Dynamics nature of the biomembrane is explained by this model.
4. This model also explains the route of both electrolytes and non-electrolytes.

1.3.1.2 Plasma membrane structure (summary) and function

1.3.1.2.1 Structure

The plasma membrane separates the internal environment of the cell from the external enviornment and regulates both exit and entry of the molecules into the cell. Steady internal enviornment is maintained through its mechanism. The membrane as seen in Fig. 3 is composed of a phopspholipid bilayer with embedded protein. Few proteins cross the membrane while others do not. Even some of the proteins are pesent on the inside surface of the membrane. Together the proteins make up a *mosaic* pattern.

While the hydrophobic non-polar tails face each other, the polar hydrophilic heads of the phospholipid molecules face the outside and inside of the cell where water is available. The cholesterol— another lipid found in animal plasma membrane but the related steroids are present plasmamembrane of plants. The fuidity of the membrane is regulated by the cholesterol.

As described the proteins in the membrane may be peripheral proteins or integral proteins. The peripheral proteins present on the inside surface of the membrane of course can move laterally back and forth but some may project from only one surface of the bilayer.

Both proteins and phospholipids can have attached carbohydrate chain as glycoprotein and glycolipids respectively. The two sides of the membrane are not identical as because carbohydrate chains occur only on the outer surface while peripheral proteins are present asymmetrically on one surface of the membrane.

1.3.1.2.2 Function :

1. The primary function of the cell membrane is to discriminate the boundaries of a cell.
2. The whole contents of a cell are maintained by the membrane.
3. Not only support, but also, the cell membrane maintains a contact with other cells.

4. Proteins present in the cell membrane carry out the function of diffusion of elements in a selective manner.

5. Proper shape is maintained and provides protection.

1.4 Transport across membrane

The plasma membrane is selectively permeable. In the sense, some substances can move across the membrane or they allow some substances to pass through, but not others. The cell would be destroyed if they were to lose this selectivity and the cell would no longer be able to sustain itself. Larger amounts of specific substances are required by some cells than other cells. They also have a way of obtaining such materials from extracellular fluids.

The ways of crossing the plasma membrane are classified into passive or active. This may happen passively, as certain materials move back and forth, or the cell may have special mechanisms that facilitate transport. Some materials are so important to a cell that it spends some of its energy (hydrolyzing adenosine triphosphate (ATP) to obtain these materials. All cells spend the majority of their energy to maintain an imbalance of sodium and potassium ions between the interior and exterior of the cell.

The most direct forms of membrane transport are passive. Passive transport is a naturally occurring phenomenon and does not require the cell to exert any of its energy to accompanish the movement. In pssive transport, substances move from an area of higher concentration to an area of lower concentration.

1.4.1 Passive transport

The passive forms of transport, includes diffusion and osmosis. Small non-changed molecules, such as carbon dioxide, oxygen, glycerol, water, alcohol can diffuse across the membrane. Substances diffuse from areas of high concentration to areas of lower concentration ; this process continues until the substance is evenly distributed in a system. In solutions containing more than one substance, each type of molecule diffuses according to its own concentration gradient, independent of the diffusion of other substances. Many factors can affect the rate of diffusion, including, but not limited to, conentration gradient, size of the particles that are diffusing, and temperature of the system.

The molecules can diffuse because they are able to slip between the hydrophilic heads of the phospholipids and pass through the hydrophobic tails of the membrane. These molecules are said to follow their concentration gradient as they move from an area where their concentration is high to an area where their concentration is low. Consider that a cell is always using oxygen during cellular respiration. Therefore the concentration of oxygen is always higher outside a cell and oxygen follows a concentration grandient when it enters a cell. On the other hand, carbon dioxide follows a concentration gradient when it moves from inside the cell to outside the cell.

Ions and polar molecules such as glucose and amino acids are often assisted through the plasma memberane by their faciliatated transport. Two major classes of membrane proteins are also available in transportation across the membrane. These are carrier proteins and channel proteins. Carrier proteins, also known as transporters bind the specific solute to be transported while undergoing a series of conformational changes for tranferring the solute through the membrane (Fig. 5.A).

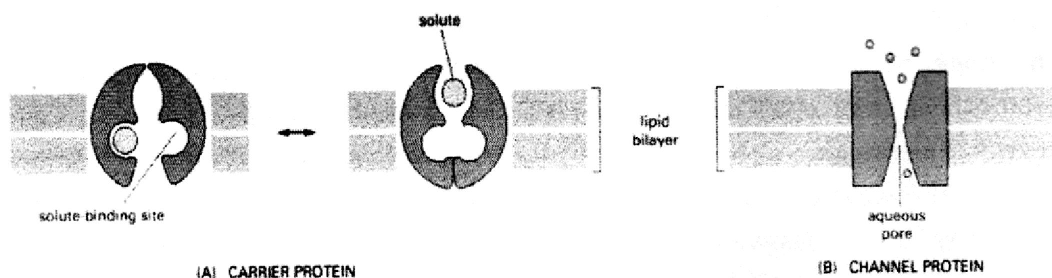


Fig. 5.A. Movement through carrier proteins
B. Movement through channel proteins

On the other hand, channel proteins interact with the solute to be tranported by forming an aqueous pore through the lipid bilayer. When these pores are open, they allow specific solutes, of course an inorganic ion of appropriate size and charge, to pass through them (Fig. 5B). It is obvious that channel proteins transport molecules at a much faster rate than carrier proteins.

1.4.2 Active transport

During active transport, ions or molecules move through the plasma membrane, accumulating either inside or outside the cell. In these instances molecules move to the region of higher concentration, exactly opposite to the process of diffusion.

1.4.2.1 Primary active transport

The primary active transport that functions with the active transport of sodium and potassium allows secondary active transport to occur. The secondary transport method is still considered active because it depends on the use of energy as does primary transport. Both carrier proteins and an expenditure of energy are needed to transport molecules against their concentration gradient. In this case, chemical energy (ATP molecules) is required for the carrier to combine with the substance to be transported.

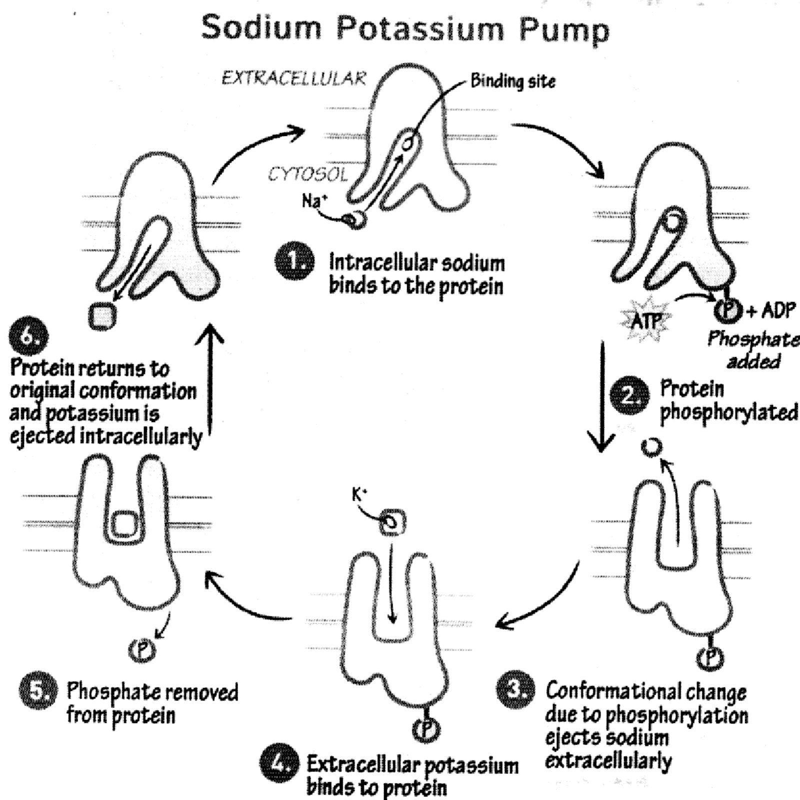


Fig.6 : The sodium potassium pump

Protein involved in active transport often are called pumps because, just as a water pump uses energy to move water against the force of gravity, proteins uses energy to move a substance against its concentration gradient.

One of the most important pumps in animals cells is the sodium-potassium pump (Na '-K' ATPase), (Fig. 6) which maintains the electrochemical gradient (and the correct concentrations of Na+ and K+) in living cells. The sodium-potassium pump

moves two K' into the cell while moving three Na+ out of the cell. The Na+ -K+ ATPase exist in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either sodium or potassium ions. The process consists of the following six steps :

- With the enzyme oriented towards the interior of the cell, the carrier has a high affinity for sodium ions. Three sodium ions bind to the protein.
- ATP is hydrolyzed by the protein carrier, and a low-energy phosphate group attaches to it.
- As a result, the carrier changes shape and re-orient itself towards the exterior of the membrane. The protein's affinity for sodium decreases and the three sodium ions leave the carrier.
- The shape change increases the carrier's affinity for potassium ions, and two such ions attach to the protein. Subsequently, the low-energy phosphate group detaches from the carrier.
- With the phosphate group removed and potassium ions attached, the carrier protein repositions itself towards the interior of the cell.
- The carrier protein, in its new configuration, has a decreased affinity for potassium, and the two ions are released into the cytoplasm. The protein now has a higher affinity for sodium ions, and the process starts again.

Several things have happened as a result of this process. At this point, there are more sodium ions outside of the cell than inside and more potassium ions inside than out. For every three ions of sodium that move out, two ions of potassium move in. This results in the interior being slightly more negative relative to the exterior. This difference in charge is important in creating the conditions necessary for the secondary process. The sodium-potassium pump is, therefore, an electrogenic pump (a pump that creates a charge imbalance), creating an electrical imbalance across the membrane and contributing to the membrane potential.

1.4.2.1.1 Moving against gradients :

To move substances against a concentration or electrochemical gradient, the cell must use energy. This energy is harvested from adenosine triphosphate (ATP) generated through the cell's metabolism. Active transport mechanisms, collectively called pumps, work against electrochemical gradients.

Two mechanisms exist for the transport of small-molecular weight material and small molecules. Primary active transport moves ions across a membrane and creates a difference in charge across that membrane, which is directly dependent on ATP. Secondary active transport describes the movement of material that is due to the electrochemical gradient established by primary active transport that does not directly require ATP.

1.4.2.1.2 Carrier proteins involved

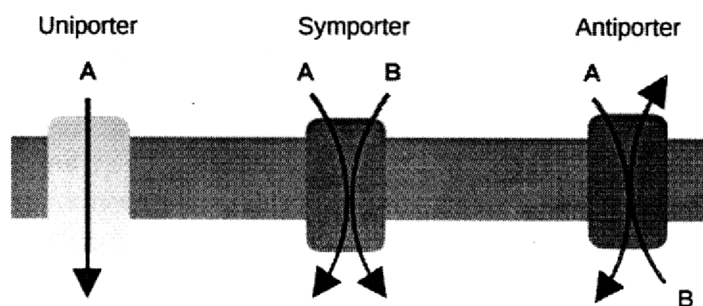


Fig.7 : Transporters

An important membrane adaption for active transport is the presence of specific carrier proteins or pumps to facilitate movement. There are three types of these proteins or transporters : uniporters, symporters, and antiporters. (Fig 7)

A uniporter carries one specific ion or molecule.

A symporter carries two different ions or molecules, both in the same direction.

An antiporter also carries two different ions or molecules, but in different directions.

All of these transporters can also transport small, unchanged organic, molecules like glucose. These three types of carrier proteins are also found in facilitated diffusion, but they do no require ATP to work in that process. Some examples of pumps for active transport are Na^+ -K^+ ATPase, which carriers sodium and potassium ions, and H^+ -K^+ ATPase, which carries hydrogen and potassium ions. Both of these are antiporter carrier proteins. Two other carrier protein pumps are Ca^{2+} ATPase and H^+ ATPase, which carry only calcium and only hydrogen ions, respectively.

1.4.2.2 Secondary active transport

In secondary active transport, a molecules is moved down its electrochemical gradient as another is moved up its concentration gradient.

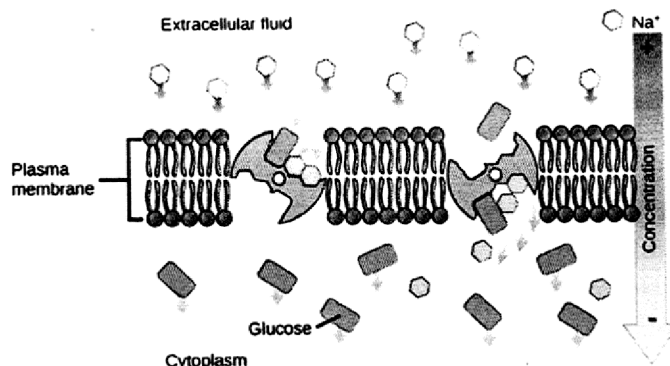


Fig.8 : Secondary active transport

In secondary active transport, (Fig. 8) ATP is not directly coupled to the molecule of interest. Instead another molecule is moved up its concentration gradient, which generates an electrochemical gradient. The molecule of interest is then transported down the electrochemical gradient. While this process still consumers ATP to generate that gradient, the energy is not directly used to move the molecule across the membrane, hence it is known as secondary active transport.

Both antiporters and symporters are used in secondary active transport. Co-transporters can be classified as symporters and antiporters depending on whether the substances move in the same or opposite directions across the cell membrane.

1.4.3 Facilitated transport

Facilitated transport explains the passage of molecules like glucose and amino acids through the plasma membrane even though they are not lipid soluble. The passage of these molecules is facilitated by their reversible combination with carrier proteins which are specific. As for example various sugar molecules of identical size might be present inside or outside the cell, but glucose can travel the membrane hundred times faster than the other sugars. Facilitated transport does not require an expenditure of energy because the molecules move down their concentration gradient in the same direction they tend to move.

1.5 Cell junctions

Cell junctions are intercellular connections between the plasma membranes of adjacent cells of animal tissues. They are formed by multiprotein complexes that provide contact between adjacent cells of between a cell and the extracellular matrix.

There are three major types of cell junction : (1) tight junctions, (2) gap junctions, and (3) anchoring junction (e.g. desmosomes).

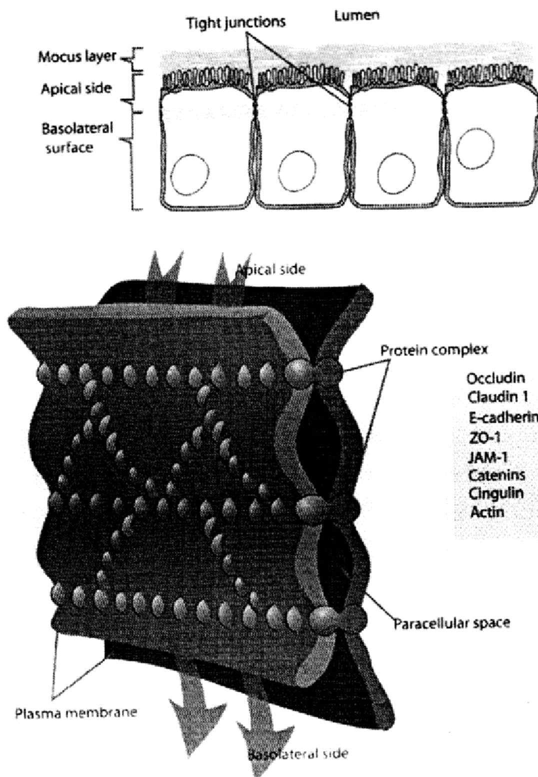


Fig.9 : Tight junction and proteins ivolved

1.5.1 Tight junctions

Tight junctions are areas where the membranes of two adjacent cells join together to form a barrier. The cell membranes are connected by strands of transmembrane proteins such as claudins and occuldins. Tight junctions bind cells together, prevent molecules from passing in between the cells, and also help to maintain the polarity of cells (Fig. 9).

They are only found in vertebrates, animals with a backbone and skeleton. Invertebrates of course have septate junctions instead.

1.5.1.1 Structure

Tight junctions are a branching network of protein strands on the surface of a cell that link with each other throughout the surface of the membrane. The strands are

formed by transmembrane proteins on the surfaces of the cell membrance that ar adjacent to each other.

There are around 40 different proteins at tight junctions. These proteins can be grouped into four main types. Transmembrane proteins are wedged in the middle of the cell membrane and are responsible for adhesion and permeability. Scaffolding proteins organize transmembrane proteins. Signaling proteins are responsible for forming the tight junction and regulating the barrier. Regulation proteins regulate what proteins are brought to the cell membrane in vesicles.

Claudins and occludins (Fig. 9) are the two main types of proteins present at tight junctions, and they are both transmembrane proteins. Claudins are important in forming tight junctions, while occludins play more of a role in keeping the tight junction stable and maintaining the barrier between cells that keeps unwanted molecules out.

1.5.1.2 Function of tight junction

Tight junctions have different function :

- To help cells form a barrier that prevents molecules from getting through, and to stop proteins in the cell membrane from moving around.
- Tight junctions are often found at epithelial cells, which are cells that line the surface of the body and line body cavities. Not only do epithelial cells separate the body and line body cavities. Not only do epithelial cells separate the body from the surrounding environment, they also separate surfaces, within the body.
- Permeability of molecules through layers of epithellal cells is thus tightly controlled.
- If molecules are blocked by tight junctions and physically unable to pass through the space in between cells, they must enter through other methods that involve entering the cells themselves. They could pass through special proteins in the cell membrane, or be engulfed by the cell through endocytosis. Using these methods, the cell has greater control over what materials it takes in and allows passing through.
- Another function of tight junctions is simply to hold cells together. The branching protein strands of tight junctions link adjacent cells together tightly so that they form a sheet.

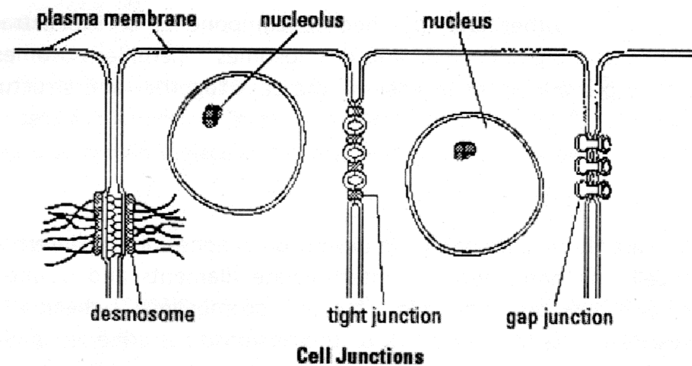


Fig.10 : Junctions between cells

1.5.2 Gap junctions

Gap junctions are cell junctions characterized by the intercellular channel that is formed between neighbouring cells that allow direct communication between cells. Small molecules, ions and electrical impulses are allowed to move between cells without passing the outside of cells. (Fig. 10)

1.5.2.1 Structure

In vertebrate cells, gap junctions are made up of connexin proteins. Groups of six connexins form a connexon, and two connexins are put together to form a channel that molecules can pass through. Other channels in gap junctions are made up of pannexin proteins. Relatively less is known about pannexins ; they were originaly thought only to form channels within a cell, not between cells. Hundreds of channels are found together as a mass of protein as a gap junction plaque at the site of a gap junction.

1.5.2.2 Function

The main function of gap junctions is to connect together so that molecules may pass from one cell to the other. This allows for cell-to-cell communication, and makes it so that molecules can directly enter neighboring cells without having to go through the extracellular fluid surrounding the cells.

Gap junctions are especially important during embryonic development, a time when neighbouring cells must communicate with each other in order for them to develop in the right place at the right time. If gap junctions are blocked, embryos cannot develop normally.

When a cell starts to die from disease or injury, it sends out signals throgh its gap junctions. These signals can cause nearby cells to die even if they are not diseased or injured. This is called the "bystander effect", since the nearby cells are innocent bystanders that become victims.

1.5.3 Desmosome

Desmosomes are protein attachments between adjacent cells. Inside the plasma membrane, a desmosome bears a disk-shaped structure from which protein fibres extend into the cytoplasm. Desmosomes act like spot welds to hold together tissues that undergo considerable stress (such as skin or heart muscle). Also known as anchoring junction as it anchored to one another attached to components of the extracellular matrix. Examples of anchoring junctions are desmosomes hemidesmosomes and adherens junctions. They are important in keeping the cells together and structural cohesion of tissues.

1.5.3.1 Structure

There are three components is desmosomal adhesion : the intermediate filaments inside the cell, the bond between intermediate filaments and desmosomal adhesion molecules, and the bond provided by the desmosomal adhesion molecules. The intermediate filaments and their link to the desmosomal adhesion molecules are both located inside the cell, while the bonds of the desmosomal adhesion molecules themselves are on the outside of the cell. Specifically, desmoglein and desmocollin are the two proteins that bind cells at desmosomes.

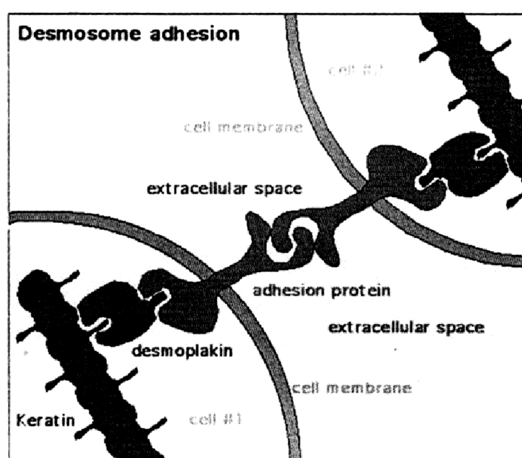


Fig.11 : Showing how cells adhere at desmosomes

1.5.3.2 Function

The function of desmosomes is to adhere cells together. They are found in high numbers in tissues that are subject to a lot of mechanical forces. For example, many are found in the epidermis, which is the outer layer of skin, and the myocardium, which is muscle tissue in the heart. They are also found in between squamous epithelial cells, which form the lining of body parts like the heart, blood vessels, air sacs of the lungs, and esophagus.

1.6 Conclusion

Membranes are made of lipids and proteins, and they serve a variety of barrier functions for cells and intracellules. Membranes keep the outside "out" and the inside "in" allowing only certain molecules to cross and relaying messages via a chain of molecules events.

1.7 Summary

(i) The fluid-mosaic model of membrane structure developed by Singer and Nicolson was preceded by several other models. E.M. of freeze-fractured membrane support the fluid mosaic model, rather than Robertson's unit membrane concept based on Danielli and Davson sandwich model.

(ii) Two components of plasma membrane are lipids and proteins. In the lipid bilayer, phospholipids are arranged with their hydrophilic (polar) heads at the surfaces and their hydrophobic (nonpolar) tails in the interior.

The hydrophobic portion of an integral protein lies in the lipid bilayer of the plasma membrane, and the hydrophilic portion lies at the surfaces.

(iii) Some molecules (lipid-soluble compounds, water and gases) simply diffuse across the membrane from the area of higher concentration to the area of lower concentration.

Other molecules are transported across the membrane by carrier proteins that span the membrane. During facilitated transport, a carrier protein assists the movement of a molecule down its concentration gradient. No energy is required.

During active transport, a carrier protein acts as a pump that causes a substance to move against its concentration gradient.

(iv) Some animal cells have junctions. Adhesion junctions and tight junctions help hold cells together ; Gap junctions allow passage of small molecules between cells.

1.8 Model questions

1. What are cellular membranes made of ?
2. What do cell membranes do ?
3. Describe the fluid-mosaic model of membrane structure.
4. How the phospholipids are arranged in the membrane ? What functions do they serve ?
5. Explain through diagram how the sodium-potassium pump work.
6. Give an account of membrane protein found in the plasma membrane.

Unit-2 □ Mitochondria

Structure

2.1. Objectives

2.2. Introduction

2.3. Structure of mitochondria

2.4. Function of mitochondria

2.5. Mitochondrial respiratory chain

2.5.1 Oxidative phosphorylation : an overview

2.5.2 The electron transport chain

2.6. Chemiosmosis

2.6.1 ATP yield

2.7. Peroxisome

2.7.1 Peroxisomes-another enzyme package

2.7.2 Peroxisomes structure

2.7.2.1 Similarity and difference with lysosomes

2.7.3 Function of peroxisomes

2.7.4 Peroxisomal disorders

2.8 Summary

2.9 Model questions

2.1 Objectives

This unit of cell biology further allows a reader to make a comprehensive idea about the one of the cellular organelles as mitochondria. After completion of the topic the reader would be able to become aware of

- the mitochondrial structural features
- function of mitochondria
- mitochondrial electron transport system and

- ATP yield
- Structure and function of peroxisomes and
- Disorders of peroxisomes

2.2 Introduction

Life is possible only because a constant energy keeps the structure of cells. Even though motochondria (singular, mitochondrion), are smaller in size, it can still be visible through light microscope. Mitochondria are one of the eukaryotic membranous oraganelles that specialize in converting energy to a form that can be used by the cell. In eukaryotes, mitochondria are necessary to the process of cellular respiration, which produces ATP. Molecules of ATP otherwise are known as energy. If a cell needs energy, ATP supplies it. The energy of ATP is used for synthetic reactions, active transport and all energy-requiring processes in cells. Cellular respiration is a process of converting chemical energy of carbohydrates into ATP (Adenosine triphosphate). The whole process can be represented by the equation :

Carbohydrates + Oxygen $\rightarrow$ Carbon dioxide + water + energy

In fact, mitochondria enable cells to produce 15 times more ATP than they could otherwise, and complex animals, like humans, need large amounts of energy in order to survive. The number of mitochondria varies in a cell from single large mitochondria to thousands of the organelles depending upon the metabolic requirement of the cell. It was first discovered in the 1800s. The name of the organelles was coined by the scientists coming from the Greek words for "thread" and "granule". For many years after their discovery, mitochondria were commonly believed to transmit hereditary information. Later, when a method for isolating the organelles intact was developed the modern understanding of mitochondrial function was worked out and confirm as "the powerhouse of the cell".

2.3 Structure of mitochondria

Typically mitochondria are rod shaped with a diameter of approx. 1mm. However their shape and size vary considerabley and in some types appear as a closest reticulated network. Mitochondria have two membranes. an outer membrane and an inner membrane. These membranes are mde of phospholipid layers, just like the cell's outer membrane. The outer membrane covers the surface of the mitochondrion, while the inner membrane is located within and has many folds called cristae that

project into the matrix. These cristae increase the surface area of the inner membrane, so much that in a liver cell they account for about one third the total membrane in the cell. The increase surface area is also important because the inner membrane holds the proteins involved in the electron transport chain. It is also where many other chemical reactions take place to carry out the mitochondria's many functions. An increased surface area creates more space for more reactions to occur and increases the mitochondria's output. The space between the outer and inner membranes is called the intermembrane space, and the space inside the inner membrane is called the matrix. The matrix contains the mitochondrial DNA and ribosomes.

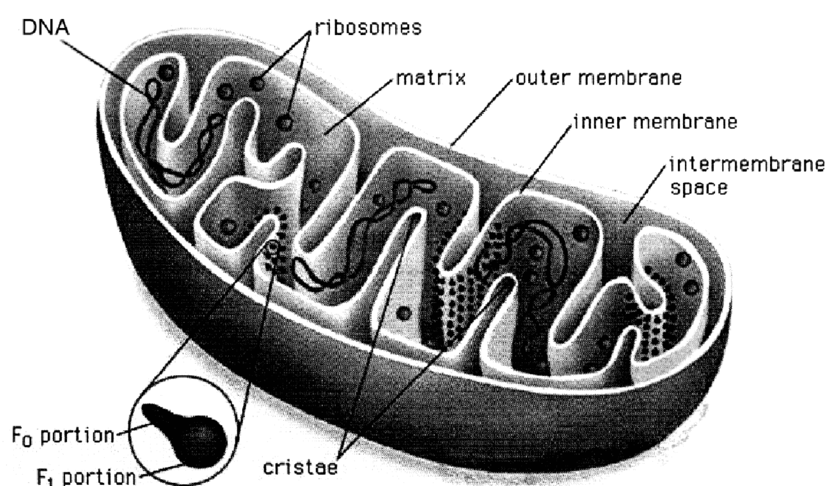


Fig.2.1 : Components of a typical mitochondria

Cell fractionation and centrifugation allowed investigators to separate the inner membrane, the outer membrane and the matrix from each other. That leads to a discovery that the matrix is a highly concentrated mixture of enzymes that break down carbohydrates and other nutrient molecule. These reactions supply the chemical energy and through a process leads to the synthesis of ATP. The entire process is called cellular respiration that involves cytoplasm.

2.4 Function of mitochondria

- Mitochondria produce ATP through process of cellular respiration—specifically, aerobic respiration, which requires oxygen.
- The citric acid cycle, or Krebs cycle, takes place in the mitochondria.
- The amount of mitochondria in a cell depends on how much energy that cell

needs to produce. As for example muscle cells, have many mitochondria because they need to produce energy to move the body, where as RBC do not need to produce energy so they have none.

- They can store calcium, which maintains homeostasis of calcium levels in the cell.
- They also regulate the cell's metabolism and have roles in apoptosis, cell signaling and thermogenesis.

2.5 Mitochondrial respiratory chain

Currently, mitochondria are thought to be composed of thousands of different types of proteins that perform a very wide range of metabolic functions. The protein complexes that form the mitochondrial membrane. The respiratory chain complexes transfer electrons from an electron donor to an electron acceptor and are associated with a proton pump to create a transmembrane electrochemical gradient that leads ultimately sysnthesis of ATP.

2.5.1 Oxidative phosphorylation : an overview

The **electron transport chain** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in thee reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis.** Together, the electron transport chain and chemiosmosis make up **oxidative phosphorylation.** The key steps of this process, shown in simplified form in the diagram below, (Fig. 2.2.) include :

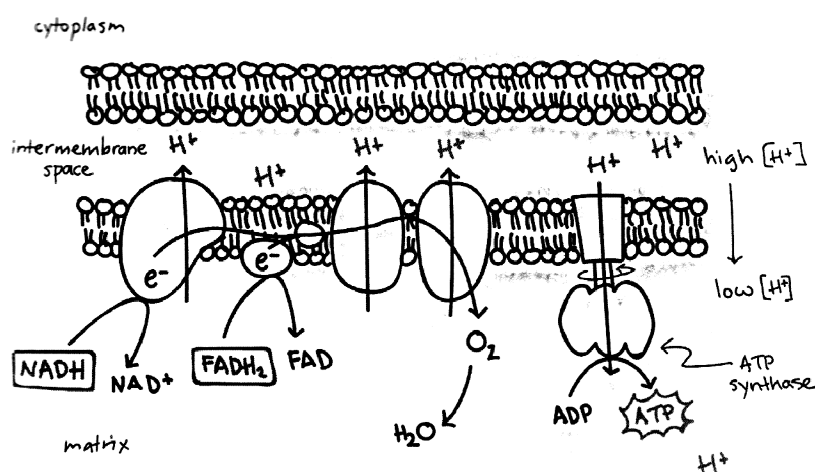


Fig.2.2 : Steps of Oxidative phosphorylation

1. **Delivery of electrons by NADH and $FADH_2$**

   Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD+ and FAD, which can be reused in other steps of cellular respiration.

2. **Electron transfer and proton pumping.**

   As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H ions, moving them out of the matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.

3. **Splitting of oxygen to form water.**

   At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up H^+ to form water.

4. **Gradient-driven synthesis of ATP.**

   As H^+ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthesis, which harnesses the flow of protons to synthesize ATP.

2.5.2 The electron transport chain

The **electron transport chain** is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these "downhill" electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the intermembrane space, forming a proton gradient. (Fig. 2.3.)

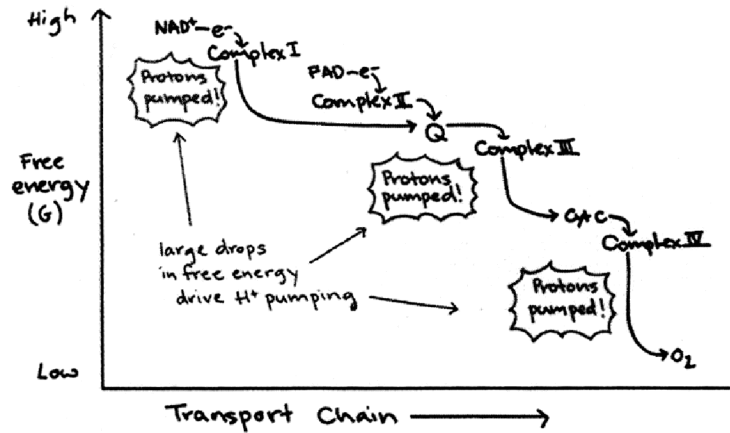


Fig.2.3 : Free emergy

All of the electrons that enter the transport chain (Fig. 2.4) come from NADH and $FADH_2$ molecules produced during earlier stages of cellular respiration : glycolysis, pyruvate oxidation, and the citric acid cycle.

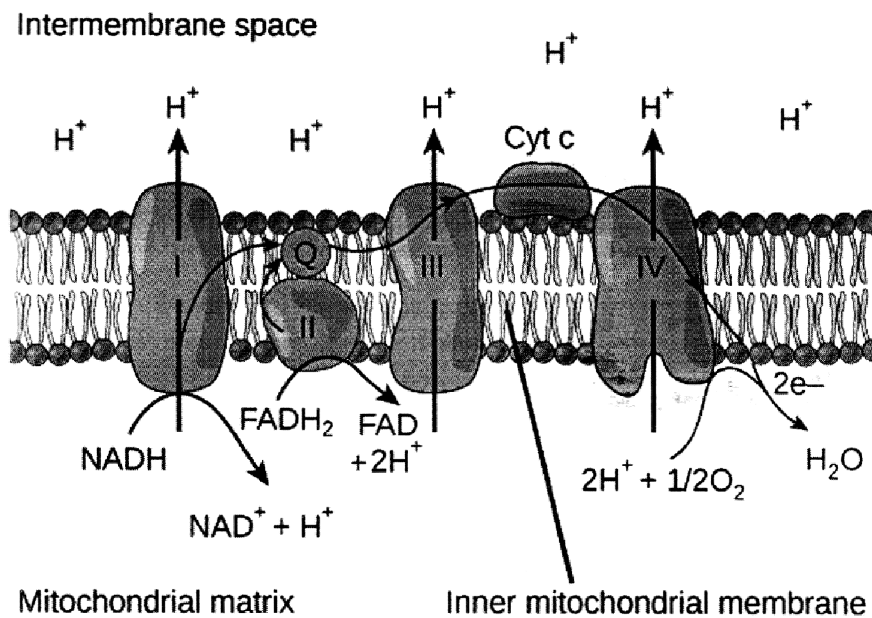


Fig.2.4 : The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and FADH2 to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

- **NADH** is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD^+. As electrons move through complex I in a series of redox reactions, energy is released and the complex uses this energy to pump protons from the matrix into the intermembrane space.
- **$FADH_2$** is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

Because of this "bypass," each $FADH_2$ molecule causes fewer protons to be pumped (and contributes less to the proton gradient) than an NADH.

Complex I, NADH transfers its electrons to complex I. Complex I is quite large, and the part of it that receives the electrons is a flavoprotein, meaning a protein with an attached organic molecule called flavin mononucleotide (FMN). FMN is a **prosthetic group,** a non-protein molecule tightly bound to a protein and required for its activity, and it's FMN that actually accepts electrons from NADH, FMN passes the electrons to another protein inside complex I, one that has iron and sulfur bound to it (called an Fe-S protein), which is turns transfers the electrons to a small, mobile carriercalled ubiquinone (Q in the diagram above).

Complex II, Like NADH, $FADH_2$ deposits its electrons in the electron transport chjain, but it does so via complex II, bypassing complex I entirely. As a matter of fact, $FADH_2$ is a part of complex II, as is the enzyme that reduces it during the citric acid cycle ; unlike the other enzymes of the cycle, it's embedded in the inner mitochondrial membrane. $FADH_2$ transfers its electrons to iron-sulfur proteins within complex II, which then pass the electrons to ubiquinone (Q), the same mobile carrier that collects electrons from complex I.

Beyond the first two complexes, electrons from NADH and $FADH_2$ travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called **ubiquinone (Q),** which is reduced to form QH_2 and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more H^+ ions are pumped across the membrane, and the electrons are ultimately delivered to another mobile carrier called **cytochrome C (cyt C).** Cyt C carries the electrons to complex IV, where a final batch of H^+ ions is pumped across the membane. Complex IV passes the electrons to O_2, which splits into two oxygen atoms and accepts protons from the matrix to form water. Four

electrons are required to reduce each molecule of O_2 and two water molecules ar formed in the process.

Complex III :

It comprises cytochrome b andcytochrome CI. Non-heme iron of complex III (FeNHR) is associated with cytochrome b.

Complex IV :

Cytochrome a and a3 and bound copper constitute this complex. It is pertinent to mention that electrons follow either the pathway of complexes I, III and IV or I or III and IV.

The electron transport chain has two main functions :

1. **Regenerates electron carriers.**

   NADH and $FADH_2$ pass their electrons to the electron transport chain, turning back into NAD^+ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.

2. **Makes a proton gradient.**

   The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H^+ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy which can be used to make ATP.

2.6 Chemiosmosis

Complexes, I, III and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H^+ ions from the matrix to the intermembrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the **proton-motive force,** and it is as a form of stored energy, kind of like a battery.

Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H^+ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.

In the inner mitochondrial membrane H^+ ions have just one channel available : a membrane-spanning protein known as **ATP synthase.** Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it's turned by the flow of H^+ ions movng down their electrochemical gradient. As ATP synthase turns, it catalyzes the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.

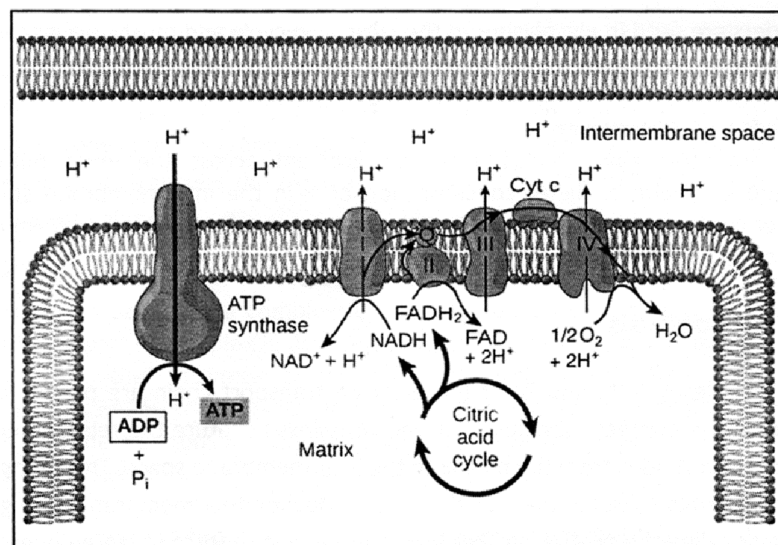


Fig.2.5 : In oxidative phosphorylation, the pH gradient formed by the electron transport chain is used by ATP synthase to form ATP

This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis.** More broadly, chemiosmosis can refer to any process in which energy stored in a proton gradient is used to do work. Although chemiosmosis accounts for over 80% of ATP made during glucose breakdown in cellular respiration, it's not unique to cellular respiration.

What would happen to the enrgy stored in the proton gradient if it weren't used to synthesize ATP or do other cellular work ? It would be released as heat, and interestingly enough, some types of cells deliberately use the proton gradient for heat generation rather than ATP synthesis. This might seem wasteful, but it's an important strategy for animals that need to keep warm. For instance, hibernating mammals (such as bears) have specialized cells known as bown fat cells. In the brown fat cells,

uncoupling proteins are produced and inserted into the inner mitochondrial membrane. These proteins are simply channels that allow protons to pass from the intermembrane space to the matrix without travelling through ATP synthese. By providing an alternate route for protons to flow back into the matrix, the uncoupling proteins allow the energy of the gradient to be dissipated as heat.

2.6.1 ATP yield

How many ATP do we get per glucose in cellular respiration ? However, most current sources estimate that the maximum ATP yield for a molecule of glucose is around 30-32 ATP (Fig. 2.6) This range is lower than previous estimates because it accounts for the necessary transport of ADP into, and ATP out of, the mitochondrion.

Where does the figure of 30-32 ATP come from ? Two net ATP are made in glycolysis, and another two ATP (or energetically equivalent GTP) are made in the citric acid cycle. Beyond those four, the remaining ATP all comes from oxidative phosphorylation. Based on a lot of expermental work, it appears that four H^+ ions must flow back into the matrix through ATP synthase to power the synthesis of one ATP molecule. When electrons from NADH move through the transport chain, about 10 H^+ ions are pumped from the matrix to the intermembrane space, so each NADH yields about 2.5 ATP. Electrons from $FADH_2$, which enter the chain at a later stage, drive pumping of only $6H^+$ leading to production of about 1.5ATP.

With this information, we can do a little inventory for the breakdown of one molecule of glucose :

Stage	Direct product (net)	Ultimate ATP yield (net)
Glycolysis	2 ATP	2ATP
	2 NADH	3-5 ATP
Pyruvate Oxidation	2 NADH	5 ATP
Citric acid Cycle	2 ATP/GTP	2 ATP
	6 NADH	15 ATP
	2 $FADH_2$	3 ATP
Total		**30-32 ATP**

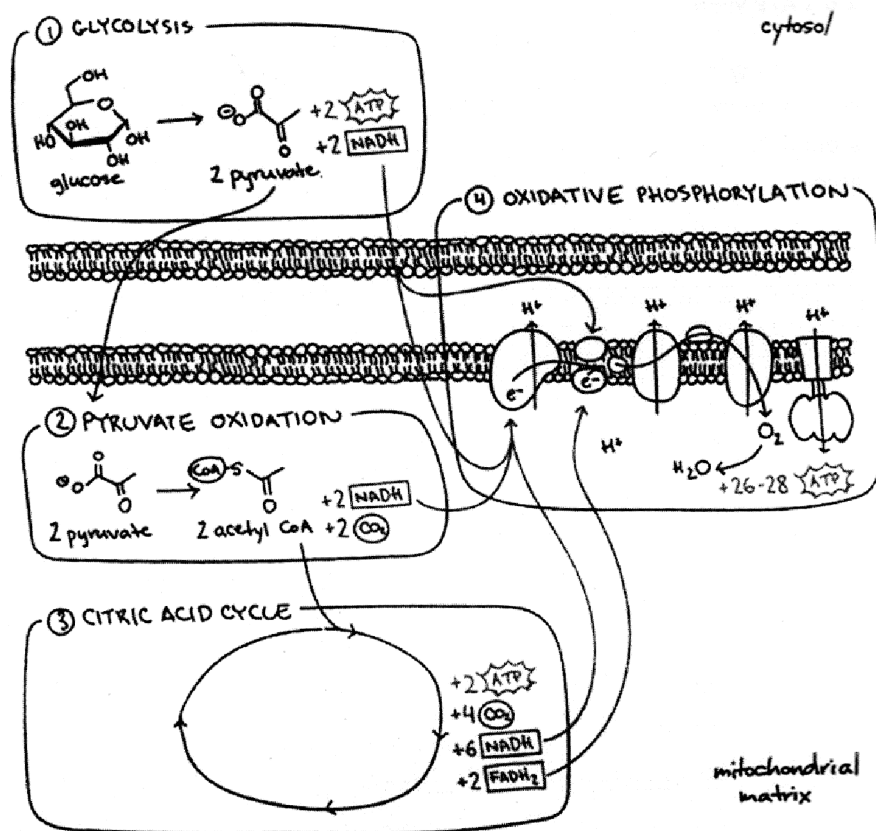


Fig.2.6 : Diagram showing ATP production

One number in this table is still not precise : the ATP yield from NADH made in glycolysis. This is because glycolysis happens in the cytosol, and NADH can't cross the inner mitochondrial membrane to deliver its electrons to complex I. Instead, it mut hand its electrons off to a molecular "shuttle system" that delivers them, through a series of steps to the electron transport chain.

- Some cells of your body have a shuttle system that delivers electrons to the transport chain via $FADH_2$. In this case, only 3 ATP are produced for the two NADH of glycolysis.
- Other cells of your body have shuttle sytem that delivers the electrons via NADH, resulting to the production of 5 ATP.

In bacteria, both glycolysis and the citric acid cycle happen in the cytosol, so no shuttle is needed and 5 ATP are produced.

2.7 Peroxisome

2.7.1 Peroxisome-another enzyme package

Peroxisomes are organelles that contain enzymes to carry out their functions and are very much similar to lysosomes. Only they differ from lysosomes in the type of enzyme they hold. Peroxisomes hold on to enzymes that require oxygen **(oxidative enzymes).** They are called "Peroxisomes" because they are the site of synthesis and degradation of Hydrogen Peroxide (H_2O_2), a highly reactive and toxic oxidizing agent.

2.7.2 Peroxisome structure

Peroxisome are found around the cell. They are membrane bound small vesicles organelles, occurring in the cytoplasm of almost all eukaryotic cells. Peroxisomes have the thickest membrane of all organalles. Also called 'Microbodies'. Peroxisomes are ovoid granules limited by a single membrane. They contain a fine, granular substance which may condense in the centre, forming an opaque and homogeneous core or nucleoid (Fig. 2.7). The average size of the peroxisomes in rat liver cells was shown to be 0.6 to 0.7 mm. The number of peroxisomes per cell varied between 70 and 100, whereas 15 to 20 lysosomes were found per liver cell. In many tissues peroxisomes show a crystal-like body made of tubular subunits.

Their existence was first discovered by J. Rhodin in— 1954. Human cells may contain upto hundred peroxisomes depending on type of cell. They are particles of about 100-500 nm in diameter. A lipid bilayer membrane surrounds which regulates what enters and exits the peroxisome. Inside there is a dense matrix containing enzymes.

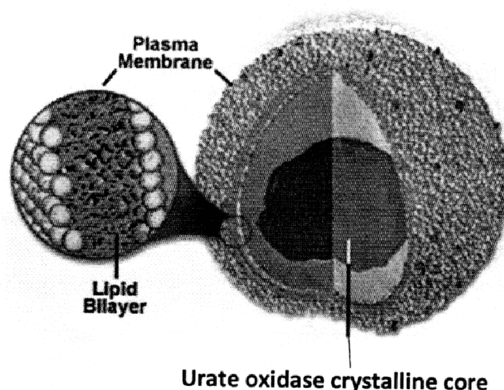


Fig.2.7 : Anatomy of peroxisome

More than 30 different enzymes are present in peroxisomes. It has a cyrstalloid core in the center, which is present in some species e.g. liver cells of rat. They are abundant in cells of liver and kidney, where they are required the most. It is absent in human liver cells. It contains urage oxidase enzyme which oxidizes uric acid. Humans do have a gene for urate oxidase, but it is nonfunctional. An enzyme catalase (a type of oxidase) present in large quantity in peroxisomes. Peroxisomes are replicated by fission. They are believed to be formed from self replication. Their life span is 1 day. And they are self assembling. There are at least 32 known peroxisomal proteins, called peroxins, which carry out peroxisomal function inside the organelle.

Most organelles are created by budding off of the endomembrane system, but that is not the case with peroxisomes. Peroxisomes are created by taking in proteins and lipids from the cytoplasm of the cell in which the organelles are suspended.

The influx of proteins and lipids makes the peroxisome grow in size. Once the peroxisome is large enough, it divides through fission to create two peroxisomes. Peroxisomes are created in this manner because they don't have their own DNA to give instructions on making the proteins that they need to function. So peroxisomes must be created already containing all the proteins that they need.

Since peroxisomes are not created from the endomembrane system, they are bound by a single membrane instead of a double membrane like most organelles. The end result is a single membrane-bound organelle with lipids and proteins that act as enzymes.

2.7.2.1 Similarity and difference with lysosomes

A. Similarity

They are similar to lysosomes, being filled with enzymes.

B. Difference

Peroxisomes	Lysosomes
• Peroxisomes contain Oxidase enzymes enzymes	• Lysosomes contain hydrolase
• They originate from Endoplasmic reticulum	• Lysosomes come from Golgi apparatus.

2.7.3 Function of Peroxisomes

1. In order to carry out their activities, peroxisomes use significant amounts of oxygen. They are involved in many diffrent activities, such as : hydrogen peroxide degradation by catalyse.

   Mechanism : First several oxidases combine oxygen and hydrogen to form H_2O_2. Then this H_2O_2 is oxidized by catalase (another oxidase) into H_2O and O_2.

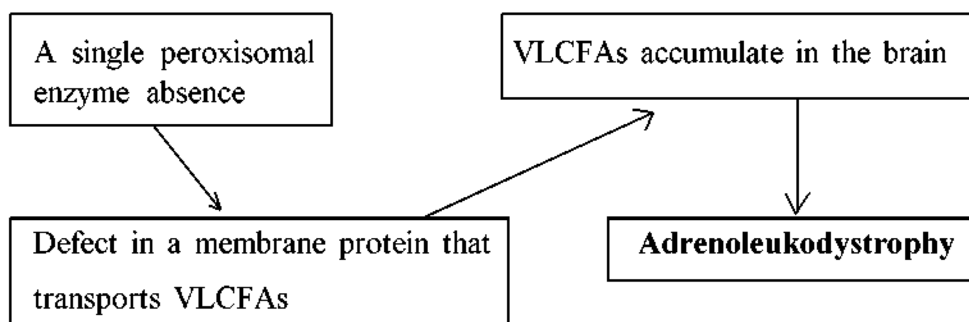
2. Detoxification of alcohol and other toxic compounds. Peroxisomes detoxify about half of the alcohol a person drinks daily.
3. β-oxidation of Very Long Chain Fatty Acids (VLCFA) provides the cell with a major soure of metabolic energy.
4. Biosynthesis of plasmogens, ether phospholipids, which are necessary for normal function of brain and lungs.
5. Synthesis of unsaturated fatty acids.
6. Participates in the synthesis of bile acids on liver cells.
7. Participates in the synthesis of cholesterol.
8. Participates in the synthesis of the lipids used to make myelin.
9. Peroxisomes are important for normal brain and lungs functioning. Absence of peroxisomes can lead to abnormalities, especially brain disorders.

2.7.4 Peroxisomal disorders

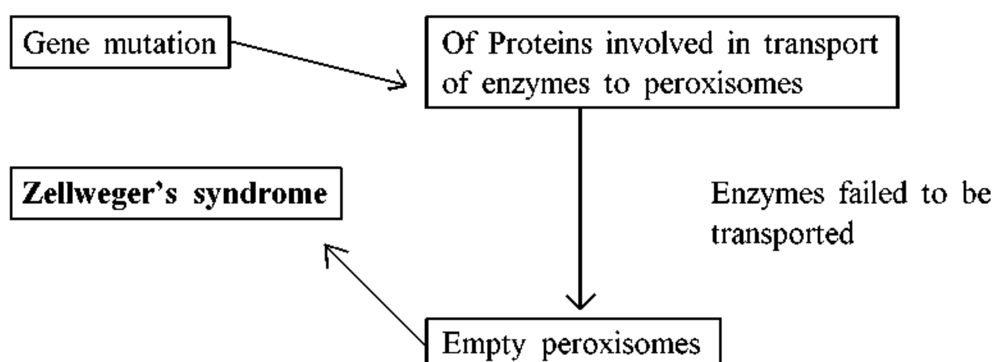
In humans, peroxisomal disorders may result due to abnormal function of single enzyme, which are necessary for normal peroxisomal function. Problems with nervous system are commonly observed. Other problems include : 1. Skeletal and craniofacial dysmorphism 2. Liver dysfunction 3. Sensorineutral hearing loss 4. Retinopathy.

Two nervous problems : 1. Adrenoleukodystrophy 2. Zellweger's syndrome.

1. Adrenoleukodystrophy : It is a fatal inherited disorder that leads to extensive brain damage and adrenal gland failure. Disorder results from a failure to metabolize Very long chain fatty acids (VLCFA) properly. One result is deterioration of the myelin sheaths of neurons. The disorder occurs in young boys because the gene is X-linked.



2. Zellweger's syndrome : also called cerebrohepatorenal syndrome, is a rare congenital disorder characterized by the reduction or absence of functional peroxisomes in the cells of an individual. It is caused by mutation of those genes that are responsible for encoding the proteins required to assembles enzymes in the peroxisomes.



2.8 Summary

(i) A mitochondria is bounded by a double membrane with an intermembranous space. The inner membrane invaginates to form the shelf like cristae.

(ii) Glycolysis takes place in the cytoplasm outsidie ithe mitochondria. The breakdown of glucose release enough energy to immediately give a net gain of two ATP by substrate-level phosphorylation. Two NADH are formed.

(iii) The final stage of glucose breakdown involves the electron transport system located in the mitochondria. The electron received from NADH and FDH_2 are passed down a chain of carriers until they are finally received by oxygen, which combines with H^+ to produce water.

(iv) Peroxisomes are organelles that contain enzymes to carry out their functions having very much similarity with lysosomes.

2.9 Model questions

1. Write down the structure of mitochondria.
2. State the function of mitochondria.
3. What are NAD^+ and FAD ? What are their functions ?
4. What are the main events of glycolysis ? How is ATP formed ?
5. What is the electron transport system and what are its functions ?
6. What is oxidative phosphorylation ?

Unit-3 □ Cytoskeleton

Structure

3.1. Objectives

3.2. Introduction

3.3. Structure of cytoskeleton

3.3.1 Eukaryotic cytoskeleton

3.4. Microtubules

3.4.1 Occurrence

3.4.2 Origin

3.4.3 Structures of microtubules

3.4.4 Assembly of microtubules

3.4.5 Dynamic instability of microtubules

3.4.6 Functions of microtubules

3.5. Microfilaments

3.5.1 Structure of microfilaments

3.5.2 Polymerization and deploymerization

3.5.3 Functions of microfilaments

3.6. Intermediate filaments

3.6.1 Definition

3.6.2 Polymerization of intermediate filaments

3.7. Microfilaments vs Microtubules

3.8. Functions of cytoskeleton

3.9. Summary

3.10 Model questions

3.1 Objectives

This specialized section of the unit of the module of Cell Biology gives a clear picture of the idea of Cytoskeleton. When the reader finishes this particular unit one will be able to understand :

- What is cytoskeleton
- Components of cytoskeleton
- Characteristics and the role of the constituent part of the cytoskeleton and
- About explanation of different components of the cytoskeleton and their individual function.

3.2 Introduction

The cell have a network of filaments known as the cytoskeleton (literally, "cell skeleton"), which not only supports the plasma membrane and gives the cell an overall shape, but also aids in the correct positioning of organelles, provides tracks for the transport of vesicles, and (in many cell types) allows the cell to move. The cytoskeleton is thus a lattice of protein fibers that maintains the shape of the cell and assists in the movement of organelles. The protein fibers serve as tracks for the transport vesicle that are taking molecules from one organelie to another. Without a cytoskeleton, a eukaryotic cell would not have an efficient of moving organelles and their products within the cell possibly could not exist. Prior to 1970, it as believed that the cytoplasm was an unorganized mixture of organic molecules. But after the invention of the high-voltage electron microscopes, which can penetrate thicker specimens, showed that the cytoplasm was instead a highly organized. Technique of immunofluorescence microscopy identified the makeup of specific protein fibres within the cytoskeletal network.

3.3 Structure of cytoskeleton

The cytoskeletn is a series of intercelular proteins that help a cell with shape, support, and movement. Under electron microscope, several types of protein filaments are visible within the eukaryotic cells forming an inter-locking three dimensional mesh work throughout the cytoplasm as cytoskeleton. **Cytoskeleton** has three main structural components : microfilaments, intermediate filaments and microtubules. Each of the cytoskeletal components is composed of simple protein subunits that

polymerize to form filaments of uniform thickness. These filaments are not permanent structure ; they undergo constant disassembly into their monomeric subunits and reassembly into filaments. Their locations in cells are not rigidly fixed, but may change dramatically with mitosis ; cytokinesis or changes in the cell shape. They have unique characteristics and perform various functions according to their nature. The cytoskeleton has both structural roles and functional roles. The former refers to the roles played by filaments in cells to maintain the cell shape and the arrangement of organelles. The latter refers to the functional roles played through the interaction with other proteins, such as muscle constraction, cell locomotion, cell division and intercellular transport.

3.3.1 Eukaryotic cytoskeleton

Eukaryotic cells contain three man kinds of cytoskeletal filaments : microfilaments which are composed of actin, intermediate filaments which have around 70 dfferent proteins as building blocks, and microtubules with tubulin as the basic subunit. The cytoskeleton mainly composed of three elements within a cell (Fig. 3.1) : (i) a network of microtubules (20-30 nm in diameter) which are long protein fibres lying mainly in the ectoplasm or cell cortex i.e. in the cytoplasm below the palsma membrane, (ii) cytoplasmic network of **actin filaments** also called **microfilaments,** about 5-7 nm in diameter, and (iii) **intermediate filaments** with a diameter of 10nm. Since microtubules, actin filaments and intermediate filaments play an important role in maintaining the structure and function of the cell, it is essential that students of biology should learn about these cellular structures, which are collectively called as **cytoskeleton.**

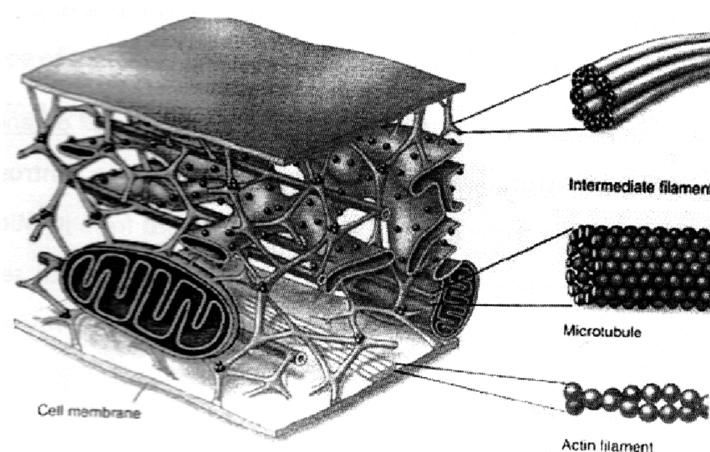


Fig. 3.1 : A model of structure of the cytoskeleton of a cell

3.4 Microtubules

Microtubules were first observed in the axoplasm of the myelinated nerve fibres by De Roberts and Franchi (1953). The exact nature of microtubules were brought into light by Sabatini, Bensch and Bernett (1963) through Electron microscopy.

3.4.1 Occurrence

Microtubules are regular components of most animals and plant cells except perhaps amoeba, molds and mature mammalian erythrocytes. The microtubules occur in a variety of cell structures including cilia and filaments, centrioles and basal bodies, nerve processes, mitotic apparatus (spindle), and such other structures which have flagella or fiber like structures. Microtubules form structures like flagella, which are "tails" that propel a cell forward.

3.4.2 Origin

Most of the microtubules in an **animal cell** come from a cell **organelle** called the **centrosome,** which is a **microtubules** organizing center (MTOC). The centrosome is found near the middle of the cell and microtubules radiate outward from it. Microtubules are important in forming the spindle apparatus (or mitotic spindle), which separates **sister chromatids** so that one copy can go to each daughter cell during cell division.

3.4.3 Structures of microtubules

Microtubules, as the word indicates are tube like cylindrical structures, which are unbranched and can be several microns in length. They are hollow tubes, 20-30 nm (200-300 A0) in thickness. In transverse section, these appear to consist of a circular array of 13 subunits, and in surface view, a microtubules may appear to consist of 13 rows of subunits. These subunits in spare 5-7 nm in diameter (same as the thickness of a single microfilament) and are called protofilaments, which are helically arranged around a central axis. (Fig. 3.2)

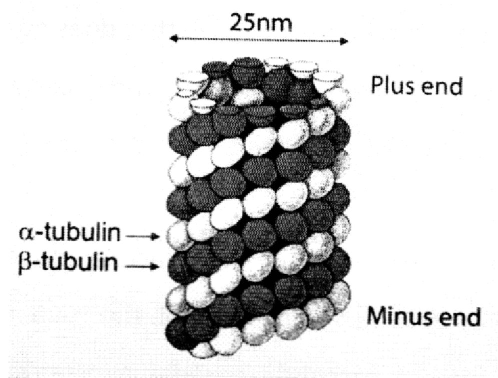


Fig. 3.2 : Microtubule schematic

Microtubules are composed of a single type of globular protein, called tubulin. These are like the strings of beads. Each unit is about 50 $A^0 \times 40\ A^0$ in size. Tubulin is a dimer consisting of two closely related 55-kd polypeptides, α-tubulin and β-tubulin. Like actin, both α-and b-tubulin are encoded by small families of related genes. In addition, a third type of tubulin (γ-tubulin) is specifically localized to the centrosome, where it plays a critical role in initiating microtubule assembly.

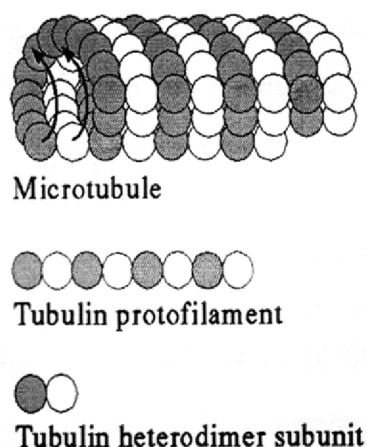


Fig. 3.3 : Microtubule and its components

An α, β-tubulin heterodimer (Fig 3.3) is the basic structural unit of microtubulin. The heterodimer does not come apart, once formed. The α and β tubulins, which are each about 55 kDa MW, are hemologous but not identical. Each has a nucleotide binding site.

- **α-Tubulin** has a bound molecule of GTP, that does not hydrolyze.
- **β-Tubulin** may have bound GTP or GDP. Under certain conditions b-tubulin can hydrolyze its bound GTP to GDP plus Pi, release the Pi, and exchange the GDP for GTP.

Electron microscopy of microtubules decorated with motor protein heads indicated a "3 start helix" in which each turn of the helix spans 3 tubulin monomers (eg. a, b, a). These result in the microtubule wall having a "seam" where, instead of the predominant aa and bb lateral contacts, a subunits are laterally adjacent to b subunits.

3.4.4 Assembly of microtubules

When intracellular conditions favour assembly, tubulin hetarodimers assembly into linear protofilaments. Protofilaments in turn assemble into microtubules. All such assembly is subject to regulation by the cell.

In the cell there are sites of orientation from which the polymerization is directed. These are the microtubule organization center. Microtubules are polar structure each with a plus (+) end, that capable of rapid growth or polymerization and the other is minus (–) end, that capable of loosing subunits or depolymerization. The minus and remains embedded in a structure called centrosomes and thus the microtubules are stabilized. The minus ends of the microtubules always tend to arrange themselves in a center. One end is assembled and the other end is disassembled.

Assembled of microtubules could be possible into three steps. (Fig. 3.4)

In the first step, free a-b tubulin dimmers associate longitudinally to form short protofilaments. These are unstable.

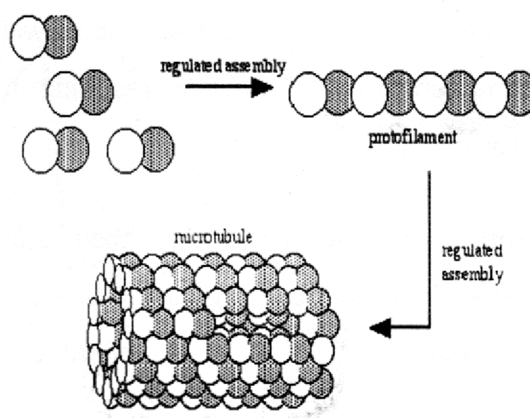


Fig. 3.4 : Assembly of microtubules

In the second step, the short protofilaments quickly associate laterally into more stable curved sheet.

In the third step thirteen such protofilaments join laterally and the curve of the sheet eventually completes into a cylinder and the microtubule then grows by the addition of the subunits to the ends of the protiofilaments. The free tubulin dimmers have GTP bound to the exchangeable nucleotide binding site on the beta tubulin monomer. After incorporation of a dimeric subunit into a microtubule, the GTP on the beta tubuline monomer. After incorporation of a dimeric subunits into a microtubule, the GTP on the beta tubule (but not the alfa tubule) is hydrolyzed to GDP. If rate of polymerization is faster than the rate of GTP hydrolysis, then a cap of GTP-bound subunits is generated at the (+) end, although bulk of the alpha tubulin in the microtubule will contain GDP. The rate of polymerization is twice as fast as the (+) end then (–) end.

3.4.5 Dynamic instability of microtubules

Microtubules undergo treadmilling, a dynamic behaviour in which tubulin molecules bound to GDP are continually lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end of the same microtubule. In microtubules, GTP hydrolysis also results in the behaviour known as **dynamic instability,** in which individual microtubules alternate between cycles of growth and shrinkage. Whether a microtubule grows or shrinks is determined by the rate of tubulin addition relative to the rate of GTP hydrolysis also results in the behaviour known as **dynamic instability,** in which individual microtubules alternate between cycles of growth and shrinkage. Whether a microtubule grows or shrinks is determined by the rate of tubulin addition relative to the rate of GTP hydrolysis. As long as new GTP-bound tubulin molecules are added more rapidly than GTP is hydrolyzed, the microtubule retains a GTP cap at its plus end and microtubule growth continues. However, if the rate of polymerization slows, the GTP bound to tubulin at the plus end of the microtubule will be hydrolyzed to GDP. If this occurs, the GDP-bound tubulin will dissociate, resulting in rapid depolymerization and shrinkage of the microtubule.

Once microtubules have assembled, their stability becomes temperature dependant. For instance, if microtubules are cooled at 4°C, they depolymerize into stable a - b tubulin, when warmed at 37°C in the presence of GTP, the tubulin dimers polymerize into microtubules. (Fig. 3.5.)

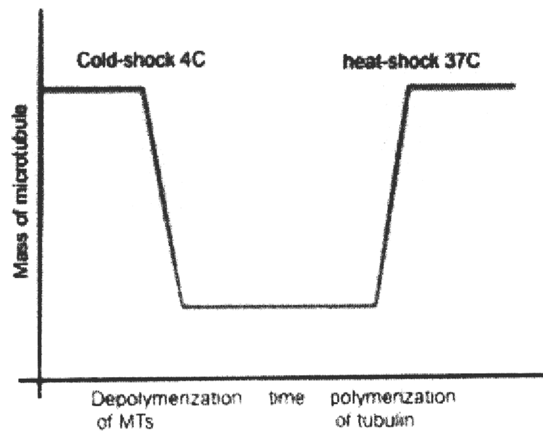


Fig. 3.5 : Polymerisation of microtubules

The assembly and disassembly of microtubule can be observed in the light microscope and their length can be plotted during the stage of assembly and disassembly. Though assembly and disassembly each proceeds at uniform rates, but there is a large difference between the rate of assembly and disassembly. The assembly is at the rate of 1mm/sec, whereas, the disassembly rate is 7 mm/min.This dynamic behaviour of microtubule is termed as **dynamic instability.** (Fig. 3.6.).

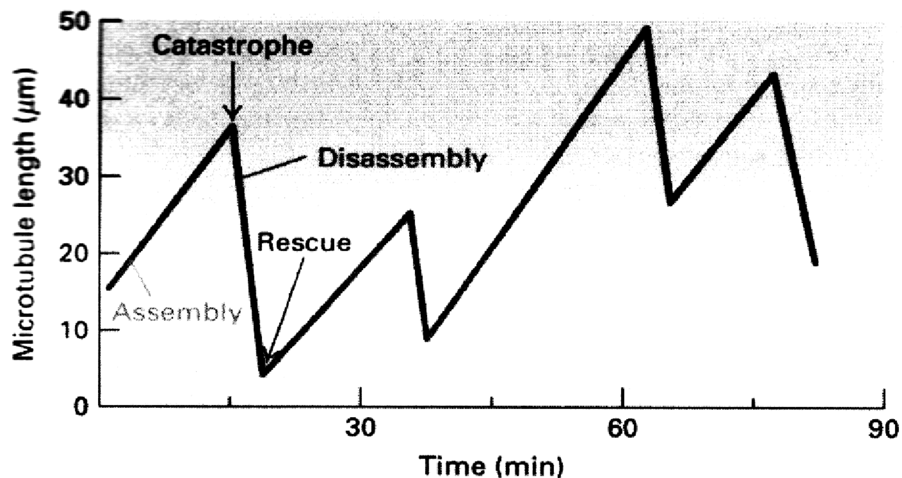


Fig. 3.6 : Dynamic instability of microtubule in vitro (after Lodish et.al).

3.4.6 Functions of microtubules

1. Mechanical function in shaping the cells through orientation and distribution of microtubules.

2. Cell membrane movement during pseudopodial activity, endocytosis and extension of microvillus.
3. Movement of cilia and flagella.
4. Chromosomal movement during cell divison.
5. Circulation and transport.
6. Morphogenesis — in shaping of the cell during the cell differentiation particularly during the formation of lens placode, spermatogenesis or muscle cell differentiation in the embryo.
7. Other functon where it may be involved in converting stimuli into nerve impulses, to drive the food in the gullet in ciliates and also cyclosis movement in the plant cells.

3.5 Microfilaments

Micfofilaments, also called actin filaments, are polymers of the protein actin that are part of a cell's cytoskeleton. The cytoskeleton is the network of protein filaments that extends throughout the cell, giving the cell structure and keeping organelle in place. Microfilaments are the smallest filaments of the cytoskeleton. They have roles in cell movement, muscle contraction, and cell division.

3.5.1 Structure of microfilaments

Microfilaments are composed of two strands of subunits of the protein actin (hence the name actin filaments) wound in a spiral. Specifically, the actin subunits that come together to form a microfilament are called globular actin (G-actin), and once they are joined together they are called filamentous actin (F-actin). The basic unit of actin filaments is a protein called **G-actin,** whose structure is very similar in many organisms including amoeba, plants and humans. G-actin polymerizes to form actin filaments with a diameter of around 7 nm (Fig. 3.7). Since G-actin molecule has plus end and minus end, the polymerized filaments also has **plus end** and **minus**

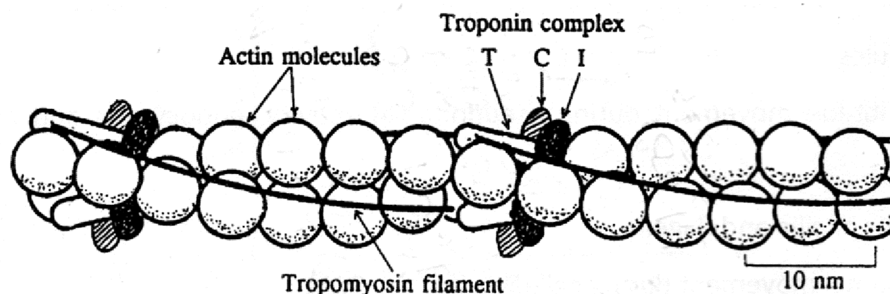


Fig. 3.7 : Schematic drawing of part of an actin filaments showing the relationship between successive pairs of actin molecule, thin filaments of tropomyosin and three types of troponin.

end.

Like microtubules, microfilaments are polar. Their positively charged, or plus end, is berbed and their negatively charged minus end is pointed. Polarization occurs due to the molecular binding pattern of the molecules that make up the microfilaments. Also like microtubules, the plus end grows faster than the minus end.

Microfilaments are the thinnest filaments of the cytoskeleton, with a diameter of about 6 and 7 nanometers. A microfilament begins to form when three G-actin proteins come together by themselves to form a trimer. Then, more actin binds to the barbed end. The process of self-asembly is ided by autoclampin proteins, which act as motors to help assemble the long strands that make up microfilaments. Two long strands of actin arrange in a spiral in order to form a microfilament.

3.5.2 Polymerization and depolymerization

G-actin has a binding site for ATP or ADP, and ATP bound G-actin molecules (ATP-G-actin) polymerize stably. However, after polymerization, when bound ATP is hydrolyzed into ADP, the polymer becomes unstable and is easily deploymerized. After depolymerization, when ADP is replaced with ATP, G-actin molecules again become able to bind to actin filaments. In this way, G-actin is recycled (Fig. 3.8)

In cells, compared with an in vitro environment, polymerization and deploymerization of actin filaments take place faster and more accurately. This is due to the action of many types of regulatory protein that bind to actin filaments to regulate their polymerization. These proteins are called **actin-binding proteins.**

G-actin (the basic unit of actin filaments) and actin filaments (polymeric of G-actin molecules) are shown here. Each actin filaments has two stranded helix of polymerized G-actin molecules. Since G-actin has polarity, an actin filaments also has polarity.

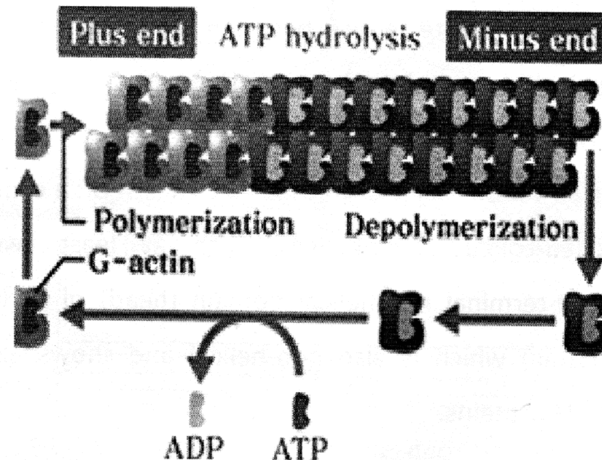


Fig. 3.8 : Formation of action filaments and the recycling of G-actin

ATP G-actin binds to the plus end of actin filaments. Hydrolysis of the ATP facilitates the deploymerization, removing G-actin molecules from its minus end. If the ADP of the dissociated G-actin is replaced with ATP, the G-actin is able to polymerize again.

3.5.3 Functions of microfilaments

- Microfilaments from the dynamic cytoskeleton, which gives structural support to cells and links interior of the cell with the surroundings to convey information about the external environment.
- Microfilaments provide cell motility. e.g., Filopodia, Lamellipodia.
- During mitosis intracellular organelles are transported by motor proteins to the daughter cells along actin cables.
- In muscle cells, actin filaments are alligned and myosin proteins generate forces on the filaments to support muscle contraction.

3.6 Intermediate filaments

3.6.1 Definition

Intermediate filaments (IF) are proteins which are primordial components of the cytoskeleton and the nuclear envelope. They generally form filamentous structures 8 to 14 nm wide and intermediate in size between microtubules and microfilaments. This family of protein includes cytokeratins, vimentin, desmin, gilai fibrillary acidic protein, neurofilament proteins and nestin.

All IF proteins are structurally similar in that they consist of : a central rod doman which is arranged in coiled-coiled alpha-helices, with at least two short characteristic interruptions ; an N-terminal non-helical domain (head) of variable length ; and an C-terminal domain (tail) which is also non-helcal and shows extreme length variation between different IF proteins.

The name intermediate comes from the diameter (10 nm) of these filaments. Like actin filaments and microtubules, intermediate filaments are polymers of elementary unit protein (Fig. 3.9). However, their polymerization mechanism differs. Polymerization does not require nucleotides such as ATP and GTP. Other differences include a lack of polarity the plus and minus ends in filaments.

Intermediate filaments in cells form complex networks with actin filaments and microtubules. They are abundant in cells to which physical tension is applied (e.g. epidermal cells and muscle cels) and neurons. Generally, intermediate filaments exist stably (degradation and reconstruction are infrequent), but when significant changes (such as cell division) occur, their degradation and reconstruction become very active.

3.6.2 Polylmerization of intermediate filaments

The polymerization steps of intermediate filaments are shown here. As the first step, two basic units (i.e., monomers) associate in the same direction to form a dimer. Two dimers running in the reverse direction are line up to form a tetramer, with the two strands slightly offset in their opposite directions. Tetramers are arranged side by

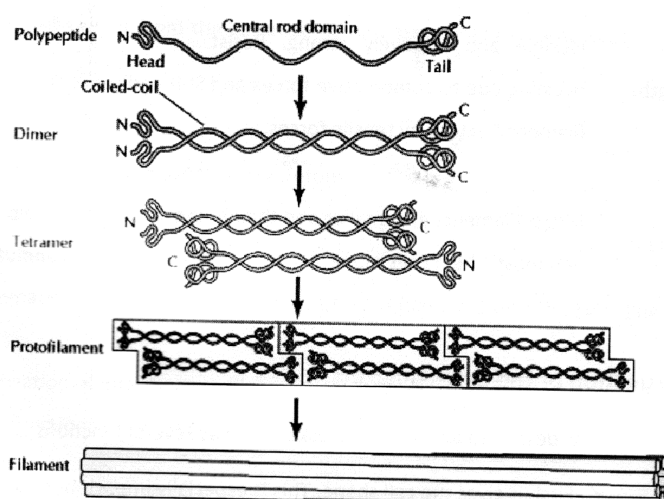


Fig. 3.9 : Intermediate filaments and their polymerization

side to form a protofilament. Eight protofilaments are bundled to form an intermediate filaments with a diameter of approximately 10 nm.

3.7 Microfilaments vs Microtubules

Microtubules and **microtubules** are key components of the cytoskeleton in eukaryotic cells. A cytoskeleton provides structure to the cell and connects to every part of the cell memebrane and every organelle. Microtubules and microfilaments together allow the cell to hold its shape, and move itself and its organelles.

Comparison chart

	Microfilaments	**Microtubules**
Structure :	Double Helix	Helical lattice
Size :	7 nm in diameter	20-25 nm in diameter
Composition :	Predominantly composed of contractile protein called actin.	Composed of subunits of protein tubulin. These subunits are termed as alpha and beta.
Strength :	Flexible and relatively strong. Resist buckling due to compressive forces and stiff and resist bending forces, filament fracture by tensile forces.	
Function :	Micro-filaments are smaller and thinner and mostly help cells move	Microtubules are shaped similarly but are larger, and help with cell functions such as mitosis and various cell transport functions.

3.8 Function of the cytoskeleton

As described above, the cytoskeleton has several functions.

- First, it gives the cell shape. This is especially important in cells without cell walls. Such as animal cells, that do not get their shape from a thick outer layer.
- It can also give the cell movement. The microfilaments and microtubules can

disassemble reassemble, and contract, allowing cells to crawl and migrate, and microtubules help form structures like cilia and flagella that allow for cell movement.

- The cytoskeleton organizes the cell and keeps the cell's organelles in place, but it also aids in the movement of organelles throughout the cell. For example, during endocytosis when a cell engulfs a molecule, microfilaments pull the vesicle containing the engulfed particles into the cell. Similarly, the cytoskeleton helps move chromosomes during cell division.

3.9 Summary

(i) The cytoskeleton contains actin filaments, intermediate filaments and microtubules.

(ii) These maintain cell shape and allow it and the organells to move.

(iii) Actin filaments, the thinner filaments interact with the mortor molecule myosin in muscle cells to bring about contraction.

(iv) Intermediate filaments support the nuclear envelope and the plasma membraneand probably participate in cell–to–cell junctions.

(v) Microtubules radiate out from the centrosome and are present in centrioles, cilia an flagella.

3.10 Model questions

1. What are the three components of cytoskeleton ?
2. State their structure and functions.
3. How muicrotubules are assembled ?
4. Give diagrammatic explanaton of formation of actin filaments and the recycling of G-actin.
5. How polymerization of intermediate filaments take place ?
6. What are the functions of cytoskeleton ?

Unit-4 □ Nucleus

Structure

4.1. Objectives

4.2. Introduction

4.3. Structure of nucleus

4.3.1 Unique characteristics

4.3.2 Nucleoplasm

4.3.3 The nucleolus

4.3.4 Ultrastructure analysis

4.3.4.1 Nuclear membrane

4.3.4.2 Nuclear lamina

4.3.4.3 Nuclear matrix

4.3.4.4 Nucleolus

4.3.4.5 Nucleoplasm

4.3.4.6 Chromatin threads or chromosomes

4.4. Nuclear envelope

4.4.1 Definition

4.4.2 Parts of the nuclear membrane

4.4.2.1 Outer membrane

4.4.2.2 Inner membrane

4.4.3 Functions of the nuclear membrane

4.5. Nuclear pore complex

4.5.1 Structure

4.5.2 Role of nuclear pore in transport

4.5.3 Function of nuclear pore

4.6. Nucleolus

4.6.1 Structure of nucleolus

4.6.2 Composition

4.6.3 Function of nucleolus

4.7. Difference between nucleus and nucleolus

4.8. Summary

4.9 Model questions

4.1 Objectives

This unit of the cell biology gives a fascinating idea about the central component of a cell. After completion of the topic the reader must be able to get an idea about

- What a nucleus is ?
- How it is structurally composed of
- The envelope surrounded in it
- The pore present and its function and
- The more about the nucluolus

4.2 Introduction

The nucleus is an important cell organelle like that of central nervous system. Since it controls the whole function or activities of the cell, it can simply be called as 'brain of the cell'. A nucleus contains many things of which the most important compound is the chromosome which further contains large amount of DNA and genes. Due to the presence of genes it is possible to control various cellular activities and to decide when, what and how to do.

Nucleus is a dense and most significant component of the cell. Animal cell nucleus has a lot of functions like controlling all the cellular activities and carrying the hereditary information of cell. It is found in both plant and animal cell. In 1831, Robert Brown first discovered nucleus in an orchid cell. All the eukaryotic animal cells have true nucleus except mature mammalian RBCs. The prokaryotes have incipient nucleus called as nucleoid or genophore or prokaryon.

4.3 Structure of nucleus

The cell nucleus is a membrane bound structure that contains the cell's hereditary information and controls the cell's growth and reproduction. It is the command center of a eukaryotic cell and is commonly the most prominent organelle in a cell. The nucleus is generally located at the centre of animal cell. It is mostly spherical in shape. However, its shape may be cuboidal, ellipsoidal, discoidal or irregular. Size of animal cell nucleus varies between 5 to 25 micrometers, depending upon the size of cell and age of nucleus.

Most of the animal cells have single nucleus while some cells like liver cells, *Paramecium,* etc have two nucleus. Some animal cells like osteoblasts even have multiple nucleus.

4.3.1 Unique characteristics

The cell nucleus is bound by a double membrane called the **nuclear evelope.** This membrane separates the contents of the nucleus from the cytoplasm. Like the cell membrane, the nuclear envelope consists of phospholipids that form a lipid bilayer. The envelope helps to maintain the shape of the nucleus and assists in regulating the flow of molecules into and out of the nucleus through **nuclear pores.** The nuclear envelope is connected with the **endoplasmic reticulum (ER)** in such a way that the internal compartment of the nuclear envelope is continuous with the lumen of the ER.

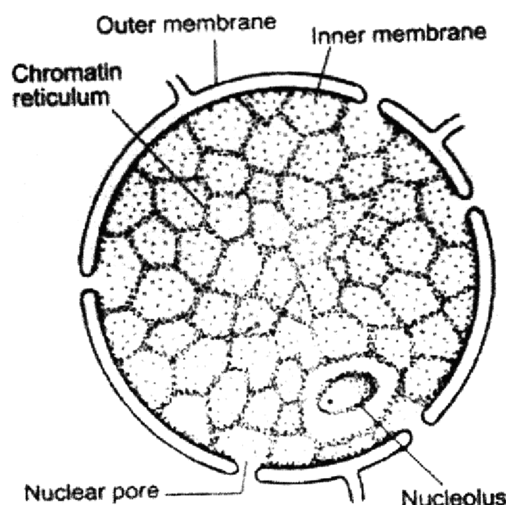


Fig. 4.1 : A schematic figure of nucleus

4.3.2 Nucleoplasm

Nucleoplasm is the gelatinous substance within the nuclear envelope. Also called karyoplasm, this semi-aqueous material is similar to cytoplasm and is composed mainly nucleolus and chromosomes are surrounded by nucleoplasm, which functions to cushion and protect the contents of the nucleus. Nucleoplasm also supports the nucleus by helping to maintain its shape. Additionally, nucleoplasm provides a medium by which materials, such as enzymes and nucleotides (DNA and RNA subunits), can be transported throughout the nucleus. Substances are exchanged between the cytoplasm and nucleoplasm through nuclear pores.

4.3.3 The nucleolus

Contained within the nucleus is a dense, membrane-less structure composed of RNA and proteins called the nucleous. The **nucleous** contains nucleolar organizers, which are parts of chromosomes with the genes for ribosome synthesis on them.

4.3.4 Ultra structure analysis

The detailed structure of nucleus can only be observed with electron microscope. From such study it reveals that the nucleus is made of six important structures :

1. Nuclear membrane or envelope or karyotheca
2. Nuclear lamina
3. Nuclear matrix
4. Nucleous
5. Chromatin thread or chromosomes

4.3.4.1 Nuclear membrane

It is the thin double layered porous outer envelope of the nucleus. Hence, it regulates nucleo-cytoplasmic interactions and acts as selective permeable membrane. Under E.M. the nuclear envelop in the interphase or prophase stage appears to consists of two concentric membranes, viz. inner nuclear membrane and outer nuclear membrane. Each membrane is about 75 to 90 A° The inter-membrane space is known as perinuclear cisternae (Fig. 4.2).

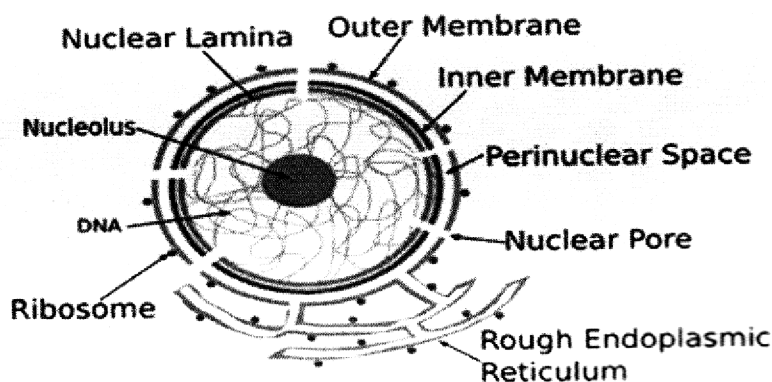


Fig. 4.2 : Showing nuclear structure

The inner membrane defines the content of nucleus itself and it contains specific peoteins that act as binding sites for the nuclear lamina. The outer membrane is rough due to presence of ribosome attached with it.

4.3.4.2 Nuclear lamina

The nuclear lamina is a proteins meshwork that lines the inner surface of the inner nuclear membrane in interphase cell forming a discreate layer of 30-100 nm thick that connects the inner membrane with chromatin. It is composed of three principal extrinsic membrane proteins, **lamina A, B and C** which together forms a fibrous network. The lamina with the lamin polypeptide carry out functions like regulating assembly and disassembly of the nuclear membrane during cell division as well as attachment of chromatin to the nuclear envelop.

4.3.4.3 Nuclear matrix

Nuclear matrix is an intranuclear framework, analogous to cytoskeleton. In EM view of nuclei from which all DNA has been removed, a network of protein fiber is left inside the nucleus. This structure is called the **nuclear matrix or scaffold.** The protein that constitute the matrix can be shown to bind specific DNA sequences called SARs or MARs (scaffold or matrix associated regions). The structural components of matrix have not yet been fully identified. So far the function are concerned, it is responsible for the determination of nuclear shape. It also gives mechanical support to resist disaggregation of nucleus in high ionic strength buffers.

4.3.4.4 Nucleolus

The nucleolus is the only organized body presents in the nucleous. In course of cell division, it usually disappears by late prophase, reforms during telophase and retains up to late prophase of the next cell cycle. In light microscope the suitable stained nucleolus is seen as a dense body of variable size and shape. It is now well established that the nucleolus is the site for the synthesis and biogenesis if ribosomal nlucleic acid (rRNA) in eukaryotes.

4.3.4.5 Nucleoplasm

It is a transport, semi-fluid homogenous and gel-like substance present inside nuclear membrane. Nucleoplasm is chemically composed of lipids, water, fat, mRNA etc. It acts as nuclear skeleton and helps in maintaining the shape of nucleus. The nuclear components such as the chromatin threads, nuclear matrix and the nucleolus remain suspended in the nucleoplasm.

Nucleoplasm is mainly composed of nuceoproteins but it also contains various inorganic and organic substances. DNA and RNA are the common nucleic acids of nucleoplasm. It also contains many enzymes that the are necessary for the synthesis of DNA and RNA. It carries out the biosynthetic functions of the nucleus.

4.3.4.6 Chromatin threads or chromosomes

It is a numerous, dark staining, thread like structure in nuceoplasm. They are coiled upon themselves, forming a mass of chromatin reticulum. It contains DNA which acts as genetic material.

4.4 Nuclear envelope

4.4.1 Definition

The nuclear membrane, also called the nuclear envelope, is a double membrane layer that separates the contents of the nucleus from the rest of the cell. It is found in both animal and plant cells. A cell has many jobs, such as building proteins, converting molecules into energy, and removing waste products. The nuclear envelope protects the cell's genetic material from the chemical reactions that take place outside the nucleus. It also contains many proteins that are used in organizing DNA and regulating genes.

4.4.2 Parts of the nuclear membrane

4.4.2.1 Outer membrane

Like the cell membrane, the nuclear membrane is a lipid bilayer, meaning that if consists of two layers of lipid molecules.The outer layer of lipids has ribosomes, structures that make proteins, on its surface. It is conneted to the endoplasmic reticulum, a cell structure that packages and transports proteins (Fig. 4.2).

4.4.2.2 Inner membrane

The inner membrane contains proteins that help organize the nucleus and tether genetic material in place. This network of fibres and proteins attached to the inner membrane is called the nuclear lamina. It structurally supports the nucleus, plays a role in repairing DNA, and regulates events in the cell cycle such as cell division and the replication of DNA. The nuclear lamina is only found in animal cells, although plant cells may have some similar proteins on the inner membrane. Both the membrane is about 75 to 90 A° thick and lipoproteinous in nature (Fig. 4.2).

4.4.3 Function of the nuclear membrane

1. The nuclear membrane is a barrier that physically protects the cell's DNA from the chemical reactions that are occurring elsewhere in the cell. If molecules that stay in the cytoplasm were to enter the nucleus, they could destroy part of the cell's DNA, which would stop it from functioning properly and could even lead to cell death.
2. The envelope also contains a network of proteins that keep the genetic material in place inside the nucleus.
3. It also manages what materials can enter and exit the nucleus. It does so by being selectively permeable. Only certain proteins can physically pass through the double layer. This protects genetic information from mixing with other parts of the cell, and allows different cellular activities to occur inside the nucleus and outside the nucleus in the cytoplasm, where all other cellular structures are located.

4.5 Nuclear pore complex

The nuclear pore complex is the organelle within the nuclear envelope that mediates the export of messanger RNA, transfer RNA, and the subunits of ribosomes, which are synthesized or assembled in the nucleus but function in the cytoplasm. The pore complex also mediates the nuclear import of proteins and protein complexes (eg. snRNP particles) that are synthesized and assembled in the cytoplasm but destined to function in the nucleus. The number of nuclear pore complees varies considerably, depending on the cell type. Their number generally reflects the biosynthetic activity of the cells. For example, the nuclear envelope of the Xenopus oocyte has the highest known concentration of pores per unit area of nuclear envelope (60 pores mm), whereas quiescent chick erythrocytes have two to four pores mm.

Nuclear pore complexes were first described by Callan and Tomlin in their electron microscopic analysis of the nuclear envelope in amphibian oocytes. Architecturally, the nuclear pore is a huge beautifully elaborate, symmetrical organelle within the nuclear envelope.

4.5.1 Structure

The nuclear pore is a large complex structure of 125 million daltons or 30 time the size of a eukaryotic ribosome. The pore is 120 nm in diameter and 50 nm in thickness. It consists of four separate elements.

(i) The scaffold, which includes majority of the pore

(ii) The central hub or tranporter, which carried out active transport (both import and export) or proteins and RNAs

(iii) Short thick filaments attached to the cytoplasmic side of the pore and

(iv) A newly discovered basket attachedd to the nucleoplasmic side of the pore.

The scaffold is a stack of three closely apposed rings, namely the cytoplasmic ring, the nucleoplasmic ring and a central ring of thick spokes. Each ring has a eight-fold symmetry. The spokes of central ring is attached to the transporter on the inner side, and to the nucleoplasmic and cytoplasmic rings on the outer side. Interspersed

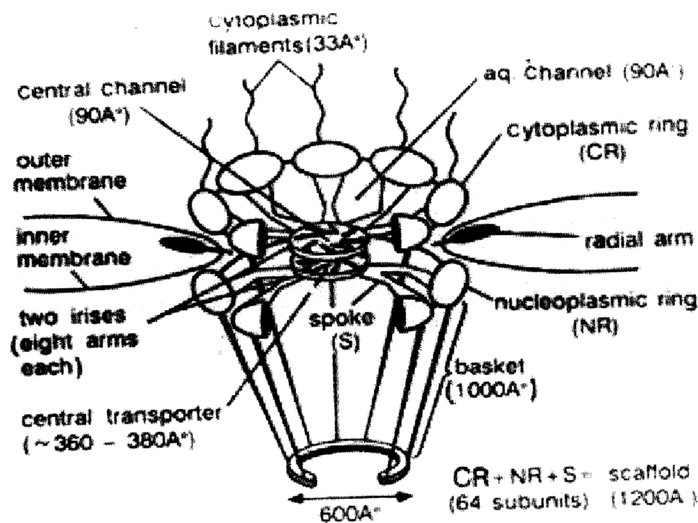


Fig. 4.3 : Detailed structure of nuclear pore

between the spokes are aqueous channels, 9nm wide, which allow diffusion of proteins and metabolites between the nucleus and the cytoplasm.

The transporter is a proteinceous ring, 36-38 nm in diameter and consists of two irises of eight arms each. The two irises are assumed to be stacked atop one another and open sequentially, each like the diaphragm of a camera, to let a nuclear protein or RNA pass through from the nucleus to the cytoplasm. On the cytoplasmic side of the pore, thick fibres (3.3 in diameter) extend into the cytoplasm. On the nuclear side, a large basket like structure is found, which consists of eight filaments (each 100 nm long), extending from nucleoplasmic ring of the pore and meeting a smaller ring (60 m in diameter) within the nucleus. The basket may play an important role in RNA export.

4.5.2 Role of nuclear pore in transport

The import and export of proteins and RNAs into and outside the nucleus are facilitated by the presence of **signal sequences.** Even gold particles 25nm in diameter, if coated the nucleus. ATP has also been found to be essential for nuclear transport. The transport (this is usually unidirectional) actually involves following two steps :

(i) an ATP-independent, but signal-sequence dependent step involving binding of **protein to the pore,** and

(ii) an ATP-requiring step, involving translocation through the pore ; this step is the only energy requiring step. It has been shown that in the absence of ATP, the protein binds to the pore, but can not be transported to the nucleus.

4.5.3 Function of nuclear pore

The function of the nuclear pore complex, as stated above, is to help transport macromolecules back and forth between the nucleus and the cytoplasm. In addition, the nuclear pore complex also serves as a barrier between the cytoplasm and nucleus to prevent harm to genetic material housed in the nucleus. Macromolecules move through the NPC through diffusion channels (up to a size of about 40 kilodaltons), the diffusion barrier is made by spreading nucleoporins that have many FG repeats. FG repeats also serve an important role in the NPC ; they act as a docking zone for transport receptors (known as karyopherins) that move molecules between the NPC. For molecules to move through the NPC, they have short localization sequences for import, and nuclear export sequences for export.

The nuclear pore complex has roles outside of nucleocytoplasmic transport between the nucleus and cytoplasm. The NPC is also involved in chromatin organization, gene expression regulation, DNA repair, and many other functions.

4.6 Nucleolus

The nucleolus is the distinct structure present in the nucleus of eukaryotic cells. It is mainly involved in assembling the ribosomes, modification of transfer RNA and sensing cellular stress. The nucleolus is composed of RNA and proteins which form around specific chromosomal regions.

4.6.1 Structure of nucleolus :

Estable and Sotelo (1951) described the structure of a nucleolus under the light microscope. According to them, nucleolus consists of a continuous coiled filament called the nucleolonema embedded in a homogeneous matrix, the pars amorpha. The first description of nucleolar ultra structure was given by Borysko and Bang (1951)

and Bernhard (1952).

They described two main nucleolar components, a filamentous one corresponding to the nucleolonema and a homogeneous one corresponding to the pars amorpha (matrix). Later on, Gonzales-Ramirez (1961) and lzard & Bernhard (1962) demonstrated that the nucleolonema consists of a spongy net work in place of a continuous filament. The ultra structure of the nucleolus have been reviewed by Day (1968), Bernhard and Granboulan (1968) and Bush and Smetana (1970).

Four chief components have been observed :

(i) An amorphous matrix or pars amorpha.

(ii) Chromatin containing abundant DNA.

(iii) Fibrils containing RNA, 80-100 A in Diameter, precursor of granules.

(iv) Granules-Ribonucleoprotein granules 1500-200 A in diameter.

4.6.2 Composition

(i) DNA

(ii) RNA

(iii) Protein

4.6.3 Functions of nucleolus

(i) Ribosome formation or biogenesis of ribosomes.

(ii) Synthesis and storage of RNA :

It produces 70-90% of cellular RNA in many cells. It is source of RNA. The chromatin in nucleolus contains genes or ribosomal DNA (rDNA) for coding ribosomal RNA. Chromatin containing DNA gives rise to fibrils containing RNA. Granules containing RNA already produces ribosomes.

(iii) Protein synthesis :

Maggis (1960) and others have suggested that protein synthesis takes place in nucleolus. Other studies confirm the above views. In eukaryotes the gene coding for RNA contains a chain of at-least 100-1000 repeating copies of

DNA. This DNA is given off from the chromosomal fibre in the forms of loops. The DNA loops are associated with proteins to form nucleoli.

The DNA seems as a template for 45S rRNA. Half the 45S is broken down to form 28S and 18S RNA. The other half is broken down fruther to nucleotide level. Within the nucleolus the 28S rRNA combines with proteins made in cytoplasm to form the 60S ribosomal sub-unit. The 185 rRNA also associates with proteins to form the 40 S subunit of the ribosome.

4.7 Difference between nucleus and nucleolus

Nucleus	Nucleolus
• Large in size	• Very small in size
• Bounded by the nuclear envelope	• It has no limiting membrane.
• It contains chromosomes	• It does not hold any chromosomes.
• It is rich in DNA, the genetic material.	• It is rich in RNA

4.8 Summary

(i) Nucleus, a specialized structure occurring in most cells (except bacteria and blue-green algee) and separated from the rest of the cell by a double layer, the nuclear membrane.

(ii) The nucleus controls and regulates the activities of the cell (e.g., growth and metabolism) and carries the genes, structures that contain the hereditary information.

(iii) Nucleoli are small bodies often seen within the nucleus ; they play an important part in the synthesis of ribonucleic acid and protein. The gel-like matrix in which the nuclear components are suspended is the nucleoplasm.

4.9 Model questions

1. Describe the structure of nucleus.
2. Discuss the structure of nuclear envelope and nuclear pore.

3. Distinguish between nuclus and nucleolus.
4. Give the structure of nucleolus.
5. State the composition and function of nucleolus.
6. What is the function of the nuclear membrane?

Unit-5 □ Cell Division

Structure

5.1. Objectives

5.2. Introduction

5.3. Cell division

5.3.1 Mitosis

5.3.1.1 Significance of mitosis

5.3.2 Meiosis

5.3.2.1 Significance of meiosis

5.4. Cell cycle

5.5. Cell cycle regulation

5.6. Cell cycle checkpoints

5.7. Summary

5.8. Model questions

5.1 Objectives

This unit of the cell biology gives an idea about the cellular division leading to growth of an individual organism. After completion of the topic the reader would be able to get a brief idea about

✓ How a cell divide

✓ What are the phases of cell cycle

✓ How this cell cycle is regulated.

5.2 Introduction

All living organism of the biological world start life as one cell, i.e. unicellular zygote the product of the union of sperm and egg. There are of course unicellular organisms those live their entire lives as one cell. But in multicellular organisms, the unicellular zygote undergoes countless divisions and produces many cells. These cells ultimately build the organism. The process by which any cell produces its own replica is known as **cell division.** Thus simply be cell division a zygote enables an

organism to grow. During this period of growth, many cells undergo a course of specialization that commits them to perform specific functions. Some cells function in cell division—either they divide to produce gametes or they divide to make new cells for growth or to replace old and damaged cells. Thus cell division is at the core of life itself. Three fundamental activities for life is possible by helping organisms to grow, reproduce, repair damaged and worn tissue.

5.3 Cell division

Primarily there are two kinds of cell divisions.

(i) Mitosis–Which is meant for multiplication of cell number and

(ii) Meiosis–Which helps in alteration of generations.

Irrespective of whether or not, products of meiosis directly take part in fertilization, meiotic division always reduces the chromosome number to half, which is restored to normal diploid number at the time of zygote formation.

5.3.1 Mitosis

Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell. Chromosomes replicated during the 5 phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome. In activity dividing animal cells, the whole process takes about one hour. The process of mitosis consists of the following stages or phases.

1. **Interphase or Interkinesis**
2. **Karyokinesis**
3. **Cytokinesis**

1. Interphase or Interkinesis

- Interphase is the phase between two successive cell division (end of one cell division to the beginning of next cell division).
- It is the longest phase in the cell cycle.
- Interphase looks dormant but it is metabolically active stage.
- It is divided into 3 sub-stages viz. G_1-phase, S-phase and G_2-phase.

(i) G_1-Phase or Gap-1 phase

- The cell grows in size due to active biosynthesis.
- Formation of structural and functional proteins.
- Synthesis of mRNA, tRNA and rRNA takes place.

(ii) S-Phase or Synthetic phase

- Replication of DNA takes place.
- Synthesis of histone proteins takes place which covers DNA.

(iii) G_2-phase or Gap-two phase or Second growth phase (Fig. 5.1)

- RNA and protein is synthesized.
- Centrioles get replicate (in case of animal cell)
- Synthesis of spindle proteins takes place.

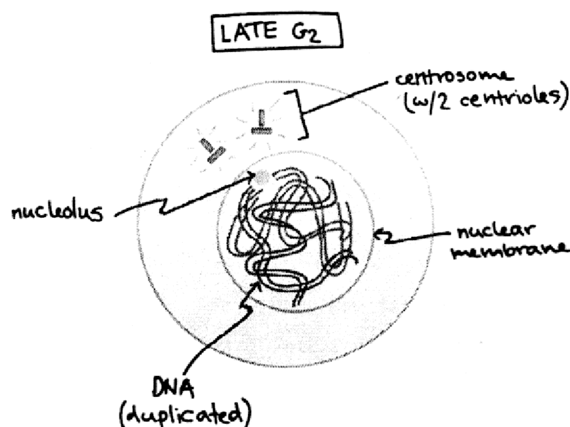


Fig. 5.1 : Late G_2 phase of mitosis

2. Karyokinesis

Karyokinesis is the division of the nucleus.
It consists of the following four phases.

(i) Prophase (Fig. 5.2 A, B)

- It is the first visible stage in karyokinesis.
- The chromosomes appear as long coiled threads called chromatids.
- The chromatin becomes shorter, thicker and visible due to the condensation of DNA.

- The chromatin are now called chromasomes.
- Stainability of nucleus is increased.
- Each chromosome starts to splits longitudinally into two sister chromatids. These sister chromatids are attached with each other at centromere.
- The nuclear membrane and nuclelus starts to disappear and by the end it will completely disappeared.

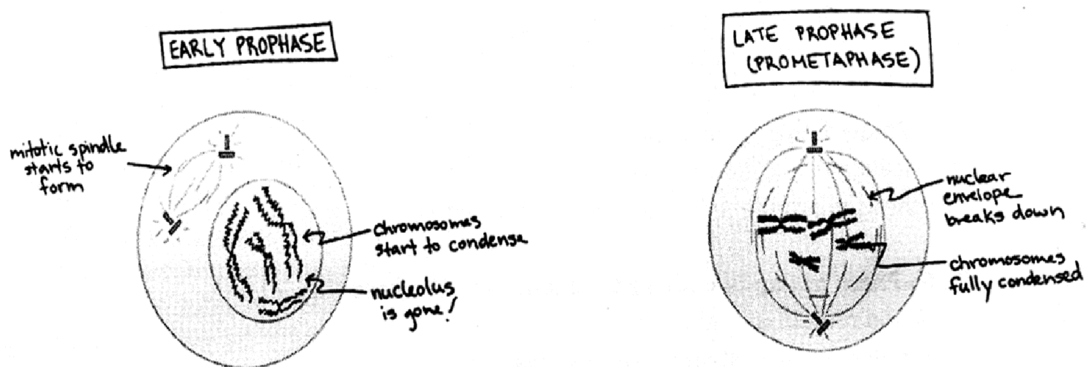


Fig. 5.2A : Early Prophse stage of mitosis **Fig. 5.2B : Late Prophse stage of mitosis**

(ii) Metaphase (Fig. 5.3)

- Nuclear membrfane and nucleolus completely disappears and simultaneously appearance of spindle fibres.
- Spindle fibres attached to the centromere of chromosome.
- The chromosomes are arranged on the equatorial plane.
- The process of gathering of chromosomes in equator is called congression and plate formed is called metaphasic plate.

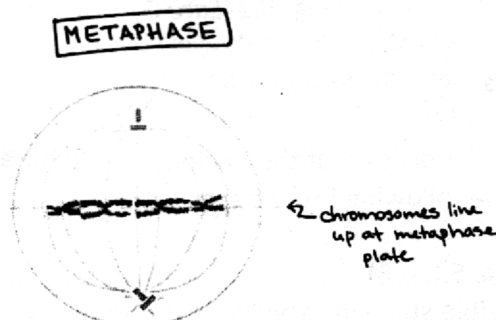


Fig. 5.3 : Metaphase stage of mitosis

(iii) Anaphase (Fig. 5.4)

- The centrosome of each chromosome splits into two sister chromatids and forms two daughter chromosomes.
- The daughter chromosomes are pulled towards the poles due to the contraction of spindle fibres and stretching of inter zonal fibres.

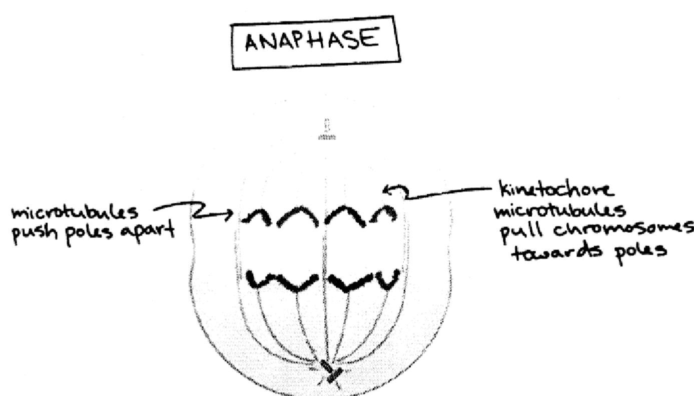


Fig. 5.4 : Anaphase stage of mitosis

- During polar movement, the chromosomes shows different shapes i.e. J, U, V, L or I shaped in appearance.
- At the end of anaphase, each pole will get one set of daughter chromosomes.
- It is shortest phase and is also known as migratory phase.

(iv) Telophase (Fig. 5.5)

- The daughter chromosomes reach respective poles and uncoil and become thin, long and visible.
- The spindle fibres start disappearing and finally disappear.
- The nuclear membrane and the nucleolus reappear.
- Two nuclei are formed at the end of telophase. Both the nuclei have the same number of chromosome as parent cell.
- It is the last visible stage of karyokinesis and is also known as reorganization phase.

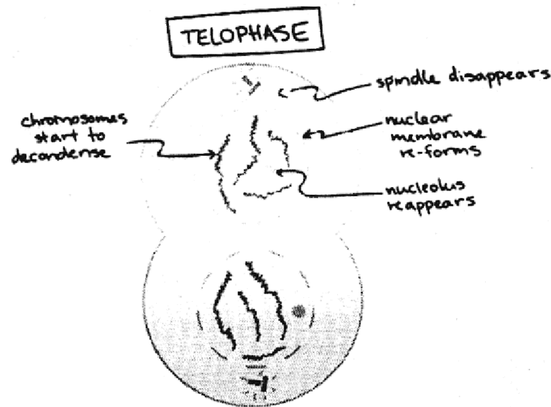


Fig. 5.5 : Telophase stage of mitosis

3. Cytokinesis (Fig. 5.6)

- Cytokinesis is the division of the cytoplasm.
- In plant cells, cytokinesis occurs by cell plate formation.
- During cytokinesis, many granular matrix formed by the golgi body and endoplasmic reticulum accumulates in the equatorial region. These granular matrix form cell plate.
- This plate divides the cell and by the end of telophase, cytokinesis is completed.
- In animal cells, cytokinesis occurs by cleavage or furrow formation.

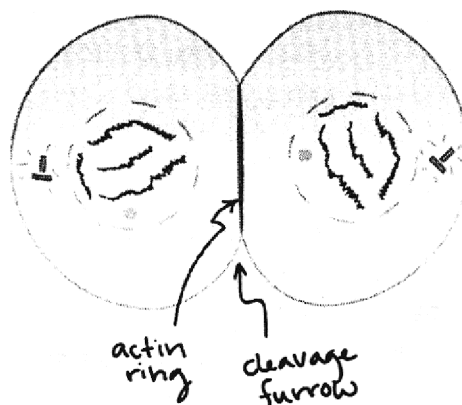


Fig. 5.6 : Cytokinesis

5.3.1.1 Significance of mitosis

- Mitosis produces 2 genetically identical cells, so mitosis maintains the genetic stability of organisms.
- DNA remains constant, so mitosis keeps the chromosomes number constant in a species.
- Mitosis helps in the development of multicellular organism.
- Mitosis helps to replacement of old, dead or damaged cells by new one.
- It helps in the recovery of wounds and injury of the body by formation of new cells.
- In unicellular organisms like Yeast, *Paramecium,* mitosis is a means of a sexual reproduction.
- Mitosis causes maturation and multiplication of germ cells and makes them ready for meiosis.

5.3.2 Meiosis

Meiosis is a cell division in which four haploid cells are formed from a single diploid cell.

It usually occurs in reproductive organs or gonads of the organisms.

Meiosis is also known as reductional cell division because four daughter cells produced contain half the number of chromosomes than that of their parent cell.

Meiosis has two nuclear division phases :

1. **Meiosis-I (Reductional or Heterotypic division)**
2. **Meiosis-II (Equational or Homotypic division)**

1. Meiosis-I (Heterolytic or Reductional division)

Meiosis-I has four different phases or stages : (Fig. 5.7)

1. **Prophase-I**
2. **Metaphase-I**
3. **Anaphase-I**
4. **Telophase-I**

1. **Prophase-I**

   It occupies the longest duration in Meiosis-I.

   It is divided into five sub-stages or sub-phases.

(i) Lepotene

- This phase starts immediately after interphase.
- The size of cell and nucleus increases.
- The chromosomes appear long, uncoiled thread-like in structure bearing many bead-like structures called chromomeres.
- The nuclear membrane and nucleolus remain as it is.

(ii) Zygotene

- Homologous chromosomes come closer and starts to pair up along their length.
- The pairing of homologous chromosomes is called Synapsis and the paired homologous chromosomes are referred as bivalents.
- The homologous chromosomes are held together by ribonuclear protein between them.

(iii) Pachytene

- The chromosome become shorter and thicker.
- Each chromosome of the bivalents splits longitudinally to form two chromatids such that bivalents is composed of our strands and is known as a tetrad.
- The process of crossing over starts (crossing over ; a small fragment of chromosome exchange between two non-sister chromatids of bivalent by breakage and rejoining).
- Crossing over is the most important genetic phenomenon of meiosis which causes variation in genetic characters in offspring.

(iv) Diplotene

- In this stage crossing over takes place.
- Bivalents (chromatids) repel each other.
- Homologous chromosome (two non-sister chromatide) begins to separates but separation is not complete, they remains attached to a point with a knot like structure called chiasmata (singular–Chiasma)
- The number of chiasma varies. Depending upon the number of chiasmata, chromosome appear different shape.
    - 1 chiasmata : cross like
    - 2 chiasmata : ring like
    - Many chiasmata : series of loop

- Nuclear membrane and nucleolus begins to disappear.

(v) Diakinesis

- The chiasma moves towards the end of the chromosomes (tetred) due to contraction of chromosome lastly slips over separating the homologous chromosome. This movement of the chiasmata towards the end of chromosome is called terminalization.
- By the end of diakinesis the nuclear membrane and nuleolus get completely disappeared spindle fibres begin to form.

2. **Metaphase-I**

- The spindle fibres organized between two poles and get attached to the centromere of chromosomes.
- Chromosomes moves to equator.
- The bivalent chromosomes are arranged in the equatorial plate in such a way that 2 metaphasic plates are formed.

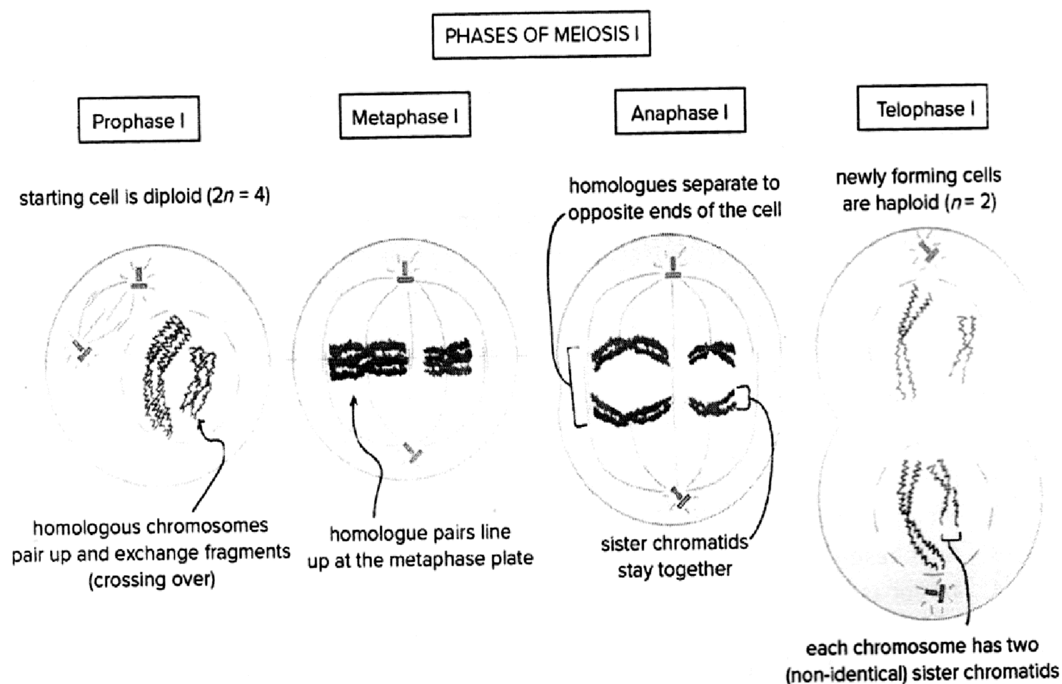


Fig. 5.7 : Showing Phases of Meiosis-I

3. **Anaphase-I**

- Spindle fibres contracts and pulls the whole chromosomes to the polar region.
- The separated chromosome is known as dyads
- No splitting of chromosomes occurs so the centromere of each homologous chromosome does not divide. Thus, the chromosome number of the daughter nuclei is reduced to half.
- Now the separated chromosome moves towards opposite poles.

4. **Telophase-I**

- Two groups of chromosome formed at each pole and organized into nuclei.
- The nuclear membrane and nucleolus reappears.
- The chromosomes get uncoiled into chromatin thread.
- The spindle fibres disappear totally.

■ **Cytokinesis I**

Cytokinesis may or may not follow nuclear division (meiosis-1 Cytokinesis occurs by cell plate formation method in plant cell and furrowing method in animal cells.

■ **Interphase II or Interkinesis**

The two cells or nuclei thus formed pass through a short stage called interphase-II. Sometimes, interphase-II is absent.

It is the resting phase between meiosis-I and meiosis-II.

It is either very short or may be absent

No DNA synthesis occurs.

2. Meiosis-II (Homolytic or equational division)

Meiosis-II is exactly similar to mitosis, so it is also known as meiotic mitosis.

In this division, two haploid chromosome splits longitudinally and distributed equality to form 4 haploid cells.

It completes in 4 stages. (Fig. 5.8)

1. **Prophase-II**
2. **Metaphase-II**
3. **Anaphase-II**
4. **Telophase-II**

1. **Prophase-II**

- The dyads chromosome become thicker and shorter.
- Nuclear membrane and nucleolus disappear.
- Spindle fibre starts to form.

2. **Metaphase-II**

- The dyads chromosome comes to equatorial plane.
- Spindle fibres organize between poles and attaches to centromere of chromosome.

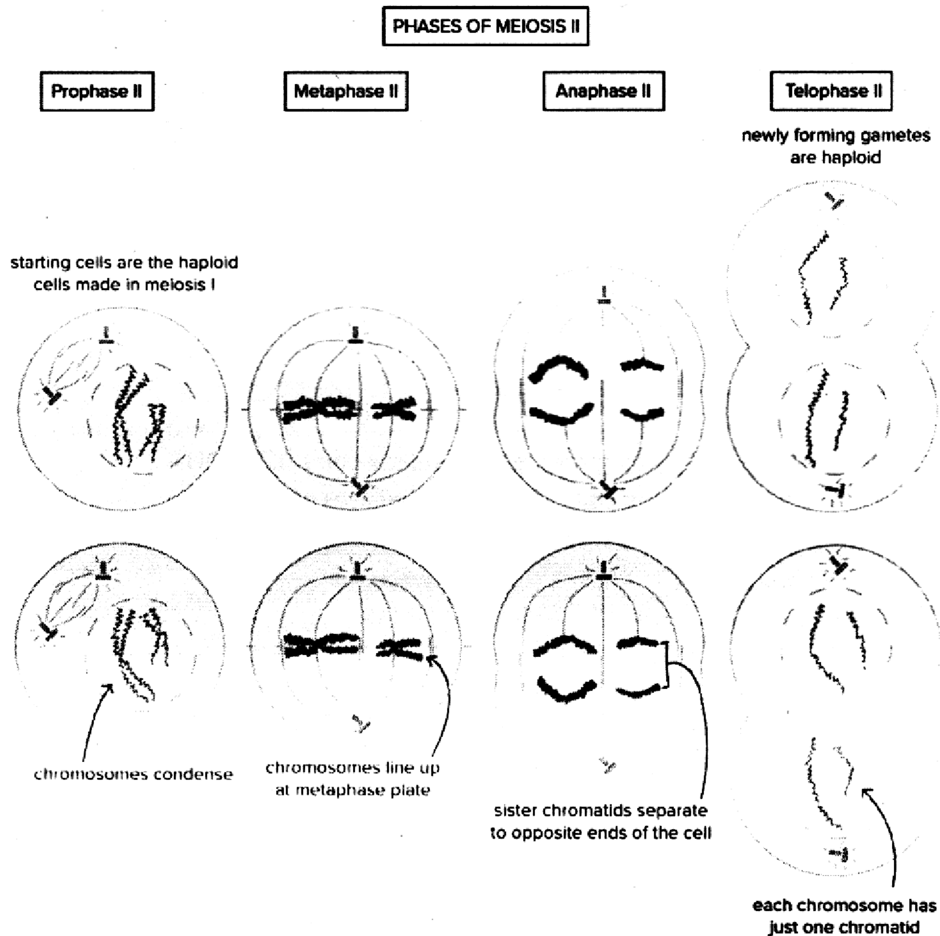


Fig. 5.8 : Showing Phases of Meiosis-II

3. **Anaphase-II**

- Centromere of each chromosome divides and sister chromatids separates to form two daughter chromosome.
- Spindle fibre contracts and pull the daughter chromosome apart towards opposite pole.

4. **Telophase-II**

- Chromosome become organize at respective pole into nuclei.
- Chromosome elongates to form thin network of chromatin.
- Nuclear membrane and nucleolus reappears.

Cytokinesis II

- The result of cytokinesis is four haploid daughter cells (gametes or spores).
- Cytokinesis takes place by cell plate formation in plant cell.
- Successive methods : Cytokinesis followed by each nuclear division resulting in 4 haploid cells. Eg. Monocot plants.
- Simultaneous methods : Cytokinesis occurs only after meiosis-II to form 4 haploid cells. Eg. Dicot plants.
- In animal cells, cytokinesis occurs by furrow formation or depression.

5.3.2.1 Significance of meiosis

1. Meiosis helps to maintain a constant number of chromosomes by reducing the chromosome number in the gametes.
2. Essential for sexual reproduction in higher animals and plants.
3. Meiosis helps in the formation haploid gametes and spores for sexual reproduction.
4. Number of chromosome remain fixed in a species from generation to generation
5. Crossing over occurring brings genetic variations in offspring which helps in evolution of organisms.
6. Failure disjunction in meiosis leads mutation to the formation of polypoid forms.
7. The random distribution of maternal and paternal chromosomes takes place into daughter cells during meiosis and it is a sort of independent assortment which leads to variation.

5.4 Cell cycle

To divide, a cell must complete several important tasks : it must grow, copy its genetic material (DNA) and physically split into two daughter cells. Cells perform these tasks in an organized, predictable series of steps that make up the cell cycle. The cell cycle is a cycle, rather than a linear pathway, because at the end of each go-round, the two daughter cells can start the exact same process over again from the beginning.

In eukaryotic cells, or cells with a nucleus, the stages of the cell cycle are divided into two major phases : **Interphase** and the **mitotic (M) phase** (Fig. 5.9).

- During interphase, the cell grows and makes a copy of its DNA.
- During the mitotic (M) phase, the cell separates its DNA into two sets and divides its cytoplasm, forming two new cells.

■ Interphase

The G_1, S, and G_2 phases together are known as **interphase.** The prefix inter-means between, reflecting that interphase takes place between one mitotic (M) phase and the next Interphase, which appears to the eye to be resting stage between cell divisions, is actually a period of diverse activities. Those interphase activities are indispensible in making the next mitosis possible. Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is contantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps :

(i) Gap 0 (G0), **(ii)** Gap 1 (G1), **(iii)** S (synthesis) phase, **(iv)** Gap 2 (G2).

Gap 0 (G0) phase : There are times when a cell will leave the cycle and quit dividing. This may be a temporary resting period or more permanent. An example of the latter is a cell that has reached an end stage of development and will no longer divide (e.g. neuron).

Gap1 (G1) phase : During G1 phase, also called the first gap phase, the cell grows physically larger, copies organelles, and makes the molecular building blocks it will need in later steps. i.e. Cells increse in size in Gap 1, produce RNA synthesize protein. An important cell cycle control mechanism activated during this period (G1 checkpoint) ensures that everything is ready for DNA synthesis.

In the great majority of cases, cells do indeed grow before division. However, in certain situation during development, cells may intentionally split themselves up into

smaller and smaller pieces over successive rounds of cell division. For instance, this happens in very early development of an African clawed frog (*Xenopus laevis*) embryo. See the end of the article for a video of cell divisions in early frog embryo.

S phase : In S phase, the cell synthsizes a complete copy of the DNA in its nucleus. It also duplicates a microtubule-organizing structure called the centrosome. The centrosome help separate DNA during M phase.

Gap 2 (G2) phase : During the second gap phase, or G_2 phase, the cell grows more, makes proteins and organelles, and begins to reorganize its contents in preparation for mitosis. G2 phase ends when mitosis begins. Actually, during the gap between DNA synthesis and mitosis, the cell will continue to grow and produce new proteins. At the end of this gap is another control checkpoint (G_2 checkpoint) to determine if the cell can now proceed to enter M (mitosis) and divide.

Interphase is composed of G1 Phase (cell growth), followed by 5 phase (DNA synthesis), followed by G2 phase (cell growth). At the end of interphase comes the mitotic phase, which is made up of mitosis and cytokinesis and leads to the formation of two daughter cells. Mitosis precedes cytokinesis, though the two processes typically overlap somewhat.

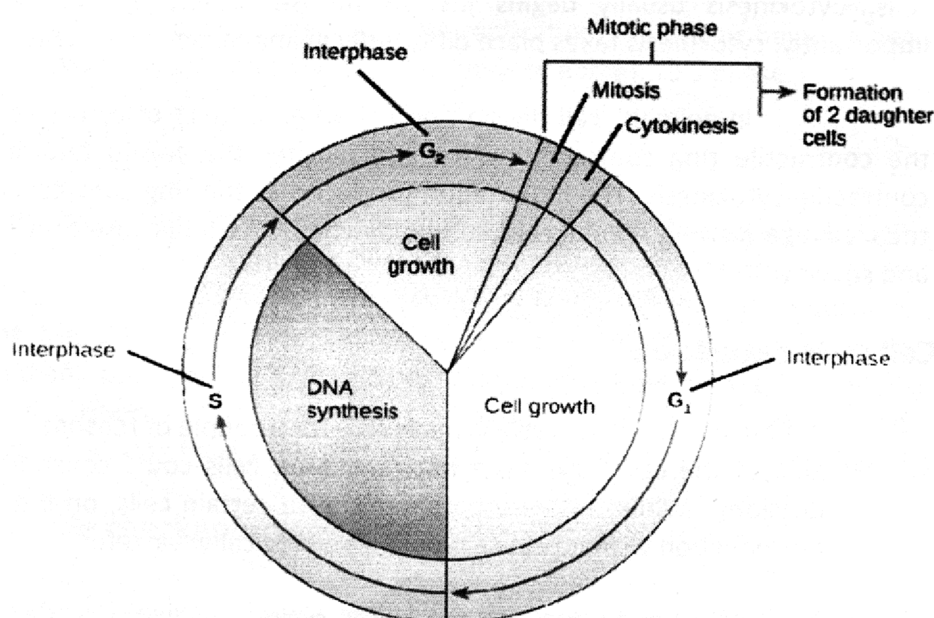


Fig. 5.9 : Showing cell cycle.

- **M. phase**

Cell growth and protein production stop at this stage in the cell cycle. All of the cell's energy is focused on the complex and orderly division into two similar daughter cells. Mitosis is much shorter than interphase, lasting perhaps only one to two hours. As in both G_1 and G_2, there is a Checkpoint in the middle of mitosis (Metaphase Checkpoint) that ensures the cell is ready to complete cell division. During the mitotic (M) phase, the cell divides its copied DNA and cytoplasm to make two new cells. M phase involves two distinct division related processes : mitosis and cytokinesis.

In **mitosis,** the nuclear DNA of the cell condenses into visible chromosomes and is pulled apart by the mitotic spindle, a specialized structure made out of microtubules. Mitosis takes place in four stages : prophase (sometimes divided into early prophase and prometaphase), metaphase, anaphase, and telophase.

In **cytokinesis,** the cytoplasm of the cell is split in two, making two new cells. Cytokinesis usually begins just as mitosis is ending, with a little overlap. importantly, cytokinesis takes place differently in animal and plant cells.

In animals, cell division occurs when a band of cytokeletal fibres called the **contractile ring** contracts inward and pinches the cell in two, a process called contractile cytokinesis. The indentation produced as the ring contracts inward is called the **cleavage furrow.** Animal cells can be pinched in two because they're relatively soft and squishy.

5.5 Cell cycle regulation

Control of the cell cycle is necessary for a couple of reasons.

- First, if the cell cycle were not regulated, cells could constantly undergo cell division. While this may be beneficial to certain cells, on the whole constant reproduction without cause would be biological wasteful.
- Second, internal regulation of the cell cycle is necessary to signal passage from one phase to the next at appropriate times. This regulation is not achieved through strict time constraints, but rather with feedback from the cell.

Two main families of proteins involved in this process— cyclin-dependent protein kinases (Cdks) and cyclins.

Cyclin-Dependent Protein Kinase (Cdks)

A Cdks is an enzyme that adds negatively charged phosphate groups to other molecules in a process called phosphorylation. Through phosphorylation, Cdks signal the cell that it is ready to pass into the next stage of the cell cycle. As their name suggests, Cyclin-Dependent Protein Kinasis are dependent on cyclins, another class of regulatory proteins. Cyclins bind to Cdks, activating the Cdks to phosphorylate other molecules.

- **Cyclins**

Cyclins are named such because they undergo a constant cycle of synthesis and degradation during cell division. When cyclins are synthesized, they act as an activating protein and bind to Cdks forming a cyclin-Cdk complex. This complex then acts as a signal to the cell to pass to the next cell cycle phase. Eventually, the cyclin degrades, deactivating the Cdk, thus signaling exit from a particular phase. There are two classes of cyclins : mitotic cyclins and G1 cyclins.

- **G1 cyclins**

G1 cyclins bind to Cdk proteins during G1. Once bound and activated, the Cdk signals the cell's exit from G1 and entry into S phase. When the cell reaches an appropriate size and the cellular environment is correct for DNA replication, the cyclins begin to degrade. G1 cyclin degradation deactivates the Cdk and leads to entry into S phase.

- **Mitotic Cyclins**

Mitotic cyclins accumulate gradually during G2. Once they reach a high enough concentration, they can bind to Cdks. When mitotic cyclins bind to Cdks in G2, the resulting complex is known as Mitosis-promoting factor (MPF). This complex acts as the signal for the G2 cell to enter mitosis. Once the mitotic cyclin degrades, MPF is inactivated and the cell exits mitosis by dividing and re-entering G1. The cellular signals that we described earlier (cell size, completion of DNA replication, and cellular environment) provide the signals that regulate the synthesis and degradation of cyclins.

5.6 Cell cycle checkpoints

The cell cycle has key checkpoints. When the cell receives key signals or information via **feedback regulation,** the cell can begin the next phase of the cell cycle. The cell can also receive signals that delay passage to the next phase of the

cell cycle. These signals allow the cell to complete the previous phase before moving forward. Three key checkpoints are the cell growth (G1) checkpoint, the G2 checkpoint, and the mitosis checkpoint. The DNA synthesis checkpoint is another checkpoint (Fig 5.10).

The cell growth (G1) checkpoint allows the cell to proceed into the S phase of the cell cycle and continue on to divide, or delay division, or enter a resting stage. The cell spends most of the cycle in the G1 phase. G1 is where the cell carries out its main functions. If the cell has performed its functions and has grown to significant size to be divided in half, key proteins will signal the cell to proceed to the S phase and stimulate DNA replication to begin. If the cells are not to divide, such as some muscle and nerve cells, the cell will stop at this checkpoint and move into a resting phase, G_0. Some cells may stay in this resting period permanently, never dividing.

The second checkpoint is located at the end of G_2 phase. Passing this checkpoint triggers the start of the mitosis. If this checkpoint is passed, the cell initiates the many molecular processes that signal the beginning of mitosis.

The mitosis checkpoint determines the end of one cycle and the beginning of the next. This checkpoint occurs at the point in metaphase where all the chromosomes should have alligned at the metaphase plate. This checkpoint signals the beginning of anaphase, allowing the cell to complete mitosis and prepare for the beginning of G1 of the next cell cycle.

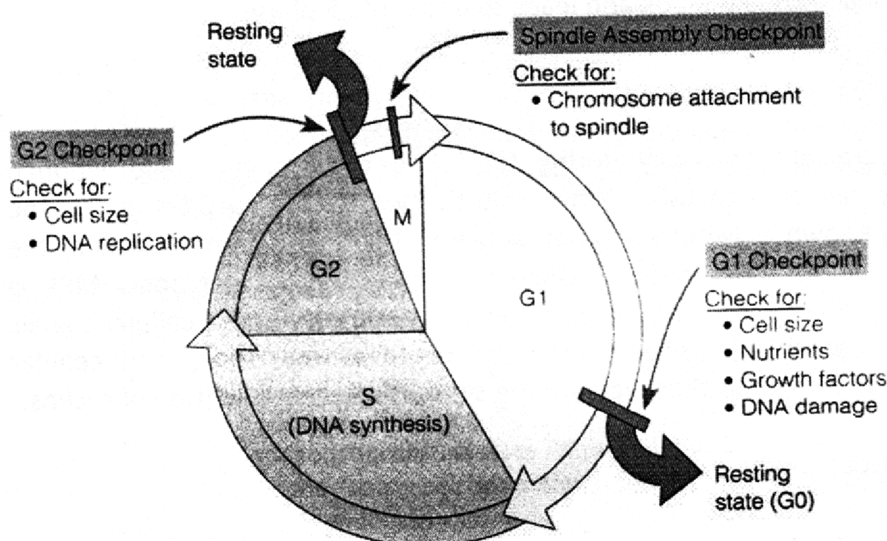


Fig. 5.10 : Showing cell cycle checkpoints

The DNA synthesis (S) checkpoint determines if the cell is ready for mitosis. DNA repair enzymes check the replicated DNA at this point. If the checkpoint is passed, the many molecular mechanisms and processes needed for mitosis will begin.

5.7 Summary

(i) Actively dividing euakryote cells pass through a series of stages known collectively as the **cell cycle :** two gap phases (G1 and G2) :an S (for synthesis) phase, in which the genetic material is duplicated ; and an M phase, in which mitosis partitions the genetic material and the cell divides.

(ii) Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell. Chomosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome. In actively dividing animal cells, the whole process takes about one hour.

(iii) Meiosis is the form of eukaryotic cell division that produces haploid sex cells or gametes (which contain a single copy of each chromosome) from diploid cells (which contain two copies of each chromosome). The process takes the form of one DNA replication followed by two successive nuclear and cellular divisions (Meiosis I and Meiosis II). As in mitosis, meiosis is preceded by a process of DNA replication that converts each chromosome into two sister chromatids.

(iv) A check point is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favourable.

(v) Damage to DNA and other external factors are evaluated at the G1 checkpoint; if conditions are inadequate, the cell will not be allowed to continue to the S phase of interphase.

(vi) The G2 checkpoint ensures all of the chromosomes have been replicated and that the replicated DNA is not damaged before cell enters mitosis.

(vii) The M checkpoint determines whether all the sister chromatids are correctly attached to the spindle microtubules before the cell enters the irreversible anaphase stage.

5.8 Model questions

1. Describe the cell cycle, including different stages.
2. Discuss three check points of cell cycle.
3. Describe the events of mitosis.
4. Describe the events of meiosis.
5. How cell cycle is regulated ?
6. What is cytokinesis ?

Unit-6 □ Nucleic Acids

Structure

6.1. Objectives

6.2. Introduction

6.3. Structure of DNA/RNA molecule

6.3.1 Structure of Polynucleotide Chain

6.3.1.1 Pentose sugar

6.3.1.2 Phosphate group

6.3.1.3 Nitrogenous bases

6.3.1.4 Polynucleotide chains

6.4. Watson and Crick model of DNA

6.4.1 The components of DNA

6.4.2 Chargaff's rules

6.4.3 The structure of DNA (Watson and Crick's model)

6.4.3.1 Antiparallel orientation

6.4.3.2 Right-handed helix

6.4.3.3 Base pairing

6.5. The impact of the double helix

6.6. Summary

6.7. Model questions

6.1 Objectives

This part of the Cell and Molecular Biology helps the reader to have a concise account about nucleic acids. After completion of the topic the reader will realize

✓ The structure of DNA

✓ The structure of RNA

✓ The model of DNA

6.2 Introduction

Nucleic acids are the most important macromolecules in the cells of all organisms. In the beginnning of the 20th century it was shown that chromosomes (consisting mainly of nucleic acids and proteins) within a cell carry the hereditary factors. However, for several decades, we did not know whether nucleic acids or proteins are the carriers of genetic information. In 1940s and 1950s, several experiments demonstrated that nucleic acids are the genetic material within the cells.

Chromosomes mainly consist of nucleoproteins having two components, nucleic acids and proteins. One of these should obviously constitute the genetic material. Transformation experiments of **Griffith** (1928) demonstrated that nucleic acids and not the proteins contain the genetic information. Further, repetition of Griffth's experiment by **Avery, Macleod and McCarthy** (1944) confirmed that DNA and not the proteins is the genetic material. In 1952, **Hershey** and **Chase** reported results of experiments which demonstrated that only DNA of the phage enters host cells and that this DNA carries all the genetic information necessary for assembly of new phage progeny.

6.3 Structure of DNA/RNA molecule

The genetic material in most organism is DNA or Deoxyribonucleic acid ; whereas in some viruses, it is RNA or Ribonucleic acid. A DNA molecule consists of two polynucleotide chains i.e. chains with multiple nucleotides. Let's understand the structure of this chain in detail.

6.3.1 Structure of Polynucleotide Chain

A nucleotide is made of the following components :

- **Pentose sugar**—A pentose sugar is a 5-carbon sugar. In case of DNA, this sugar is deoxyribose whereas, in RNA, it is ribose.
- **Phosphate group**
- **Nitrogenous base**—These can be of two types—Purines nd Pyrimidines. Purines include Adenine and Guanine whereas pyrimidines include Cytosine and Thymine. In RNA, thymine is replaced by Uracil.

Nitrogenous base + pentose sugar (via N-glycosidic linkage) = Nucleoside.

Nucleoside + phosphate group (via phosphoester linkage) = Nucleotide.

Nucleotide + Nucleotide (via 3'-5' phosphodiester linkage) = Dinucleotide.

Many nucleotides linked together = Polynucleotide.

A polynucleotide has a free phosphate group at the 5' end of the sugar and this is called the 5' end. Similarly, the sugar also has a free 3'-OH group at the other end of the polynucleotide which is called the 3' end. The backbone of a polynucleotide chain consists of pentose sugars and phosphate groups ; whereas the nitrogenous bases project out of this backbone.

6.3.1.1 Pentose Sugar

Each nucleic acid has a 5-carbon (pentose) sugar as a part of its polymer backbone. For DNA this sugar is deoxyribose, while for RNA it's ribose : (Fig. 6.1).

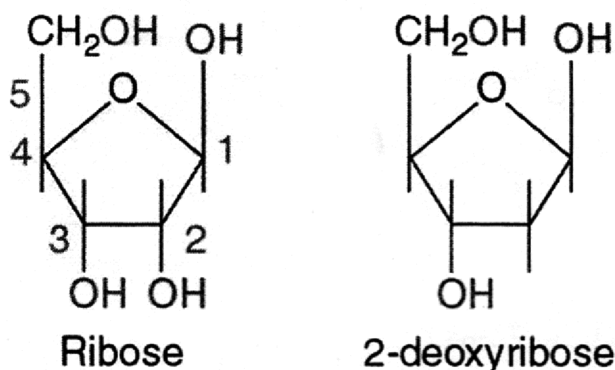


Fig. 6.1 : Chemical structure of sugars in nucleic acids

Notice the numbering convention here. For now, since there are no attachments to these sugars, the carbons are numbered 1 through 5. Later, when we attach the base, the sugar carbon numbers will change to 1' through 5'. In a nucleotide, the sugar occupies a central position, with the base attached to its 1' carbon and the phosphate group (or groups) attached to its 5' carbon.

The difference between ribose and deoxyribose is the presence of the OH on the 2(2') position. DNA is 2'-deoxy. Because of this difference, RNA is an inherently less stable molecule than DNA. For instance, RNA can readily be hydrolyzed at high pH (> 8.0). The reaction involves the 2' OH group of the polymer.

6.3.1.2 Phosphate group

The phosphoric acid (H_3PO_4) is biologically called phosphate. The other repeating part of the DNA backbone is a phosphate group. Phosphoric acid (Fig. 6.2) has three

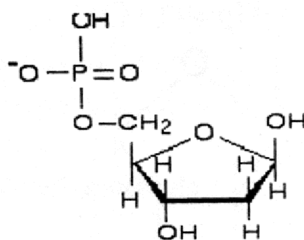


Fig. 6.2 : Phosphoric acid with ribose sugars

reactive hydroxyl groups (–OH) of which two are involved in forming sugar phosphate backbone of both DNA and RNA. A phosphate group is attached to the sugar molecule in place of the -OH group on the 5' carbon.

6.3.1.3 Nitrogenous bases

The nitogenous bases of nucleotides are organic (carbon-based) molecules made up of nirogen-containing ring structures.

Each nucleotide in DNA contains one of four possible nitrogenous bases : adenine (A), guanine (G) cytosine (C), and thymine (T). Adenine and guanine are **purines**, meaning that their structures contain two fused carbon-nitrogen ring. RNA nucleotides may also bear adenine, guanine and cytosine bases, but instead of thymine they have another pyrimidine base called uracil (U).

As shown in the figure 6.3 above, each base has a unique structure, with its own set of functional groups attached to the ring structure.

In molecular biology shorthand, the nitrogenous bases are often just referred to by their one-letter symbols, A, T, G, C, and U. DNA contains A, T, G, and C, while RNA contains, A, U, G, and C (that is, U is swapped in for T).

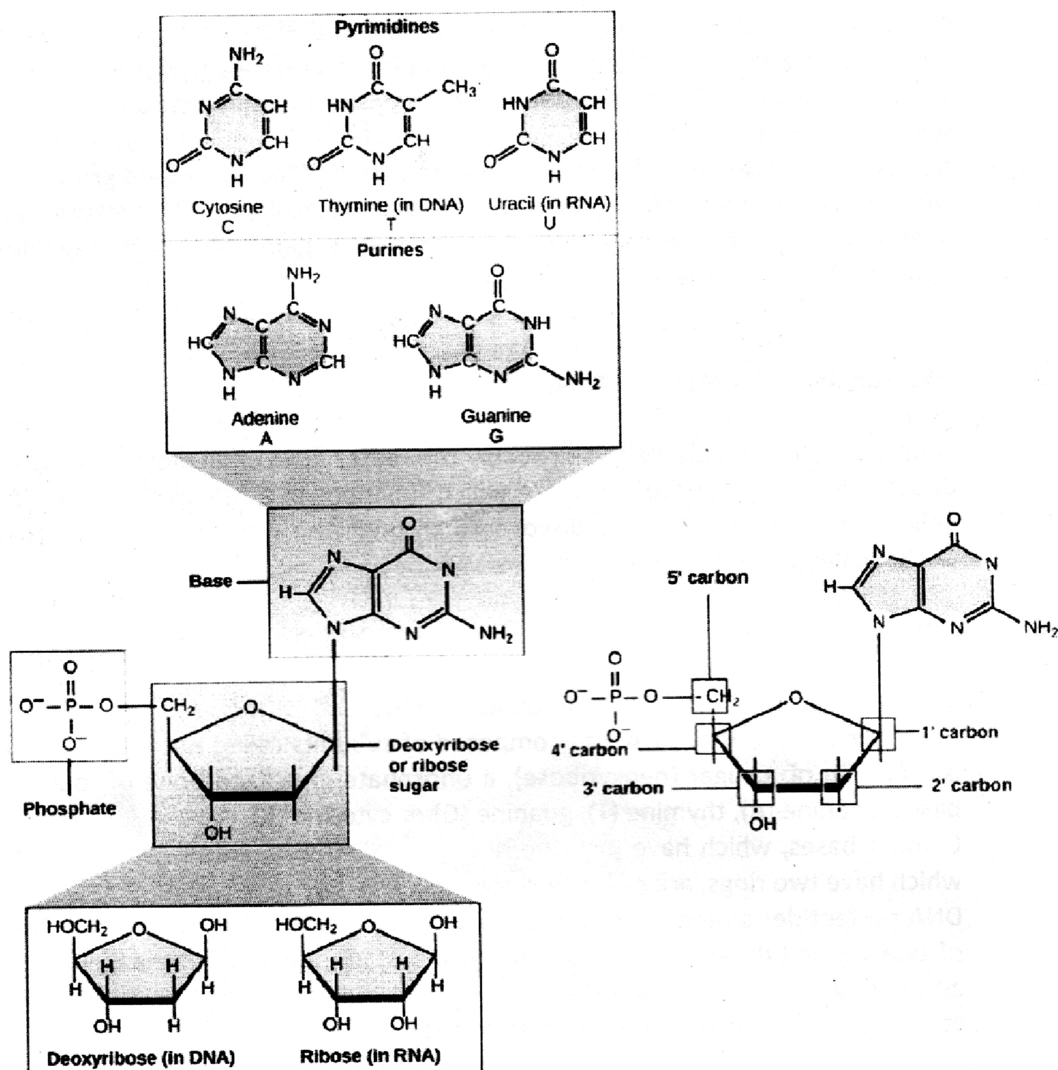


Fig. 6.3 : Showing the chemical configuration of the bases, phosphates, nucleotides, base sugar linkage and sugar

6.3.1.4 Polynucleotide chains

A consequence of the structure of nucleotides is that a polynucleotide chain has **directionality**—that is, it has two ends that are different from each other. At the **5′ end,** or beginning, of the chain, the 5′ phosphate group of the first nucleotide in the chain sticks out. At the other end, called the **3′ end**, the 3′ hydroxyl of the last

nucleotide added to the chain is exposed. DNA sequences are usually written in the 5′ to 3′ direction, meaning that the nucleotide at the 5′ end comes first and the nucleotide at the 3C end comes last.

As new nucleotides are added to a strand of DNA of RNA, the strand grows at the 3C end, with the 5C phosphate of an incoming nucleotide attaching to the hydroxyl group at the 3C end of the chain. This makes a chain with each sugar joined to its neighbors by a set of bonds called a **phosphodiester linkage.**

6.4 Watson and Cricks model of DNA

Today, the DNA double helix is probably the most iconic of all biological molecules. The double helix is a beautiful structures which functions in a remarkable way.The double-helical structures of DNA was discovered through the work of James Watson, Francis Crick, Rosalind Franklin, and others.

6.4.1 The components of DNA

From the work of biochemist Phoebus, and others, scientists in Watson and Crick's time knew that DNA was composed of subunits called **nucleotides.** A nucleotide is made up of a sugar (deoxyribose), a phosphate group, and one of four nitrogenous bases : adenine (A), thymine (T), guanine (G) or cytosine (C).

C and T bases, which have just one ring, are called **pyrimidines,** while A and G bases, which have two rings, are called **purines.**

DNA nucleotides assemble in chains linked by covalent bonds, which from between the of one nucleotide and the phosphate group of the next. This arrangement makes an alterning chain of deoxribose sugar and phosphate groups in the DNA polymer, a structure known as the **sugar-phosphate backbone.**

6.4.2 Chargaff's rules

One other key piece of information related to the structure of DNA came from Austrian biochemist Erwin Chargaff. Chargaff analyzed the DNA of different species, determining its composition of A, T, C, and G bases. He made several key observations :

- A, T, C, and G were not found in equal quantities (as some models at the time would have predicted)
- The amounts of the bases varied among species, but not between individuals of the same species.

- The amount of A always equalled the amount of T, and the amount of C always equalled the amount of G (A = T and G = C)

These findings, called **Chargaff's rules,** turned out to be crucial to Watson and Crick's model of the DNA double helix.

6.4.3 The structure of DNA (Watson and Crick's model)

The structures of DNA, as represented in Watson and Crick's model, is a double stranded, antiparallel, right-handed helix. The sugar-phosphate backbones of the DNA strands make up the outside of the helix, while the nitrogenous bases are found on the inside and from hydrogen-bonded pairs that hold the DNA strands together.

6.4.3.1 Antiparallel orientation

Double-stranded DNA is an **antiparallel** molecule, meaning that it's composed of two strands that run alongside each other but point in opposite directions. In a

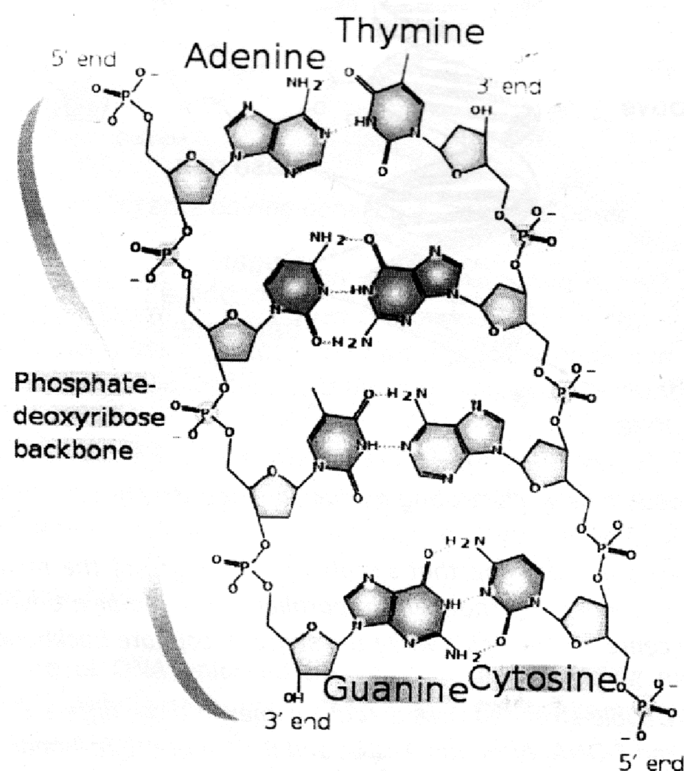


Fig. 6.4 : Illustration of the antiparallel structures of DNA.

double-stranded DNA molecule, the 5C end (phosphate-bearing end) of one strand alingns with the 3C end (hydroxyl-bearing end) of its partner, and vice versa (Fig. 6.4).

A short segment of DNA double helix is shown composed of two DNA strands held together by hydrogen bonds between the bases. The strands on the left has a phosphate group exposed at its top (5C end) and a hydroxyl group exposed at its bottom (3C end). The strands on the right has the opposite orientation, with a phosphate group exposed at its bottom (5C end) and hydroxyl exposed at its toip (3C end). The 5C end of one stand thus ends up next to the 3C end of the other, and vice versa.

6.4.3.2 Right-handed helix

In Watson and Crick's model, the two strands of DNA twist around each other to form a **right-handed helix.** All helices have a handedness, which is a property that describes how their grooves are oriented in space (Fig. 6.5).

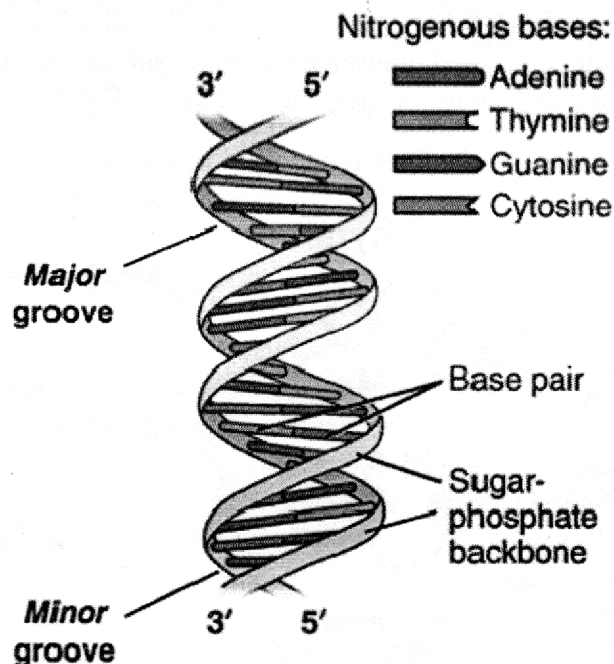


Fig. 6.5 : Showing a DNA double helix, illustrating its right-handed structure.

The major groove is a wider gap that spirals up the length of the molecule, while the minor groove is a smaller gap that runs in parallel to the major groove. The base

pairs are found in the center of the helix, while the sugar-phosphate backbones run along the outside.

Not necessarily, Double-stranded DNA actually comes in three different forms, known as A-DNA, B-DNA, and Z-DNA. Although A-DNA and B-DNA are right-handed helices, Z-DNA is a left-handed helix.

The twisting of the DNA double helix and the geometry of the bases creates a wider gap (called the **major groove)** and a narrower gap (called the **minor groove)** that run along the length of the molecule, as shown in the figure above. These grooves are important binding sites for proteins that maintain DNA and regulate gene activity.

6.4.3.3 Base pairing

In Watson and Crick's model, the two strands of the DNA double helix are held together by hydrogen bonds between nitrogeneous bases on opposite strand. Each pair of bases lies flat, forming a "rung" on the ladder of the DNA molecule.

Base pairs aren't made up of just any combination of bases. Instead, if there is an A found on one strand, it must paired with a T on the other (and vice versa). Similarly, a G found on one strand must always have a C for a partner on the opposite strand. These A-T and G-C associations are known as **complementary base pairs (Fig. 6.6).**

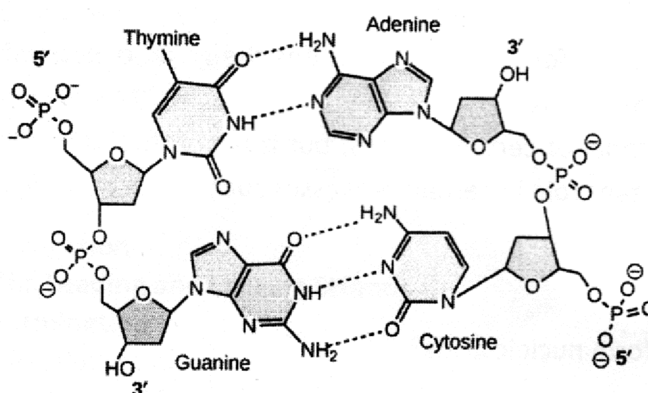


Fig. 6.6 : Diagram illustrating base pairing between A-T and G-C bases

A and T are found opposite to each other on the two strands of the helix, and their functional groups form two hydrogenbonds that hlol the stands together. Similarly, G and C are found oppoiste to each other on the two strands, and their functional groups form three hydrogen bonds that hold the strands together.

Although Watson and Crick's original model proposed that there were two hydrogen bonds between the bases of each pair, we know today that G and C form an additional bond (such that A-T pairs form two hydrogen bonds total, while G-C pairs form three.

6.5 The impact of the double helix

The structure of DNA unlocked the door to understanding many aspects of DNA's function, such as how it was copied and how the information it carried was used by the cell to make proteins.

6.6 Summary

(i) Nucleic acid, naturally occurring chemical compound that is capable of being broken down to yield phosphoric acids, sugars, and a mixture of organic bases (purines and pyrimidines).

(ii) Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing.

(iii) The two main classes of nucleic acids are deoxyribonulcleic acid (DNA) and ribonucleic acid (RNA).

(iv) DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses.

(v) RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

6.7 Model questions

1. How do nucleotides bond to form nucleic acids ?
2. State the structure of DNA.
3. State the structure of RNA.
4. What is Watson and Crick model ?
5. What are the components of DNA ?
6. Distinguish nucleosides with nucleotides.

Unit-7 □ DNA Replication

Structure / Contents

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7.2. Introduction

7.3. Basic idea of DNA replication

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7.7. RNA priming

7.8. Replication of circular dsDNA

7.9. Replication of linear DNA

7.10. Replication of telomeres

7.11. Telomerase and aging

7.12. Summary

7.13. Model questions

7.1 Objectives

This interesting topic in molecular biology will help the reader to have a summarizing account of DNA replication. After completion of the topic the reader will be able understand.

- How DNA replication takes place in both prokaryotes and also in eukaryotes
- About the enzymes and proteins involved in DNA replication
- Role played by DNA polymeres enzyme
- Replication of circular and linear dis-DNA and
- Replication of telemeres

7.2 Introduction

One of the most important properties of DNA functioning is that it can make exact copies of itself forming the basis for transformation of hereditary characters it controls. This process is called replication. Replication of a DNA molecules gives rise to two identical daughter molecules, fulfilling the criterion of autocatalytic function.

Every time a cell divides–just prior to cell division, the DNA of the cell must duplicate, so that each of the two newly forming cells receives exactly the same complement of DNA and therefore the same set of genes—both quantitatively and qualitatively— as was content in the parent. Replication of the DNA leads to the duplication of the entire chromosome once for every cell division cycle. As a result each chromosome exists as a pair of chromotids joined together by a centromere and they are separated equally during anaphase into newly forming daughter cells. Hence the total amount of DNA is similar kind of cells and the chemical nature, remain constant in every generation.

7.3 Basic idea of DNA replication

The mechanism of DNA replication is best understood in the Watson— Crick model of DNA. The two chains of DNA are united by hydrogen bonds. When the hydrogen bonds break the two chains part and unwind. It starts from one end of DNA to the other ends of DNA. One by one each purine base separates from its partner

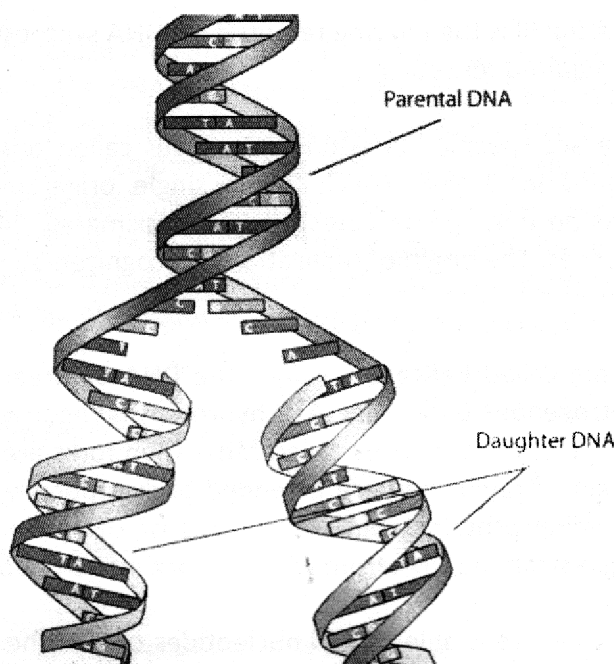


Fig. 7.1 : Showing DNA replication (General view)

pyrimidine base in each base pair, but the sugar—phosphate backbones do not break. It looks much like a zipper opening up. Each parential chain of the DNA then serves as template or mould for the syntehsis of its complementary new chain on itself and forms two daughter identical DNA double helices (Fig. 7.1).

7.4 DNA replication

7.4.1 Mode of replication

DNA replication uses a semi-conservative method that results in a double-stranded DNA with one parential strandand a new daughter strand. In semi-conservative replication, each of the two parential DNA strands would act as a template for new DNA strands to be synthesized, but after replication, each parential DNA strand would base pair with the complementary newly-synthesized strand just synthesized, and both double-stranded DNAs would include one parential or "old" strand and one daughter or "new" strand.

7.4.2 In prokaryotes

DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, which adds nucleotides one by one to the grwoing DNA chain that are this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known : **DNA pol I, DNA pol II, and DNA pol III.** DNA pol III is the enzyme required for DNA synthesis ; DNA pol I and DNA pol II are primarily required for repair.

There are specific nucleotide sequences called origins of replication where replication begins. In E. call, which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site.

An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks at the origin of replication are extended bi-directionally as replication proceeds. Single-strand binding proteins coat the strands of DNA near the replicatioan fork to prevent the single-stranded DNA from winding back into a double helix.

DNA polymerase is able to add nucleotides only in the 5C to 3C direction (a new DNA strand can be extended only in this direction). It also requires a free 3C OH group to which it can add nucleotides by forming a phosphodiester bond between the 3C-OH end and the 5C phosphate of the next nucleotide. This means that it cannot add nucleotides if a free 3C-OH group is not available.

Another enzyme, RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA, priming DNA synthesis. A primar provides the free 3C-OH end to start replication. DNA polymerase then extends this RNA primer, adding nucleotides one by one that are complementary to the template strand.

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can ony extend in the 5C to 3C direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel ; that is, one strand is in the 5C to 3C direction and the other is oriented in the 3 to 5 direction. One strand (the leading strand), completmentary to the 3 to 5 parental DNA strand,

is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. The other strand (the lagging strand), complementary to the 5 to 3 parental DNA, is extended away from the replication fork in small fragments known as **Okazaki fragments,** each requiring a primar to start the synthesis. Okazaki fragments are named after the japanese scientist who first discovered them.

The leading strand can be extended by one primar alone, whereas the lagging strand needs a new primar for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3 to 5, while that of the leading strand will be 5 to 3. The sliding clamp (a ring-shaped protein that binds to the DNA) holds the DNA polymerase in place as it continuous to add nucleotides.

Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up ; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, while the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly-synthesized DNA (that replaced the RNA primer) and the previously-synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of phosphodiester linkage between the 3′-OH end of one nucleotide and the 5C phosphate end of the other fragment.

7.4.2.1 Summary of DNA replication in E. Coli

How the enzymes and protein involved n replication work together to synthesize new DNA. summarized below (Fig. 7.2)

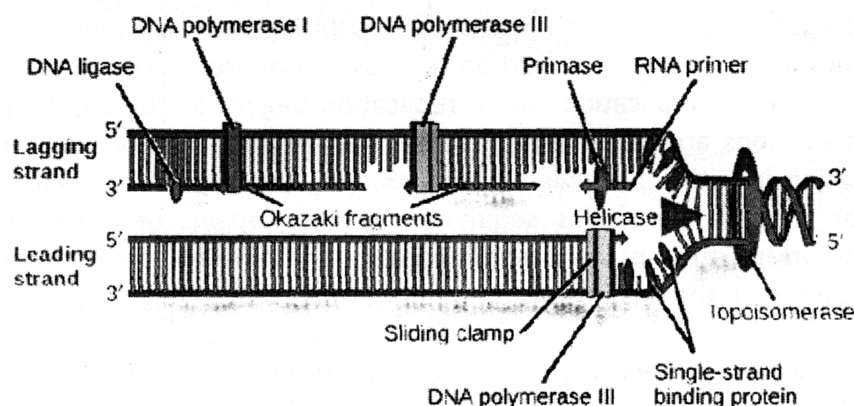


Fig. 7.2 : Showing involvement of proteins and enzymes in DNA replication in Prokaryotes

A replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteinsbind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer, DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the logging strand, DNA is synthesized in short sketches colled Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule.

- **Helicase** opens up the DNA at the replication fork.
- **Single-strand binding proteins** coat the DNA around the replication fork to prevent rewinding of the DNA.
- **Topoisomerase** works at the region ahead of the replication fork to prevent supercoiling
- **Primase** synthesizes RNA primers complementary to the DNA strand.
- **DNA polymerase III** extends the primers, adding on to the 3C end, to make the bulk of the new DNA
- RNA primers are removed and replaced with DNA by **DNA polymerase I**
- The gaps between DNA fragments are sealed by **DNA ligase.**

7.4.3 In Eukaryotes

Because eukjaryotic genomes are quite complex, DNA replication is a very complicated process that involves several enzymes and other proteins. It occurs in three main stages initiation, elongation, and termination.

7.4.3.1 Initiation

Eukaryotic DNA is bound to proteins known as histones to form structures called nulceosoms. During initiaton, the DNA is made accessible to the proteins and enzymes involved in the replication process. There are specific chromosomal locations called origins of replication where replication begins. In some eukaryotes, like yeast, these locations are defined by having a specific sequence of basepairs to which the replication initiation proteins bind. In other eukaryotes, like humans, there does not

appear to be a consensus sequence for their origins of replication. Instead, the replication initiation proteins might identify and bind to specific modifications to the nucleosomes in the origin region.

Certain proteins recognize and bind to the origin of replication and then allow the other proteins necessary for DNA replication to bind the same region. The first proteins to bind the DNA are said to "recruit" the other protein. Two copies of an enzymes called **helicase** are among the proteins recruited to the origin. Each helicase unwinds and separates the DNA helix into single-stranded DNA.

As the DNA opens up, Y-shaped structures called replication forks are formed. Because two helicases bind, two replication forks are formed at the origin of replication ; these are extended in both directions as replication proceeds creating a replication bubble. There are multiple origins of replication on the eukaryotic chromosome which allow replication to occur simultaneously in hundreds to thousands of locations along each chromosome.

7.4.3.2 Elongation

During elongation, an enzyme called DNA polymerase adds DNA nucleotides to the 3' end of the newly synthesized polynucleotide strand. The template strand specifies which of the four DNA nucleotides (A, T, C, or G) is added at each position along the new chain. Only the nucleotide complementary to the template nucleotide at the position is added to the new stand.

DNA polymerase contains a groove that allows it to bind to a single-stranded template DNA and travel one nucleotide at time. For example, when DNA polymerase meets an adenosine nucleotide on the template strand, it adds a thymidine to the 3' end of the newly synthesized strand, and then moves to the next nucleotide on the template strand. This process will continue until the DNA polymerase reaches the end of the template strand.

DNA polymerase cannot initiate new strand synthesis ; it only adds new nucleotides at the 3 end of an existing strand. All newly synthesized polynucleotide strands must be initiated by a specialized RNA polymerase called primase. Primase initiates polynucleotide synthesis and by creating a short RNA polynucleotide strand complementary to template DNA strand. This short stretch of RNA nucleotides is called the primar. Once RNA primer has been synthesized at the template DNA, primase exits and DNA polymerase extends the new strand with nucleotides complementary to the template DNA.

Eventually, the RNA nucleotides in the primer are removed and replaced with DNA nucleotides. Once DNA replication is finished, the daughter molecules are made entirely continuous DNA nucleotides, wth no RNA portions.

7.4.3.3 The leading and lagging Strands

DNA polymerase can only synthesize new strands in the 5 to 3 direction. Therefore, the two newly-synthesized strands grow in opposite directions because the template strands at each replication fork are antiparallel. The "leading strand" is synthesized continuously toward the replication fork as helicase unwinds the template double-stranded DNA.

The "lagging strand" is synthesized in the direction away from the replication fork and away from the DNA helicase unwinds. This lagging strand is synthesized in pieces because the DNA polymerase can only synthesize in the 5 to 3 direction, and so it constantly encounters the previously-synthesized new strand. The pieces are called Okazaki fragments, and each fragment begins with its own RNA primer.

7.4.3.4 Termination

Eukaryotic chromosomes have multiple origins of replication, which initiate replication almost simultaneously. Each origin of replication forms a bubble of duplicated DNA on either side of the origin of replication. Eventually, the leading strand of one replication bubble reaches the lagging strand of another bubble, and the lagging strand will reach the 5 end of the previous Okazaki fragment in the same bubble.

DNA polymerase halts when it reaches a section of DNA template that has already been replicated. However, DNA polymerase cannot catalyze the formation of a phosphodiester bond between the two segments of the new DNA strand, and it drops off. These unattached sections of the sugar-phosphate backbone in an otherwise full-replicated DNA strand are called nicks.

Once all the template nucleotides have been replicated, the replication process is not yet over. RNA primers need to be replaced with DNA, and nicks in the sugar-phosphate backbone need to be connected.

The group of cellular enzymes that remove RNA primers include the proteins FEN1(flap endonuclease 1) and RNase H. The enzymes FEN1 and RNase H remove RNA primers at the start of each leading strand and at the start of each Okazaki fragment, leaving gaps of unreplicated template DNA. Once the primers are removed,

a free-floating DNA polymerase lends at the 3' end of the preceding DNA fragment and extends the DNA over the gap. However, this creates new nicks (unconnected sugar-phosphate backbone).

In the final stage of DNA replication, the enzyme ligase joins the sugar-phosphate backbones at each nick site. After ligase has connected all nicks, the new strand is one long continuous DNA strand, and the daughter DNA molecule is complete.

7.5 DNA polymerase

One of the key molecules in DNA replication is the enzyme **DNA polymerase.** DNA polymerase are responsible for synthesizing DNA ; they add nucleotides one by one to the growing DNA chain, incorporating only those that are complementary to the template (Fig. 7.3).

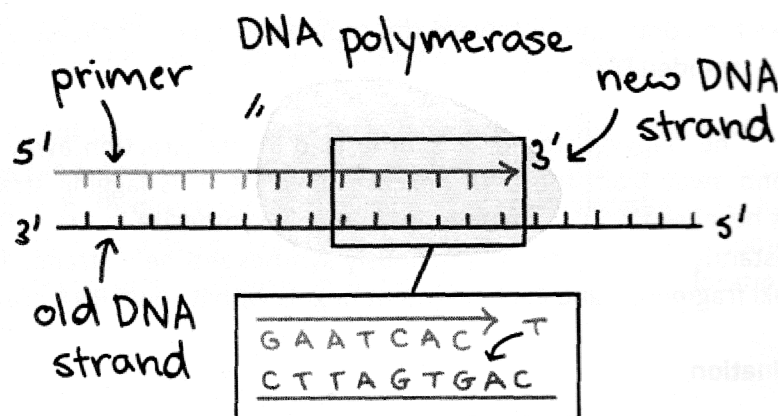


Fig. 7.3 : Showing DNA plymerase action

Here are some **key features** of DNA polymerases :

- They always need a template
- They can only add nucleotides to the 3 end of a DNA strand
- They can't start making a DNA chain from scratch, but require a pre-existing chain or short stretch of nucleotides called a **primer**.
- They **proof read,** or check their work, removing the vast majority of "wrong" nucleotides that are accidentally added to the chain.

7.5.1 Prokaryote DNA Polymerases

Prokaryotes contain five different types of DNA polymerase. These are described below.

Pol I

Polymerase I is a DNA repair enzyme from the family A polymerases that has a 5' to 3' and 3' to 5' activity. Pol I accounts for more than 95% of polymerase activity in *E.Coli*, although cells that lack this polymerase have been found and its activity can be replaced by the other four types of polymerase has a poor processivity rate, adding around 15 to 20 nucleotides per second. Pol I begins the process of DNA elongation at a point called the "origin of replication" and about 400 base pairs downstream of this point, Pol III takes over replication, which it performs at a much higher speed.

Pol II

Polymerase II is a DNA repair enzyme with a 3' to 5' exonuclease activity. Pol II is a family B polymerase and provides support to Pol III. When DNA acquires damage in the form of short gaps, which block Pol III activity, Pol II helps to remedy this problem by restarting DNA synthesis downstream of these gaps.

Pol III

This holoenzyme is the main polymerase in *E.Coli* DNA replication and is one of the family C polymerases. Polymerase III is made up of the clamp-loading complex, the beta sliding clamp processivity factor and the Pol III core. The core comprises three subunits – the a subunits which is the polymerase activity hub, the d subunits which is the exonucleotic proof reader, and the d subunit which may stabilize d. The core and the beta sliding clamp are present in duplicate, to allow for processing of both the leading and lagging DNA strands.

Pol IV

The enzyme belongs to the Y family fo DNA polymerases. Pol IV is an error-prone polymerase that has no 3 to 5 proofreading activity and is involved in mutagensis or the altering of DNA to give rise to a mutation. The enzyme is expressed by a gene (dinB) that is switched on when polymerases stall at the replication fork. This interferes with the processivity of Pol III which acts as a checkpoint, stopping replication and allowing time for DNA to be repaired. Cells that lack dinB are at an increased risk of developing mutations caused by agents that damage DNA.

Pol V : Pol V also belongs to the Y family of polymerases and allows DNA damage to be bypassed in order for replication to continue.

7.5.2 Eukaryote DNA polymerases

There are four DNA polymerases in eukaryotes called a, b, g and d.

Each polymerase has specific role (s) in DNA replication and repair, shown in the table below :

Polymerase	Function	Exonuclease activity
α	Synthesizes the RNA primer initiations DNA synthesis ad the lagging strand	None
β	Repair DNA	None
γ	Replicate mitochodrial DNA	3' to 5'
δ	Synthesizes the leading strand, filling	3' to 5'
ε	DNA gaps after removal of primar Repair DNA	3' to 5'

7.5.3 Application

Polymerase does not create a novel DNA strand from scratch. Instead it synthesizes a new strand of DNA based on the template of two existing DNA strands. It does this with the help of another enzyme, called helicase, which unwinds the double helix structure of the DNA molecule into two single DNA strands.

In addition to a template strand, polymerases require a primer to function. This is a fragment of nucleic acid that serves as the starting point for DNA replication. The primer, often a short strand of RNA, needs to be complementary to the template.

DNA polymerase works by sliding along the single strand template of DNA reading its nucleotide bases as it goes along and inserting new complementary nucleotides into the primer so as to make a sequence complementary to the template.

DNA polymerase is thought to be able to replicate 749 nucleotides per second. By the end of the replication process two new DNA molecules will have been made, each identical to the other and to the original parent molecule. Such accurate

replication is helped by the fact that DNA polymerase has an inbuilt capacity to detect and correct any mistakes it makes inthe replication process.

7.6 Primosome

Primosome is a complex of two proteins a primase and a helicase that initiates RNA primers on the lagging DNA strand during DNA replication. This can characterized by :

- A group of proteins, that bind to replication origin site and synthesize primers to initiate replication, is called Primosomes.
- Constituents of Primosomes differ from one system to the other.
- In E-coli assembly Dna-A binding leads to Dna-B and Primase G binding all together form the primosome complex.
- In the case of phiX174 phage DNA, the primosome complex is made up of Pri-A, Pri-B, Pri-C, Dna-B, Dna-C and Dna-G.
- The said components assemble at a particular site called "pas" ; means primosome assembling site.
- Such assemblies are found in Origin-C of E-coli, Col E-1 Ori, phiX174 phage origin (phi ori), m13 phage DNA and few other plasmid DNAs for that matter all chromosomal DNAs have their own specific origins and their own specific primosomes.
- Based on the sequence, structures and the components assembled to form primosomes ; they are grouped in to DNA-A dependent and Pri-A dependent primosomes.

Primosomes are complex of proteins that produce RNA primer at the time of DNA replication. Enzymatic analyses of primosome assembly at chromosomal and plasmid origins as well as that at single-stranded DNA replication origins revealed that the presence of two distinct primosomes in Escherichiacoli for primer RNA synthesis and duplex unwinding.

The DnaA-dependent primosome is assembled at oriC, the chromosomal origin of *Escherichia coli*, as well as at the A site, a single-stranded DNA hairpin containing a DnaA box sequence within its stem.

In contrast, PriA protein recognizes a small stem-loop, called n-pass (primosome assembly site), and initiates assembly of the phiX174-type PriA-primosome in

conjunction with other prepriming proteins. Genetic analyses of the prempriming proteins required specifically for the latter primosome strongly suggested that it is responsible for RecA-dependent, DnaA/oriC-independent replication.

Furthermore, primosome assembly in replication of various plasmids may also be classified into either DnaA-dependent of PriA-dependent types. Escherichia coli possess two distinct, mutually exclusive primosomes which are differentially utilized by the chromosome as well as by the plasmids. PriA protein appears to be conserved in a wide range of prokaryotic species, and the same will be discussed and its possible biological function ex. PriA-dependent primosome in responses to DNA damages.

7.7 RNA priming

RNA priming means by which the synthesis of DNA strands is initiated, that is by which DNA polymerase is provided with a 3' hydroxyl group to which incoming nuceotides are added. Thus a primer is a short single strand of RNA or DNA (generally about 18-22 bases) that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes, that catalyze this process, DNA polymerase, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3'-end of the primer, and copies the opposite strand.

RNA priming is catalyzed by the enzymes primase, which is a DNA-templated RNA polymerase. While the resulting RNA initially forms a portion of the synthesized DNA strand at some point that RNA primer must be removed and replaced with DNA.

This process, both priming and removal, occurs repeatedly on the lagging strand in association with Okazaki fragment synthesis.

7.8 Replication of circular dsDNA

This kind of replication is used by many dsDNA viruses (Fig. 7.4)

1. DNA replication begins at specific locations in the genome, called "origins". A viral endonuclease creates a nick in the origin of replication.
2. The replication machinery assembles with the DNA polymerase on the 3' extremity.

3. The DNA polymerase and associated factors begins to proceed to a strand displacement synthesis, producing a concatemer linear single stranded DNA with one genome copy per turn of replication. On the concatemer strand, Okazaki fragments are elongated after sequencial RNA primer synthesis by the primase, theus turning it into dsDNA.

   The concatemer strand RNA primer is removed and okazaki fragments ligated.

4. The replication forks go on and produce a long linear concatemer which will be processed into linear genomes and encapsidated.

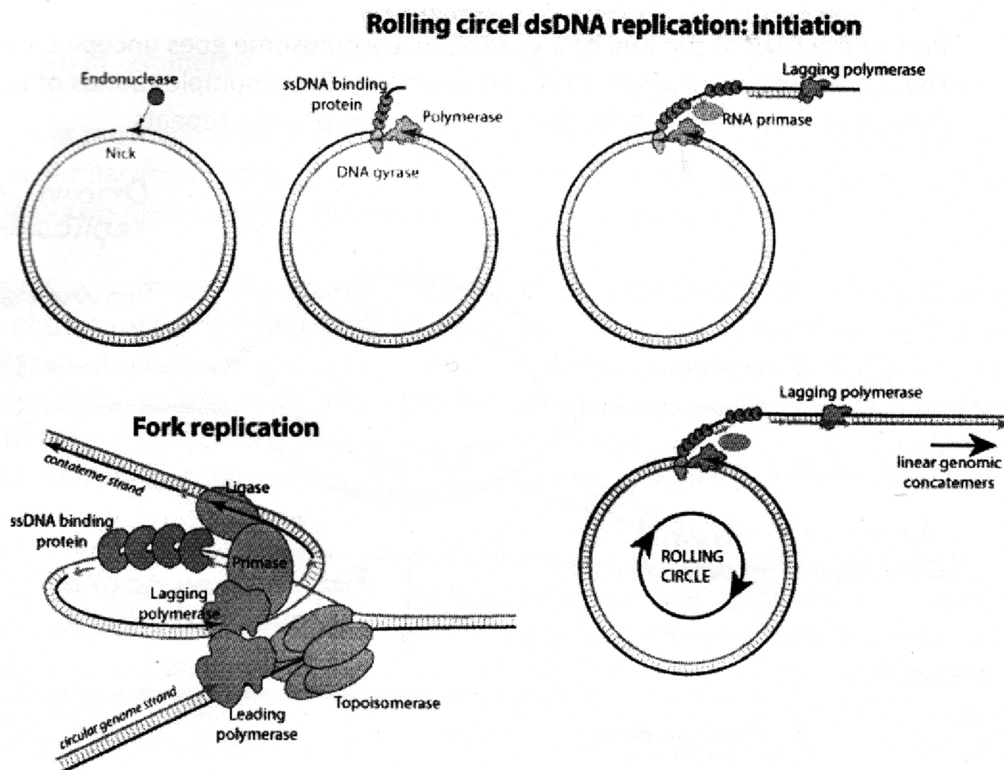


Fig. 7.4 : Showing circular dsDNA replication

7.9 Replication of linear DNA

Unlike bacterial chromosomes, the chromosomes of eukaryotes are linear (rod-shaped), meaning that they have ends. These ends pose a problem for DNA

replication. The DNA at the very end of the chromosome cannot be fully copied in each round of replication, resulting in a slow, gradual shortening of the chromosome.

Why is this the case ? When DNA is being copied, one of the two new strands of DNA at a replication fork is made continuously and is called the **leading strand.**

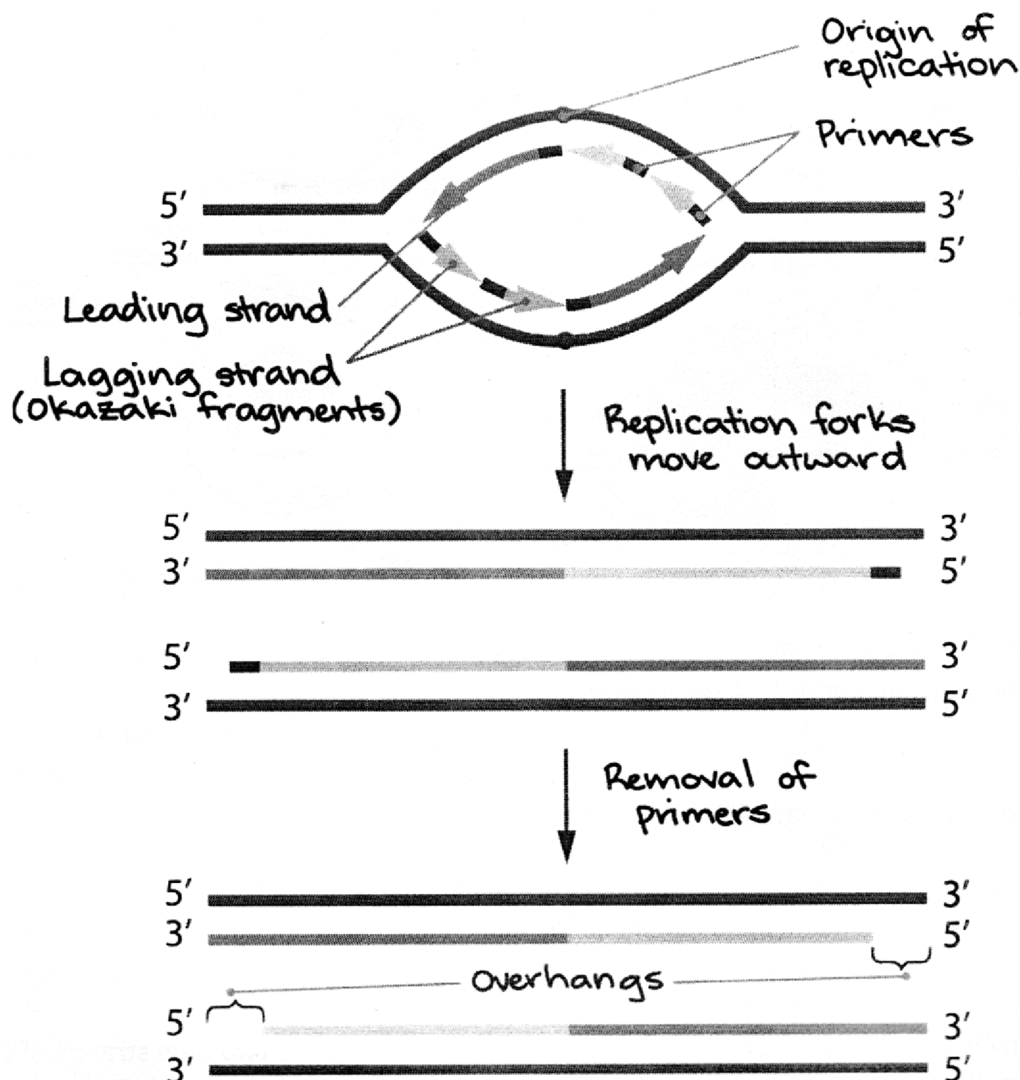


Fig. 7.5 : A real eukaryotic chromosome would have multiple origins of replication and multiple replication bubbles, but the end-replication problem would be the same as shown above.

The other strand is produced in many small pieces called Okazaki fragments, each of which begins with its own RNA primer, and is known as the **lagging strand**.

In most cases, the primers of the Okazaki fragments can be easily replaced with DNA and the fragments connected to form an unbroken strand. When the replication fork reaches the end of the chromosome, however, there is (in many species, including humans) a short stretch of DNA that does not get covered by an Okazaki fragment— essentially, there's no way to get the fragment started because the primer would fall beyond the chromosome end. Also, the primer of the last Okazaki fragment that does get made can't be replaced with DNA like other primers. Part of the DNA at the end of a eukaryotic chromosome goes uncopied in each round of replication, leaving a single-stranded overhang. Over multiple rounds of cell division, the chromosome will get shorter and shorter as this process repeats.

In human cells, the last RNA primer of the lagging strand may be positioned as much as 70 to 100 nucleotides away from the chromosome end. Thus, the single-stranded overhangs produced by incomplete end replication in humans are fairly long, and the chromosome shortens significantly with each round of cell division.

7.10 Replication of telomeres

The ends of the linear chromosomes are known as telomeres ; repetitive sequences that code for no particular gene. These telomeres protect the important genes from being deleted as cells divide and as DNA strands shorten during replication.

In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times. After each round of DNA replication, some telomeric sequences are lost at the 5' end of the newly synthesized strand on each daughter DNA, but because these are noncoding sequences, their loss does not adversely affect the cell. However,even these sequences are not unlimited. After sufficient rounds of replication, all the telomeric repeats are lost, and the DNA risks losing coding sequences with subsequent rounds.

The discovery of the enzyme telomerase helped in the understanding of how chromosome ends are maintained. The telomerase enzyme attaches to the end of a chromosome and contains a catalytic part and a built-in RNA template. Telomerase adds complementary RNA bases to the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficient elongated. DNA polymerase adds the

complementary nucleotides to the ends of the chromosomes ; thus, the ends of the chromosomes are replicated (Fig. 7.6).

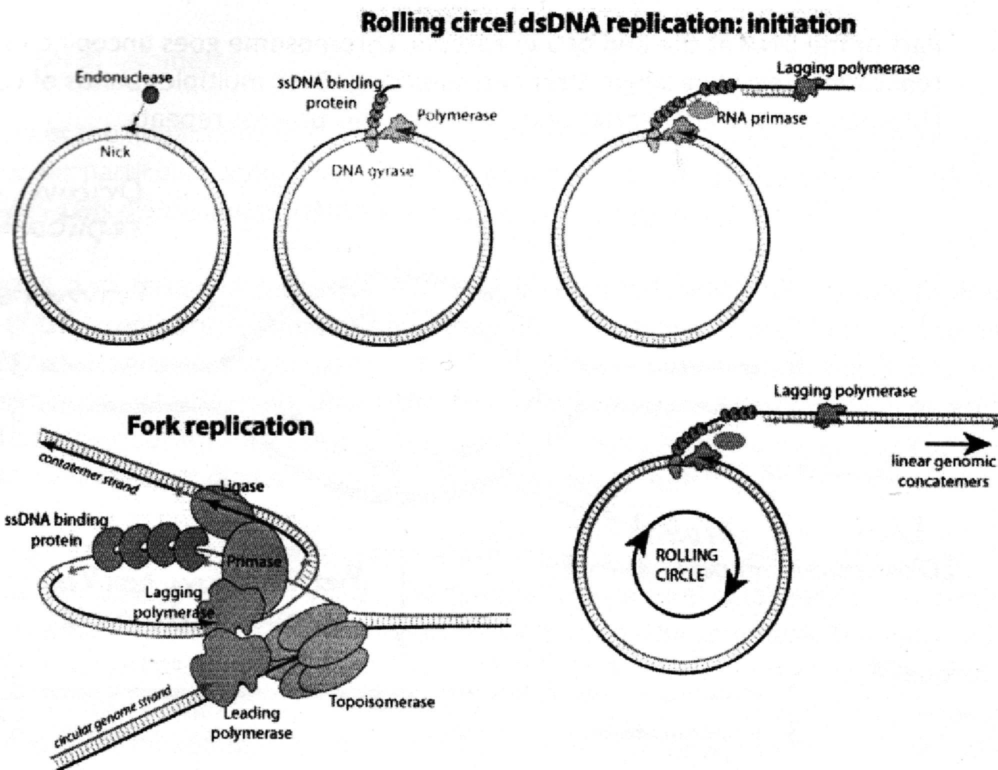


Fig. 7.6 : Telomere replication

Telomerase is important for maintaining chromosome integrity : The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

7.11 Telomerase and aging

Telomerase is typically active in germ cells and adult stem cells, but is not active in adult somatic cells. As a result, telomerase does not protect the DNA of adult somatic cells and their telomeres continually shorten as they undergo rounds of cell division.

In 2010, scientists found that telomerease can reverse some age-related conditions in mice. These findings may contribute to the future of regenerative medicine. In the

studies, the scientists used telomerase-deficient mice with tissue atrophy, stem cell depletion, organ failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegenration, and improved the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

7.12 Summary

(i) DNA replication is the production of identical DNA helices from a single double-stranded DNA molecule. Each molecule consists of a strand from the original molecules and a newly formed strand.

(ii) Prior to replication, the DNA uncoils and strands separate. A replication fork is formed which serves as a template for replication.

(iii) Primers bind to the DNA and DNA polymerases add new nucleotide sequences in the 5' to 3' direction.

(iv) This addition is continuous in the leading strand and fragmented in the lagging strand.

(v) Once elongation of the DNA strands is complete, trhe strands are checked for errors, repairs are made, and telomere sequences are added to the ends of the DNA.

7.13 Model questions

1. How DNA replicates ?
2. Give an outline view of DNA replication in prokaryotes.
3. What is RNA priming ?
4. Give a note on DNA polymerase.
5. Describe replication of circular DNA.
6. What is telomeres ? Give its significance.

Unit-8 □ Transcription and Translation

Structure

8.1. Objectives

8.2. Introduction

8.3. RNA polymerase

8.3.1 Bacterial RNA polymerase

8.3.2 Eukaryotic RNA polymerase

8.4. Transcription unit

8.5. Mechanism of transcription

8.5.1 In prokaryotes

8.5.1.1 RNA polymerase

8.5.1.2 Initiation

8.5.1.3 Elongation

8.5.1.4 Termination

8.5.2 In eukaryotes

8.5.2.1 Initiation

8.5.2.2 Elongation

8.5.2.3 Termination

8.6. Transcription factors

8.6.1 Salient points

8.6.2 Working principle of transcription factor

8.6.2.1 Activators

8.6.2.2 Repressors

8.6.2.3 Binding sites

8.7. Split genes

8.7.1 Concept of introns and exons

8.7.2 Splicing mechanism

8.7.2.1 RNA splicing

8.7.2.2 Spliciosome

8.7.2.3 Mechanism of splicing

8.7.2.4 Self splicing

8.7.3 Genetic code

8.7.3.1 Codon

8.7.3.2 Properties of genetic code

8.7.3.3 Wobble hypothesis and degeneracy of the genetic code

8.8. Mechanism of protein synthesis in prokaryotes

8.8.1 Ribosome : structure and assembly

8.8.1.1 RNA content

8.8.1.2 Protein content

8.8.2 Assembly of prokaryotic ribosome

8.8.3 Aminocylation of RNA

8.8.3.1 Activation of amino acid and attachment wth tRNA.

8.8.4 Initiation of protein synthesis

8.8.5 Elongation of polypeptide chain

8.8.6 Termination of polypeptide chain

8.8.7 Dissociation of initiation factors from the initiation complex

8.9 Summary

8.10 Model questions

8.1 Objectives

This unit gives a very attractive feature about the basic mechanism of protein synthesis within the body of an individual. After finishing the topic the reader will be able to get an idea about

✓ How the protein synthesis takes place ?

✓ What are the different factors associated with the protein synthesis

✓ The ribosome structure

✓ The initiation to termination of the polypeptide chain.

8.2 Introduction

In this module of the previous two units we have discussed different aspects of nucleic acids molecules including their replication and organization. The information stored in the nucleic acids, however needs to be used for the synthesis of proteins which determine the phenotype of an organism. For this purpose a very precise mechanism is used within the cell. For proten syntheis, within the cell a protein synthesis apparatus is present that operates in a precise manner, synthesizing individual proteins, untilizing the information encoded within the nucleic acid molecules. Protein synthesis takes place on organelles called ribosome. Another important component of protein synthesis appratus is a set of transfer RNA (t-RNA molecules). These tRNA molecules need to be aminoacylated, before they take part in protein synthesis. This aminocylation is facilitated by the presence of different aminoacyl t-RNA synthetase enzymes, one each for individual amino acids used for protein synthesis.

8.3 RNA polymerase

The main enzyme involved in transcription is **RNA polymerase,** which uses a single-stranded DNA template to synthesize a complementary strand of RNA. Specifically, RNA polymerase builds an RNA strand in the 5' to 3' direction, adding each new nucleotide to the 3' end of the strand (Fig. 8.1)

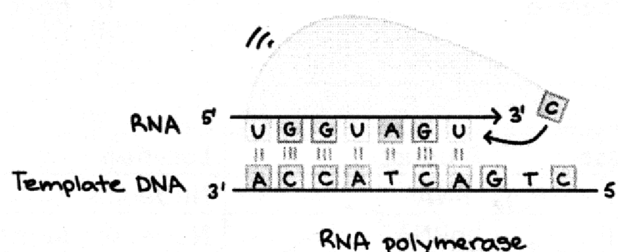


Fig. 8.1 : Use of RNA Polymerase

8.3.1 Bacterial RNA polymerase

Single RNA polymerase synthesizes all types of RNA in prokaryotes.

RNA polymerase is a holozyme made of five polypeptide chains : 2 a (helps in initiation of chain and interaction with regulatory proteins) and b (catalytic center-chain initiation and elongation), b' (DNA binding) and s factors. Sigma (s) factor recognizes the promoter to which RNA polymerase binds and the 's' factor is released immediately after initiation. RNA polymerase without 's' factor is core polymerase (Fig.8.2).

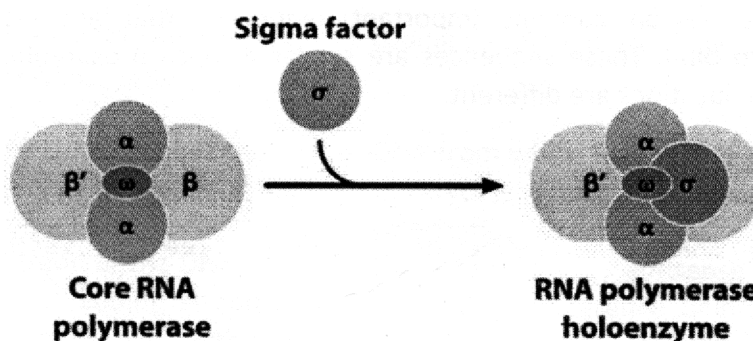


Fig. 8.2 : Showing different subunits of RNA Polymerase

In short the characteristic features of RNA polymerase are the following :

1. protein is about 480 kd in size
2. four subunits : **alpha, beta, beta' and sigma ;** only the first three subunits are required for polymerase activity and are considered the core enzyme
3. the sigma factor is required for RNA polymerase to bind to the promoter ; the enzyme has a loose affinity for DNA but when the sigma factor is present it wll bind only at a promoter.

8.3.2 Eukaryotic RNA polymerase

1. Three types exist

Type of polymerase	Product	Location
RNA Polymerase I	rRNA	Nucleolus
RNA Polymerase II	hnRNA	Nucleoplasm
RNA Polymerase III	tRNA	Nucleoplasm

2. protein is great than 500 kd in size
3. two large subunits : <10 small subunits ; largest subunits homolougous to beta', second largest subunits is homolougous to the beta subunit
4. many non-polymerase factors required for binding of the enzyme to DNA

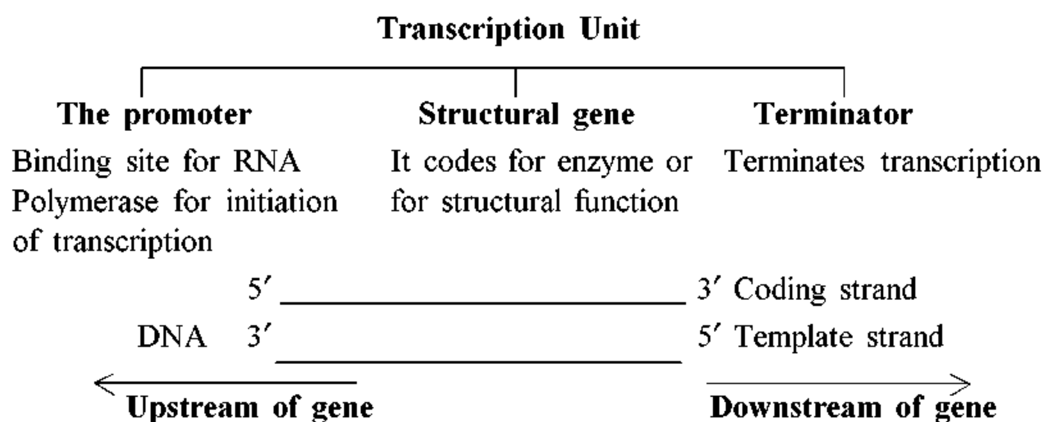
RNA polymerase produces a **transcription unit** that extends from the promoter to the termination sequences. The gene is defined in reference to the start site-those sequences before the start site are called the **upstream sequences,** those after the start site are called **downstream sequences.** The immediate product is the **primary transcript.**

The promoter region important sequences that are required for RNA polymerase to bind. These sequences are similar in both prokaryotic and equkaryotic genes, but the locations are different.

8.4 Transcription unit

A transcription unit is DNA is defined primarily by the three regions in the DNA :

1. A Promoter
2. The Structural gene
3. A Terminator



To define the two strands of DNA in the structural gene of transcription unit there is a convention. The DNA strand which takes part in synthesis of RNA is called antisense or template strand and is oriented in 3′→5′ direction while the other strand, not involved in RNA synthesis is called sense or coding strand. The promoter and

terminator flank the structural gene in a transcription unit. The promoter is said to be located towards 5′-end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides binding site for RNA polymerase, and it is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with terminator, the definition of coding and template strands could be reversed. The terminator is located towards 3′-end (downstream) of the coding strand and it usually defines the end of the process of transcription.

Structural gene is the part of DNA from which RNA is formed. It is polycistronic in prokaryotes but monocistronicn eukaryotes. Transcription occurs with the help of DNA dependent RNA polymerase. Polymerization occurs in 5′→3′ direction. The 3′ →5′ strand of the DNA acts as the template strand / antisense strand. The 5′→3′ strand is the coding strand / sense strand. All reference points while defining the transcript unit is made with coding strand.

Lying towards 5′ end (upstream) of structural gene is the promoter. It is the binding site for RNA polymerase for transcription to start. Lying towards 3′ end (downstream) of structural gene is the terminator, where transcription ends.

8.5 Mechanism of transcription

8.5.1 In prokaryotes

Both prokaryotes and eukaryotes perform fundamentally the same process of transcription, with the important difference of the membrane-bound nuclus in eukaryotes. With the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm. In prokaryotes, which lack membrane-bound nuclei and other organelles, transcription occurs in the cytoplasm of the cell.

8.5.1.1 RNA polymerase

RNA polymerase is the enzyme that produces the mRNA molecule (just like DNA polymerase produced a new DNA molecule during DNA replication). Prokaryotes use the same RNA polymerase to transcribe all of their genes. In *E.coli*, the polymerase is composed of five polypeptide subunits. These subunits assemble every time a gens is transcribed, and they disassemble once transcription is complete. Each subunit has a unique role (which you do no need to memorize). The polymerase comprised of all five subunits is called the **holoenzyme.**

8.5.1.2 Initiation

Transcription in prokaryotes (and in eukaryotes) requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a transcription bubble. The DNA sequence on to which the proteins and enzymes involved in transcription bind to initiate the process is calleda **promoter.** In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all of the time, some of the time, or hardly at all. The structure and function of a prokaryotic promoter is relatively simple (Fig. 8.3). One important sequences in the prokaryotic promoter is located 10 bases before the transcription start site (-10) and is commonly called the TATA box.

To begin transcription, the RNA polymerase heloenzyme assembles at the promoter. The dissociation of σ allows the core enzyme to proceed along the DNA

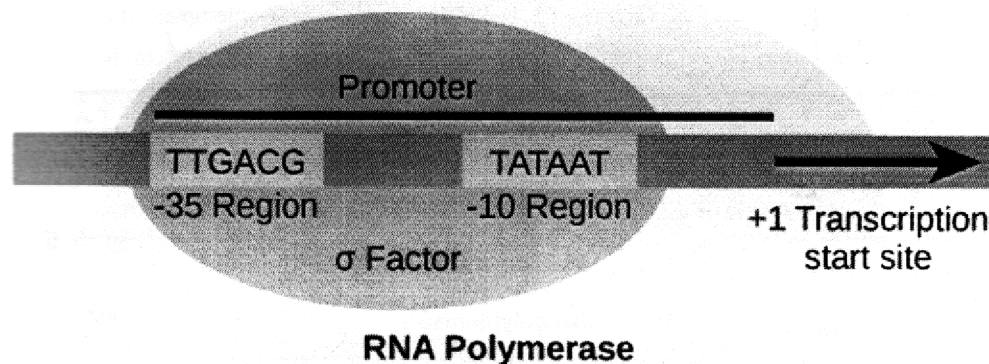


Fig. 8.3 : The general structure of a prokaryotic promoter

template, synthesizing mRNA by adding RNA nucleotides according to the base pairing rules, similar to the way a new DNA molecule is produced during DNA replication. Only one of the two DNA strands is transcribed. The transcribed strand of DNA is alled the **template strand** because it is the template for mRNA production. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the **non-template strand,** with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA. Like DNA polymerase, RNA polymerase adds new nucleotides onto the 3′-OH group of the previous nucleotide. This means that the growing mRNA strand is being synthesized in the 5′ to 3′ direction. Because DNA is antiparallel, this means that the

RNA polymerase is moving in the 3′ to 5′ directon down the template strand (Fig. 8.4).

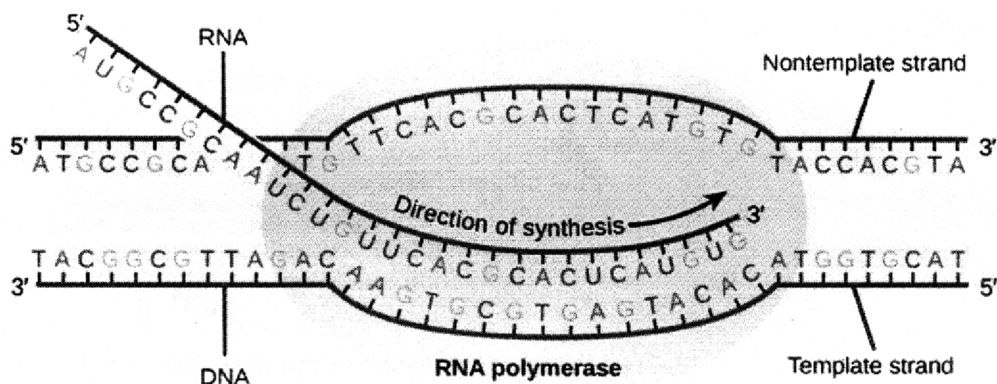


Fig. 8.4 : During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to3' direction, and unwinds then rewinds the DNA as it is read.

8.5.1.3 Elongation

As elongation proceeds, the DNA is continuously unwound ahead of the core nzyme as the hydrozen bonds tha tconnect the complementary base pairs in the DNA double helix are broken (Fig.8.4). The DNA is rewound behind the core enzyme as the hydrogen bonds are reformed . The abse pairing between DNA and RNA is not stable enough to maintain the stability of the mRNA synthesis components. Instead, the RNA polymerase acts as a stable linker between the DNA template and the newly forming RNA strand to ensure that elongation is not interrupted prematurely.

8.5.1.4 Termination

Once a gene is transcribed, the RNA polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals. One is protein-based and the other is RNA-based. Both termination signals rely on specific sequences of DNA near the end of the gene that cause the polymerase to release the mRNA.

In a prokaryotic cell, by the time transcription ends, the transcript would already have been used to begin making copies of the encoded protein because the processes of transcription and translation can occur at the same time since both occur in the cytoplasm (Fig. 8.5). In contrast, transcription and translation cannot occur simultaneously in eukaryotic cells since transcription occurs inside the nucleus and translation occurs outside in the cytoplasm.

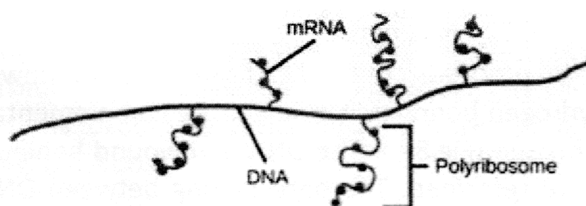


Fig. 8.5 : Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

8.5.2 In Eukaryotes

8.5.2.1 Initiation

The eukaryotic promoters that we are most interested in are similar to prokaryotic promoters in that they contain a TATA box (Fig. 8.6). However, initiation of transcription is much more complex in eukaryotes compared to prokaryotes. Unlike the prokaryotic RNA polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called **transcription factors,** to first bind to the promoter region and then help recruit the appropriate polymerase.

In addition, there are three different RNA polymerases in eukaryotes, each of which is made up of 10 subunits or more. Each eukaryotic RNA polymerase also requires a distinct set of transcription factors to bring it to the DNA template.

RNA polymerase I is located in the nucleous, a specialized nuclear substructures in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes. The rRNA molecules are considered structural RNAs because they have a cellular role but are not translated into protein. The rRNAs are components of the ribosome and are essential to the process of translation. RNA polymerase I synthesizes most of the rRNAs.

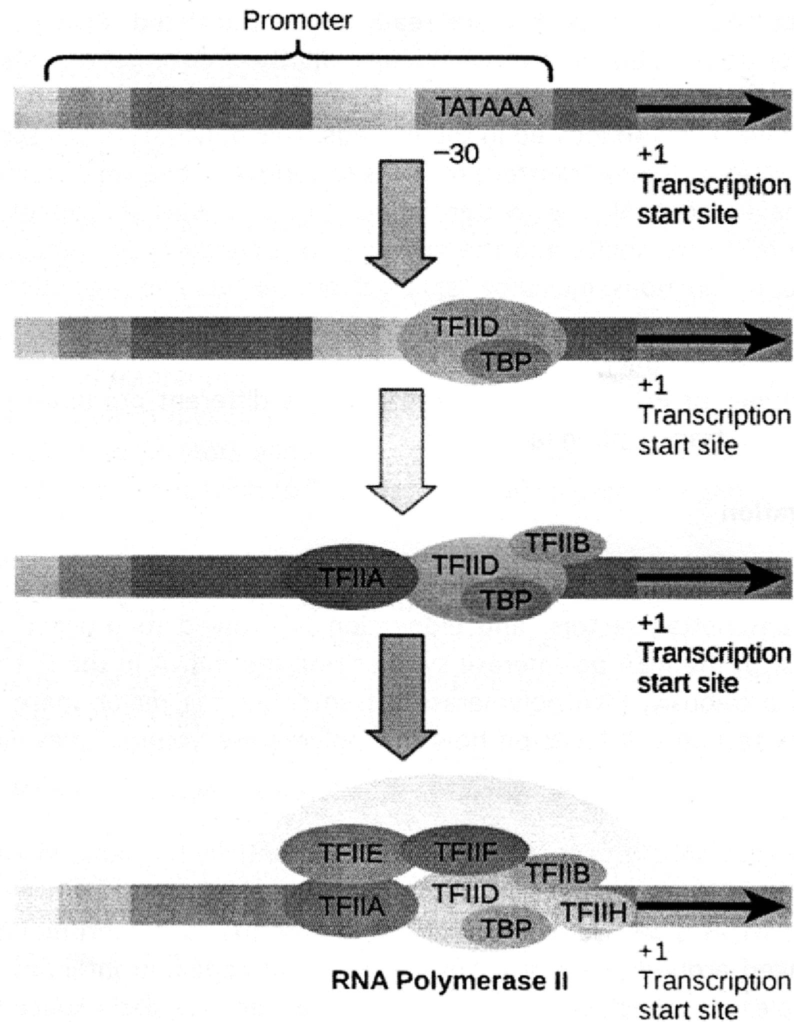


Fig. 8.6 : The generalized structure of a eukaryotic promoter and transcription factors.

RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs. Eukaryotic pre-mRNAs undergo extensive processing after transcription but before translation. For clarity, the term "mRNA" will only be used to describe the mature, processed molecules that are ready to be translated. RNA polymerase it is responsible for transcribing the overwhelming majority of eukaryotic genes.

RNA polymerase III is also located in the nucleus. This polymerase transcribes a variety of structural RNAs including transfer pre-RNAs (pre-tRNAs), and small nuclear pre-RNAs. The tRNAs have a critical role in translation ; they serve as the

adaptor molecules between the mRNA template and the growing polypeptide chain. Small nuclear RNAs have a variety of functions, including "splicing"pre-mRNAs and regulating transcription factors.

Each of the types of RNA polymerase recognizes a different promoter sequence and requires different transcription factors.

8.5.2.2 Elongation

Following the formation of the preinitiation complex, the polymerase is released from the other transcription factors, and elongation is allowed to proceed as it does in prokaryotes with the RNA polymerase synthesizing pre-mRNA in the 5' to 3'direction. As discussed previously, RNA polymerase II transcribes the major share of eukaryotic genes, so this section will focus on how this polymerase accomplishes elongation and termination.

Although the enzymatic process of elongation the same in eukaryotes and prokaryotes, the DNA template is more complex. When eukaryotic cells are not dividing, their genes exist as a diffuse mass of DNA and proteins called chromatin. The DNA is tightly packaged around charged histone proteins at repeated intervals. These DNA-histone complexes, collectively called nucleosomes, are regularly spaced and include 146 nucleotides of DNA wound around eight histones like thread around a spool.

For RNA synthesis to occur, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome. This is accomplished by a special protein complex called FACT, which stands for "facilitates chromatin transcription." This complex pulls histones away from the DNA template as the polymerase moves along it. Once the pre-mRNA is synthesized, the FACT complex replaces the histones to recreate the nucleosomes.

8.5.2.3 Termination

The termination of transcription is different for the different polymerases. Unlike in prokaryotes, elongation by RNA polymerase II in eukaryotes takes place 1,000-2,000 nucleotides beyond the end of the gene being transcribed. This pre-mRNA tail is removed during mRNA processing. RNA polymerases I and III require termination signals. Genes transcribed by RNA polymerase I contain a specific 18-nucleotide sequence that is recognized by a termination protein. The process of termination in RNA polymerase III involves an mRNA hairpin that causes the mRNA to be released.

8.6 Transcription factors

8.6.1 Salient points

- **Transcription factors** are proteins that help turn specific genes "on" or "off" by binding to nearby DNA.
- Transcription factors that are **activators** boost a gene's transcription. **Repressors** decrease transcription.
- Groups of transcription factor binding sites called **enhancers** and **silencers** can turn a gene on/off in specific parts of the body.
- Transcription factors allow cells to perform logic operations and combine different sources of information to "decide" whether to express a gene.

Transcription factors are protein that regulate the transcription of genes—that is, their copying into RNA, on the way to making a protein.

The human body contains many transcription factors. So does the body of a bird, tree or fungus ! Transcription factors help ensure that the right genes are expressed in the right cells of the body, at the right time.

8.6.2 Working principle of transcription factor

A typical transcription factor binds to DNA at a certain target sequence. Once it's bound, the transcription factor makes it either harder to easier for RNA polymerase to bind to the promoter of the gene.

8.6.2.1 Activators

Some transcription factors **activate** transcription. For instance, they may help the general transcription factors and/or RNA polymerase bind to the promoter, a shown in the diagram below (Fig.8.7).

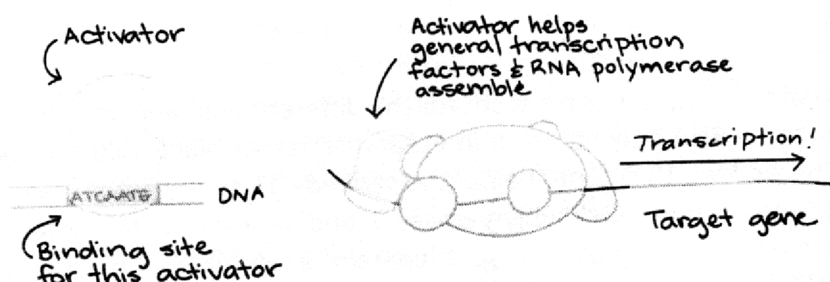


Fig. 8.7 : Showing activator action

8.6.2.2 Repressors

Other transcription factors **repress** transcription. This repression can work in a variety of ways. As one example, a repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can't bind to the promoter or begin transcription (Fig. 8.8).

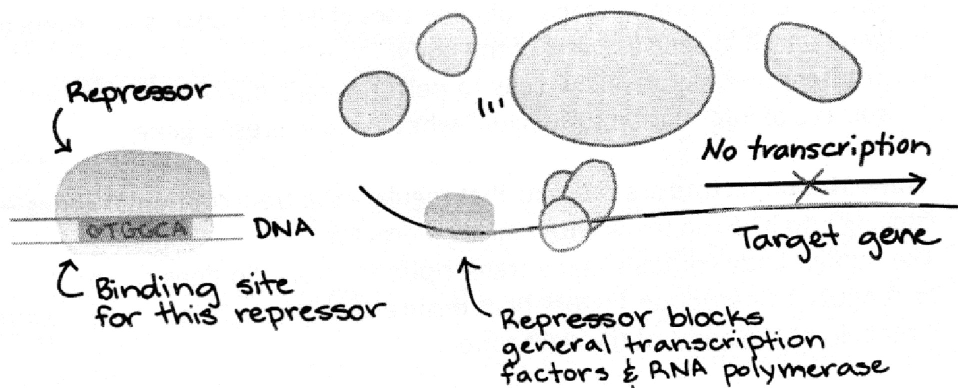


Fig. 8.8 : Repressor in oction

8.6.2.3 Binding sites

The binding sites for transcription factors are often close to a gene's promoter. However, they can also be found in other pars of the DNA, sometimes very far away from the promoter, and still affect transcription of the gene (Fig. 8.9).

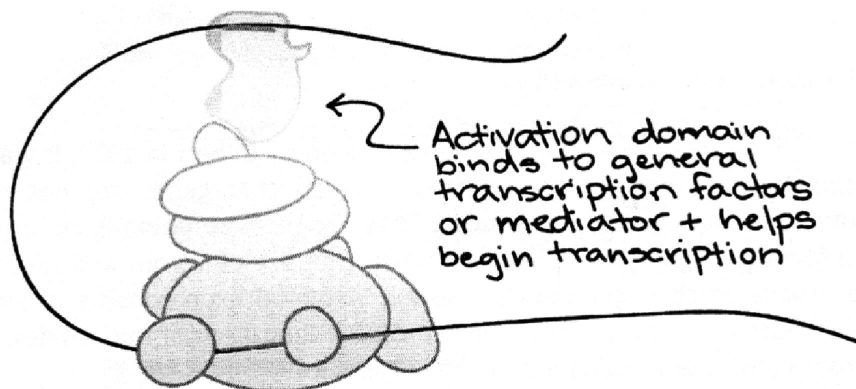


Fig. 8.9 : The parts of an activator protein : The DNA binding domain (which attaches to the recognition site in the DNA) and the activation domain, which is the "business end" of the activator that actually promotes transcription, e.g., by facilitating formation of the transcription initiation complex.

The flexibility of DNA is what allows transcription factors at distant binding sites to do their job. The DNA loops like cooked spaghetti to bring far-off binding sites and transcription factors close to general transcription factors or "mediator" proteins.

8.7 Split genes

Split genes is a gene that when transcribed results in pre-mRNA containing exons interrupted by introns. Splicing is generally performed by endonuclease enzymes cleaving the introns at both ends. Phosphodiester bond between sugar and phosphate at the junction between intron and exon is cleaved. The freed 5'-end of the intron joins the branch point sequence of form lariat or loop.

Splicing is performed by a large complex called splicesome. The spliceosme is made up of small nuclear ribonuclear proteins (sn RNP) called snurps. These consist of RNAs which are rich in Uracil and are of several types U1, U2, U4, U5 and U6 which are collectively called small nuclear RNAs (sn RNA).

Split genes are first observed in eukaryotes. No split genes are reported yet in prokaryotes. Prokaryotes genes are contiguous. Eukaryotic genes, in contrast, contain segments of DNA that are expressed (called exons) interrupted by segments that are not expressed (called introns). During gene expression, the resulting mRNA contains both exons and introns. It then undergoes splicing by which introns are removed so that it would only contain exon sequences before it is transported to the cytoplasm.

8.7.1 Concept of introns and exons

The terms exons and intons were given by Gilbert in 1977. It was discovered in Amphibia, mammals and some other animals that genes are not represented by continuous sequence of nucleotides. They are most common in eukaryotes. They are also found in viruses but rarely in bacteria. The size of introns and exons varies. Introns are usually much larger than the exons. A typical exon consists of small number of nucleotides whereas an intron may consist of thousands of nucleotides. Moreover, the introns constitute a large portion of the genome.

Now it has been discovered that coding sequence of most of eukaryotic genes is split into stretches of codons interrupted by stretches of non-coding sequences. Most human genes are discontinuous.

The coding sequences of DNA of the gene are called exons. In between exons, there are intervenig non-coding sequences called introns. This type of genes is called

split genes or interrupted genes. During transcription, the entire gene is copied into a pre-mRNA, which includes exons and introns. During the process of RNA splicing, introns are removed and exons joined to form a contiguous coding sequence. This "mature" mRNA is ready for translation.

The differences between intron and exon could be enlisted as follows :

- intron sequence will be transcripted but not translated while exon sequence will be transcripted and translated, both.
- meaningful genetic codes are present in exons, not in introns.
- after transcription, intron parts are removed but exons are not.
- only exons become part of the functional RNA, introns fail to do so.
- a mutation in exon of gene may adversely affect the protein the gene codes for ; a mutation in intron will not affect the protein in any way.

8.7.2 Splicing mechanism

8.7.2.1 RNA Splicing

Splicing is generally performed by endonuclease enzymes cleaving the introns at both ends. Phosphodiester bond between sugar and phosphate at the junction between intron and exon is cleaved. The freed 5'-end of the intron joins the branch point sequence of form lariat (Fig. 8.10).

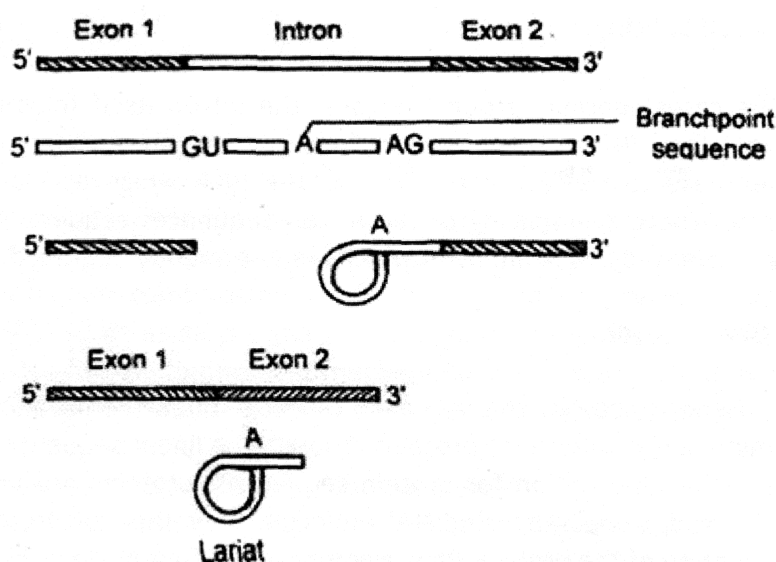


Fig. 8.10 : showing RNA splicing

8.7.2.2 Spliceosome

Splicing is performed by a large complex called spliceosome. The spliceosome is made up of small nuclear ribonuclear proteins (sn RNP) called snurps. These consist of RNAs which are rich in uracil and are of several types U1, U2, U4, U5 and U6 which are collectively called small nuclear RNAs [sn RNA] (Fig. 8.11).

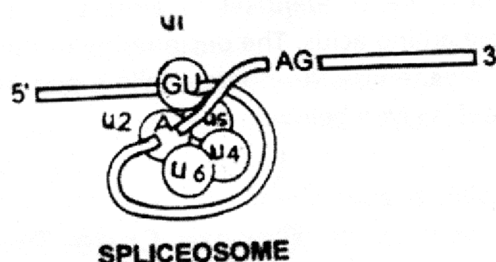


Fig. 8.11 : A Spliceosome stucture

8.7.2.3 Mechanism of splicing

At first U1 sn RNP recognize and break the 5' splice site of the intron and bring it closer to branch site. Then the complex of sn RNP of U2, U4, U5 and U6 bind to the intron. The complex of snRNPs and precursor mRNA of the intron is called spliceosome. The spliceosome is looped out. This loop of intron is called lariat which is discarded and degraded. The exons on either side of the removed intron are brought closer and ligation seals them together.

8.7.2.4 Self splicing

In some cases specially group I introns, the intron itself folds and catalyzes its own splicing. Here RNA of the intron functions as an enzyme and behaves like an endonuclease to splice out the intron. The RNA which acts as an enzyme is called ribozyme. There are splicing of the intron sequences requires no other enzyme. The intron is released in a linear form which is subsequently degraded.

8.7.3 Genetic code

Genetic code is the sequence of nucleotides in DNA and RNA that determines the amino acid sequence of proteins. Though the linear sequence of nucleotides in DNA contains the information for protein sequences, proteins are not made directly from DNA. Instead, a messanger (mRNA) molecule is synthesized from the DNA and directs the formation fo the protein. RNA is composed of four nucleotides :

adenine (A), guanine (G), cytosine (C), and uracil (U). Three adjacent nucleotides constitute a unit known as the codon, which codes for an amino acid. For example, the sequence AUG is a codon that specifies the amino acid methionine.

There are 64 possible codons, three of which do not code for amino acids but indicate the end of a protein. The remaining 61 codons specify the 20 amino acids that make up proteins. The AUG codon, in addition to coding for methionine, is found at the beginning of every mRNA and indicates the start of a protein. Because most of the 20 amino acids are coded for by more than one codon, the code is called degenerate.

The genetic code is identical in almost all species, with the same codons specifying the same amino acids.The deciphering of the genetic code was accomplished by the American biochemists Nirenberg, Holley, and Khorana in the early 1960s. The genetic code table-I is given below.

Genetic Code- Table

Second Letter

1st letter	U		C		A		G		3rd letter
U	UUU	Phe	UCU		UAU	Tyr	UGU	Cys	U
	UUC		UCC	Ser	UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU		CCU		CAU	His	CGU		U
	CUC	Leu	CCC	Pro	CAC		CGC	Arg	C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU		ACU		AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU		GCU		GAU	Asp	GGU		U
	GUC	Val	GCC	Ala	GAC		GGC	Gly	C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Table 1

8.7.3.1 Codons

RNA codons designate specific amino acids. The order of the bases in the codon sequence determines the amino acid that is to be produced. Any of the four nucleotides in RNA may occupy one of three possible codon positions. Therefore, there are 64 possible codon combinations. Sixty-one codons specify amino acid and three **(UAA, UAG, UGA)** serve as **stop signals** to designate the end of proten synthesis. The codon AUG codes for the amino acid **methionine** and serves as a **start signal** for the beginning of translation. Multiple codons may also specify the same amino acid. For example, the codons UCU, UCC, UCA, UCG, AGU, and AGC

all specify serine. The RNA codon table above lists codon combinations and their designated amino acids. Reading the table, if uracil (U) is in the first codon position, adenine (A) in the second, and cytosine (C) in the third, the codon UAC specifies the amino acid tyrosine.

8.7.3.2 Properties of genetic code

1. There is no *ambiguity* in the genetic code. This means each triplet codes for only one amino acid.
2. The genetic code is *degenerate,* which means there is more than one triplet code for many of the amino acids. Methionine and tryptophan each are coded by just one triplet. Arginine, leucine, and serine each are coded by six triplets. The other 15 amino acids are coded by two, three and four triplets.
3. There are 61 triplet codes for amino acids. Three other triplets (UAA, UAG and UGA) are stop sequences. The stop sequences signal chain termination, telling the cellular machinery to stop synthesizing a protein.
4. The degeneracy of the code for the amino acids coded by two, three and four triplets is only in the last base of the triplet code. As an example, glycine is coded by GGU, GGA, GGG, and GGC.
5. Experimental evidence indicates the genetic code is *universal* for all organisms on Earth. Viruses, bacteria, plants, and animals all use the same genetic code to form proteins from RNA.

8.7.3.3 Wobble hypothesis and degeneracy of the genetic code

Crick (1966) proposed the 'wobble hypothesis' to explain the degeneracy of the genetic code. Except for tryptophan and methionine, more than one codons direct the synthesis of one amino acid. There are 61 codons that synthesise amino acids, therefore, there must be 61 tRNAs each having different anticodons. But the total number of tRNAs is less than 61.

This may be explained that the anticodons of some tRNA read more than one codon. In addition, identification of the third codon seems to be unimportant. For example CGU, CGC, CGA and CGG all code for arginine. It appears that CG specifies arginine and the third letter is not important. Conventionally, the codons are written from 5′ end to 3′ end.

Therefore, the first and second bases specify amino acids in some cases. According to the Wobble hypthesis, only the first and second bass of the triple codon on 5′→3′ mRNA pair with the bases of the anticodon of tRNA i.e. A with U, or G with C.

The pairing of the third base varies according to the base at this position, for example G may pair with U. The conventional pairing (A = U, G = C) is known as Watson-Crick pairing (Fig. 8.12) and the second abnormal pairing is called wobble pairing.

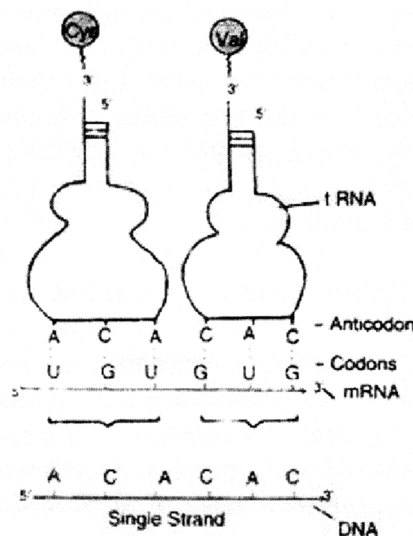


Fig. 8.12 : DNA triplet, mRNA codons and tRNA anticodons showing Watson-Crick pairing

This was observed from the discovery that the anitcodon of yeast alanine-tRNA contains the nucleoside inosine (a deamination product of adenosine) in the first position (5′→3′) that paired with the third base of the codon (5′→3′). Inosine was also found at the first position in other tRNAs e.g. isoleucine and serine.

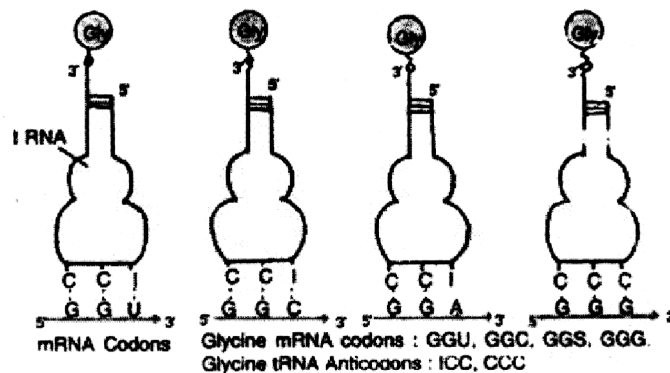


Fig. 8.13 : Wobble pairing of one glycine tRNA with three codons of mRNA due to Wobble in 5' – 3' direction

The purine, inosine, is a wobble nucleotide and is similar to guanine which normally pairs with A, U and C. For example a glycine-tRNA with anticodon 5′-ICC -3′ will pair with glycine codons GGU, GGC, GGA and GGG (Fig 8.13). Similarly, a seryl-tRNA with anticodon 5′-IGA-3′ pairs with serine codons UCC, UCU and UCA (5-3′). The U at the wobble position will be able to pair with an adenine or a guanine.

According to Wobble hypothesis, allowed base pairing are given in Table-II

Third position codon base	First position anticodon base
A	U, I
G	C, U
U	G, I
C	G, I

Table–II Wobble base pairings

Due to the Wobble base pairing one tRNA becomes able to recognise more than one codons for an individual amino acid. By direct sequence of several tRNA molecules, the wobble hypothesis is confirmed which explains the pattern of redundancy in genetic code in some anticodons (e.g. the anticodons containing U, I and G in the first position in 5′→3′ direction).

The seryl-tRNA anticodon (UCG) 5′-GCU-3′ base pairs with two serine codons, 5′-AGC-3′ and 5′-AGU-3′. Generally, Watson-Crick pairing occurs between AGC and GCU. However, in AGU and GCU pairing, hydrogen bonds are formed between G and U. Such abnormal pairing called 'Wobble pairing' is given in Table II.

Three types of Wobble pairings have been proposed :

(i) U in the wobble position of the tRNA anticodon pairs with A or G of codon.

(ii) G pairs with U or C, and

(iii) 1 pairs with A, U or C.

8.8 Mechanism of protein synthesis in prokaryotes

Protein synthesis in the cell is conducted by ribosomes that are found attached

to the membrane of endoplasmic reticulum and microsomes, as well as in free state in the ground plasm.

The main components that take part in protein synthesis at cellular level are : 20 different amino acids, different types of RNAs, enzymes, aminoacid activating enzymes, polypeptide-polymerase and energy liberating molecules, such as ATP and GTP.

DNA which contains genetic information synthesizes three kinds of RNA :

(i) Messanger RNA (mRNA)

(ii) Ribosomal RNA (rRNA) and

(iii) Transfer RNA (tRNA) or soluble RNA (sRNA).

mRNA is copied from DNA molecule. The specific locus of DNA molecule where mRNA is formed is referred to as a structural gene. tRNAs come probably from special genes called determinants for tRNAs.

8.8.1 Ribosomes : Structure and assembly in prokaryotes

Most of the work on prokaryotic ribosomes has been carried out using *Escherichia coli*. Although some variations are observed among the prokaryotes, findings using E. coli are generally representative.

In prokaryotic cells, ribosomes are typically about 70 S (MW about 2.7×10^6) and are formed from 30S and 50S subunits.

The complete ribosome formed by combination of the subunits is also referred to as a monomer. Although ribosomes from both prokaryotic and eukaryotic sources are about 30 to 45% protein (by weight), with the remainder being ribonucleic acid, the specific protein and RNA components of these two major classes of ribosomes differ (Table III) ; carbohydrate and lipid are virtually absent.

Table III Properties and composition of eukaryotic and prokaryotic ribosomes

Composition	Eukaryotic	Prokaryotes
Monomers		
Sedimentation coefficient	80 S	70 S
Number of RNAs	4	3
Number of proteins	70	55

Small subunit		
Sedimentation coefficient	40 S	30 S
RNAs present	18 S	16 S
Number of proteins	30	21
Large subunit		
Sedimentation coefficient	60 S	50 S
RNAs present	5 S 5.8 S 28 S	5 S 23 S
Number of proteins	40	34

8.8.1.1 RNA content

The small subunit of prokaryote ribosomes contains one molecule of an RNA called 16S RNA (MW 0.6×10^6), and the large subunit contains two RNA molecules, a 23S RNA (MW 1.1 × 106) and a 5S RNA (MW 3.2×10^4) (see Table III). All three rRNAs are products of closely linked genes transcribed by RNA polymerase in the sequence 16S 23S 5S. This assures an equal proportion of each RNA.

The rRNA operon also contains genes for some tRNAs. the transription product of the rRNA operon consists of a 30S RNA ; this transcript is successively cleaved and trimmed to pruduce the final 16S, 23S, and 5S RNAs that are incorporated into the small and large ribosomal subunits. Figure 22-2 presents the scheme of maturation of the prokaryotic rRNAs.

8.8.1.2 Protein content

Ribosomal proteins combine with the rRNAs at various stages of subunit assembly : some are incorporated during transaction, others following release of the primary transcript and during processing, and still others once the mature rRNA products are formed. Certain proteins bind to the rRNAs only transiently and are not found in the fully assembled subunits.

8.8.2 Assembly of prokaryotic ribosome

Because all of the proteins and RNAs of the prokaryotic robosome subunits may be isolated, it is possible through recombination studies to examine the assembly process. Nomure and others have shown that the assembly of individual subunits and their association to form functional ribosomes (i.e., ribosomes capable of transiating mRNA into protein) occur spontaneously in vitro when all the individual rRNAs and protein components are available.

Thus the ribosome is capable of self-assembly, and this is believed to be the mechanism in situ. The assembly is promoted by the unique and complementary structures of the ribosomal protein and RNA molecules and proceeds through the formation of hydrogen bonds and hydrophobic interactions. There is order to the assembly in that certain proteins combine with the rRNAs prior to the addition of others. Cooperativity also exists, because addition of certain proteins to the growing subunit facilitates addition and binding of others.

No self-assembly takes place when I. proteins are added to 16 S RNA or when S proteins are added to 5 S and 23 S RNA. However, it is interesting to note that RNA from the 30 S subunit of one prokaryotic species will combine with the S proteins of another prokaryote to form functional subunits. The same is true for 50S subunit proteins and RNAs from different prokaryotes.

Assembly of hybrid subunits and formation and formation of functional monomers from these occur in spite of the fact that ribosomal proteins and RNAs from different prokaryotes have different primary structures. It is clear that their secondary and tertiary structures, which are very similar, are more important in guiding rRNA-protein interactions. Although some proteins from yeast, reticulocyte, and rat liver cell ribosomes can be replaced by *E. coli* ribosomal proteins, hybrid monomers formed from these prokaryotic-eukaryotic subunits will not function in protein synthesis.

8.8.3 Aminoacylation of tRNA

Transfer RNA molecules play a key role in the process by delivering amino acids to the ribosome in an order specified bythe mRNA sequence ; this ensures that the amino acids are joined together inthe correct order. Cells usually contain many species of tRNA, each of which binds specifically to one of the 20 amino acids.

Consequently, there may be more than one tRNA for each amino acid. Transfer RNAs that bind the same amino acid are called iso-acceptors.

Before translation begins, amino acids become covalently linked to their tRNAs which then recognize codons in the mRNA specifying that amino acid. The attachment of an amino acid to its tRNA is called amino acylation or charging. The amino acid is covalently attached to the end of the acceptor armof the tRNA which always ends with the base sequence 5′ CCA 3′.

A bond forms between the carboxyl group of the amino acid and the 3′-hydroxyl of the terminal adenine of the acceptor arm. Charging is catalyzed by enzymes called aminoacyl tRNA synthetizes in a reaction requiring the hydrolysis of ATP. A separate enzyme exists for each amino acid and each enzyme can charge all the iso-acceptors tRNAs for that amino acid.

The aminoacyl tRNA synthetase recognizes both the appropriate amino acid and the corresponding tRNA.When the correct amino acid has been attached to the tRNA, it recognizes the codon for that amino acid in the mRNA allowing it to place the amino acid in the correct position, as specified by the sequence of the mRNA. This ensures that the amino acid sequence encoded by the mRNA is translted faithfully.

Codon recognition takes place via the anticodon loop of the tRNA and specifically by three nucleotides in the loop known as the anticodon which binds to the codon by complementary base-paring.

The entire codon—anticodon fitting is comparable to recognition of a 3-pin plug with the socketed base. Both the pin and the socket are highly specific. The four bases present in DNA can combine as 64 codons. Three codons act as signals for translation to stop and the remaining 61 encode the 20 amino acids present in proteins. Consequently, most amino acids are represented by more than one codon.

8.8.3.1 Activation of amino acid and attachment with tRNA

Amino acid in cytoplasm occurs in inactive state. They are activated by gaining energy which comes from ATP. The reaction is brought about by the binding of amino acid with ATR. The step is mediated by specific activating enzymes known as aminoacyl RNA synthetase.

A high energy acyl bond is formed between the a-phosphate of ATP and the carboxyl group of amino acid with the formation of aminoacyl adenylate. The b and g phosphate of ATP break awy as inorganic pyrophosphate.

$$\textbf{Met} - \textbf{tRNA}\boldsymbol{f}\ \textbf{met} + \textbf{formate} \xrightarrow{\textbf{transformylase}} \boldsymbol{f}\ \textbf{met} - \textbf{tRNA}\boldsymbol{f}\ \textbf{met}$$

The activated amino acid is transferred to its specific t-RNA. A high energy estar

bond is formed between the carboxyl group of the amino acid and the 3'-hydroxyl group of the terminal adenosine of tRNA. The aminoacyl AMP-enzyme complex reacts with the specific tRNA to form an aminoacyl-tRNA complex.

8.8.4 Initiation of protein synthesis

The messager RNA always has first tirplet as AUG or GUG at its 5'end and these triplets code for aminoacids N-formyl methionine (F. met) which usually initiates a protein chain. Thus, in all proteins formyl methionine occupies the first place, i.e., at, amino end and when the protein molecules are completely synthesised formyl methionine may be detached from the protein molecules by activity of hydrolytic enzyme deformylase.

In formyl methionine-tRNA complex the amino group is blocked by formyl group leaving only COOH group free to react with NH2 group of the second amino acid (AA_2). In this way, polypeptide chain always grows from amino end toward-COOH end.

When one tRNA-aminoacid complex attaches to mRNA at starting end, then the second tRNA-aminoacid complex also comes just afger the first and finally the two adjacent amino acids form peptide linkage. Like this several molecules of amino acids will join in a definite order through peptide bonds to form specific protein molecule (Fig. 8.14).

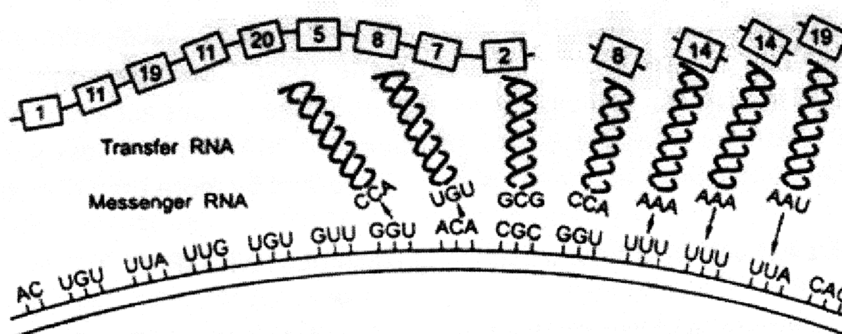


Fig. 8.14 : Synthesis of protein from amino acid through tRNAs and messanger RNA

8.8.5 Elongation of polypeptide chain

The peptide chain elongates by regular addition of aminoacids and relative movement of ribosome along with messanger RNA in presence of GTP (guanosine triphosphate) in the following sequence :

1. According to W.D. Stanfield (1969) there are three presumed sites in the ribosome (Fig. 8.15). These are :
   (i) Decoding site or 'A' site which binds the loaded AA~tRNA complex with the mRNA by base pairing.
   (ii) A condensing site or 'P' site or peptidyl site which joins the aminoacid to the growing polypeptide chain.
   (iii) An exit site or 'E' site at which tRNA detaches from the polypeptide, messanger RNA and ribosome.

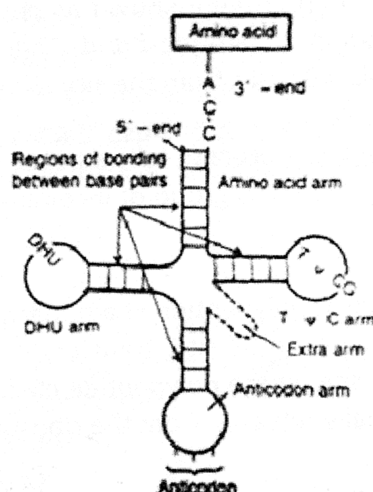
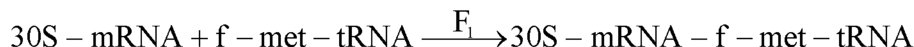


Fig. 8.15 : Aminoacyl-tRNA (amino acid is attached to CCA – OH end)

tRNA with their associated aminoacids will enter ribosomal site 'A' and will be checked by a 'checking factor' tosee if there is a correct fit between the codon on the messengers RNA and the anticodon of tRNA. If the fit is incorrect, the tRNA is rejected and presumabley other / RNAs will continue to be tried until the correct one is found.

2. As the initiaing codon AUG or GUG has entered the ribosome and is in position facing the site 'A', the correct tRNA, i.e., f-met-tRNA is checked against codon. This reaction is facilitated by the presence of initiation factor F1.



$$30S - mRNA + f - met - tRNA \xrightarrow{F_1} 30S - mRNA - f - met - tRNA$$

3. The 30S f-met-tRNA along with messenger RNA, then moves from decoding site ('A' site) to peptidyl site ('P' site) and with this the next codon of mRNA enters 'A' site where it finds the second correct aminoacyl-tRNA.

Aminoacyl-tRNA (AA_2–tRNA) binds with the codon of 'A' site in presence of GTP and two proteins called transfer factor Tu and Ts which remain associated with ribosomes.

In this binding process, a complex is formed from GTP, the transfer factors and the incoming aminoacyl-tRNA which ultimately fixes aminoacyl-tRNA (AA_2 tRNA) at the 'A' site of ribosome and at the same time releases transfer factors – GTP complex and inorganic phosphate.

4. Due to the relative movement of ribosome and mRNA in presence of single GTP molecule the next codon enters the 'A' site. The A site is now occupied by another aminoacyl-tRNA (AA3 – tRNA) corresponding to the next codon of mRNA and f-met-tRNA reaches at the exit site (E-site) and AA_2-tRNA occurs at the P site. Now an enzyme known as transferase I kicks off tRNA from formyl methionie and flips the formyl methione (AA1,) to AA_2-tRNA bound at the 'P' site.

   According to Monro (1967) an enzyme known as peptidyl synthetase found in SOS, sub-unit helps in the formation of peptide bond. The 'G' factor is supposed to release the discharged or deacetylated tRNA from the site 'E' of ribosome.

5. The next stage of elongation process follows that involves establishment of peptide bond by reaction between free NH_2 group of incoming amino acid and carboxyl group of the polypeptide.

   Thus, during the elongation of polypeptide chain, each charged tRNA (aminoacyl-tRNA) enters the decoding site, moves to 'P' site, transfers its aminoacid to the carboxyl end of polypeptide, moves to exis site where polypeptide chain is transferred to adjacent tRNA bound at 'P' site and then finally released from the ribosome.

8.8.6 Termination of polypeptide chain

The synthesis of polypeptide chain is completed to the codons of messanger RNA and the process comes to an end abruptly where any one of the three non-sense triplets UAG, UAA and UGA is present in the messanger RNA. Generally, no tRNA has anticodon for any of these three 'nonsense codons' but some suppressor mutations produce tRNA with any of these three codons.

8.8.7 Dissociation of initiation factors from the initiation complex

The polypeptide chain, still bound to the RNA is attached to mRNA. The chain is released from the ribosome under the direction of three distinct proteins which are called released factors R_1, R_2 and S.

These factors are bound to the ribosome and control the hydrolysis of ester linkage between tRNA and the polypeptide chain. Reproduction of a primary polypeptide chain according to specification of mRNA is called translation.

After the completion of chain the two sub-units of ribosomes separate.

8.9 Summary

(i) Transcription is the process of making an RNA copy of a gene sequence. This copy called a messenger RNA (mRNA) molecule, leaves the cell nucleus and enters the cytoplasm, where it directs the synthesis of the protein, which it encodes.

(ii) Translation is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis.

(iii) The genetic code describes the relationship between the sequence of base pairs in a gene and the corresponding amino acid sequence that it encodes.

(iv) In the cell cytoplasm, the ribosome reads the sequence of the mRNA in groups of three bases to assemble the protein.

(v) Transcription is performed by enzyme called RNA polymerases, which link nucleotides to form an RNA strand (using a DNA strand as a template).

(vi) Transcription has three stages : initiationm, elongation, and termination.

(vii) In eukaryotes, RNA molecules must be processed after transcription : they are spliced and have a 5' cap and poly-A tail put on theri ends.

(viii) Transcription is controlled separately for each gene is genome.

8.10 Model questions

1. Describe in detail the mechanism of transcription in prokaryotes.
2. Describe in detail the mechanism of transcription in eukaryotes.
3. What are transcription factors ?
4. Explain introns and exons.
5. Explain genetic code
6. What is ribosome ? Describe ribosome structure.

Unit-9 □ Gene Regulation

Structure / Contents

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9.1 Objectives

This particular unit of cell and developmental biology helps the reader to have a concrete idea of regulation of gene expression in both prokaryotes and eukaryotes as well. Finally, after finishing the topic the reader will be able to understand.

- How the genes activity are regulated
- The operon model particularly the *lac* operon for transcriptional regulation
- The roles of activators, repressor and inducers in gene regulation
- About enhancers in controlling gene activity in eukaryotes

9.2 Introduction

Different genes in an organism are means for the synthesis of different proteins. All these proteins are not needed at one time. Further, the same proteins are not

needed in different tissues. Specific enzymes are needed at different times and in different tissues in the life cycle of an organism. However, at all times in the life cycle, and in different tissues/organs, every cell contain same set of genes. It would be necessary, therefore, to have mechanisms which would allow only the desired genes to function at a particular time. The activity of other genes will have to be restricted. This will require regulation of expression of individual genes. A variety of mechanisms are now known, which regulate gene expression at different levels including transcription processing of mRNA and translation.

9.3 Transcription regualtion in prokaryotes

The DNA of prokaryotes is organized into a circular chromosome supercoiled in the nucleoid region of the cell cytoplasm. Proteins that are needed for a specific function are encoded together in blocks called **operons.** For example, all of the genes needed to use lactose as an energy source are coded next to each other in the lactose (or lac) operon.

9.3.1 Different components of gene regulation

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons :

1. Repressors
2. Activators and
3. Inducers

Repressors are protein that suppress transcription of a gene in response to an external stimulus, whereas **activators** are proteins that increase the transcription of a gene in response to an external stimulus. Finally, **inducers** are small molecules that either activate or repress transcription depending on the needs of the cell and the availability of substrate.

9.3.2 Gene regulation in Prokaryotes

In bacteria and archaea, structural proteins with related functions— such as the genes that encode the enzymes that catalyze the many steps in a single biochemical pathway— are usually encoded together within the genome in a block called an **operon** and are transcribed together under the control of a single **promoter.** This

forms a polycistronic transcript (Fig. 9.1). The promoter then has simultaneous control over the regulation of the transcription of these structural genes because they will either all be needed at the same time, or none will be needed.

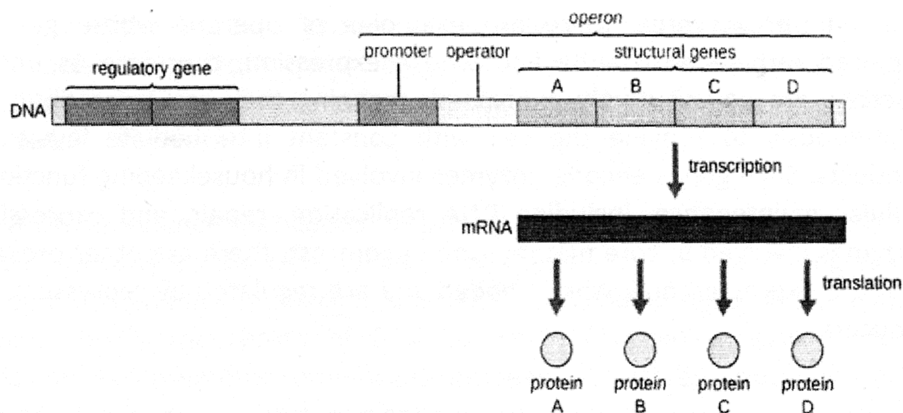

Fig. 9.1 : Showing polycistronic transcript

In prokaryotes, structural genes of related function are often organized together on the genome and transcribed together under the control of a single promoter. The operon's regulatory region includes both the promoter and the operator. If a repressor binds to the operator, then the structural genes will not be transcribed. Alternatively, activators may bind to the regulatory region, enhancing transcription.

French scientists Fracois **Jacob** (1920-2013) and Jacques **Monod** at the Pasteur Institute were the first to show the organization of bacterial genes into operons, through their studies on the ***Iac* operon** of E.Coli. They found that in E. Coli, all of the structural genes that encode enzymes needed to use lactose as an energy source lie next to each other in the lactose (or lac) operon under the control of a single promoter, the *Iac* promoter. For this work, they won the Nobel Prize in Physiology or Medicine in 1965.

Each operon includes DNA sequences that influence its own transcription ; these are located in a region called the regulatory region. The regulatory region includes the promoter and the region surrounding the promoter, to which **transcription factors,** proteins encoded by regulatory genes, can bind. Transcription factors influence the binding of **RNA polymerase** to the promoter and allow its progression to transcribe structural genes. A **repressor** is a transcription factor that suppresses transcription of a gene in response to an external stimulus by binding to a DNA

sequence within the regulatory region called the **operator,** which is located between the RNA polymerase binding site of the promoter and the transcriptional start site of the first structural gene. Repressor binding physically blocks RNA polymerase from transcribing structural genes. Conversely, an **activator** is a transcription factor that increases the transcription of a gene in response to an external stimulus by facilitating RNA polymerase binding to the promoter. An **inducer,** a third type of regulatory molecule, is a small molecule that either activates or represses transcription by interacting with a repressor or an activator.

In prokaryotes, there are examples of operons whose gene products are required rather consistently and whose expression, therefore, is unregulated. Such operons are **constitutively expressed,** meaning they are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products. Such genes encode enzymes involved in housekeeping functions required for cellular maintenance, including DNA replication, repair, and expression, as well as enzymes involved in core metabolism. In contrast, there are other prokaryotic operons that are expressed only when needed and are regulated by repressors, activators, and inducers.

9.3.3 The *trp* operon

It is considered as a repressor operon. Bacteria such as E. coli need amino acids

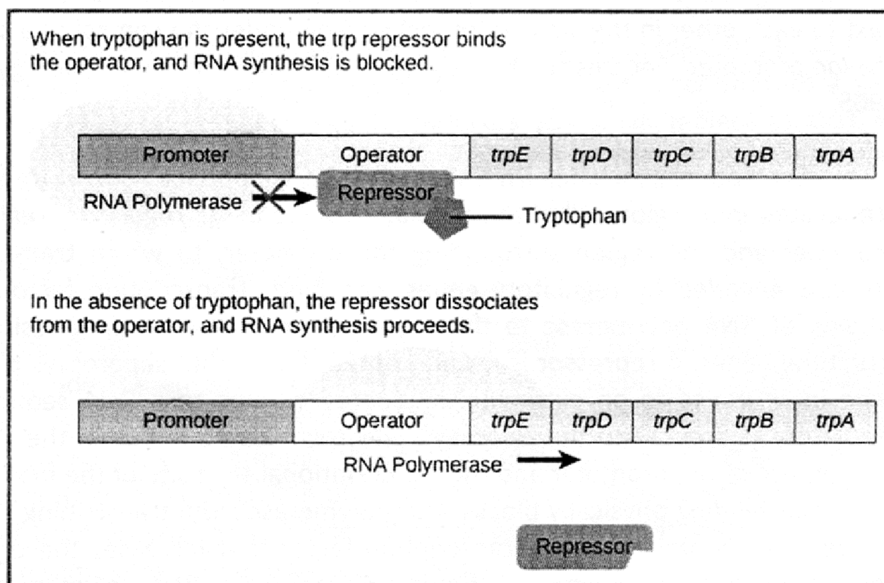

Fig. 9.2 : *trp* operon

to survive. **Tryptophan** is one such amino acid that *E. coli* can ingest from the environment. *E. coli* can also synthesize tryptophan using enzymes that are encoded by five genes. These five genes are next to each other in what is called the **tryptophan (*trp*) operon** (Fig. 9.2). If tryptophan is present in the environment, then *E. coli* does not need to synthesize it and the switch controlling the activation of the genes in the *trp* operon is switched off. However, when trypotophan availability is low, the switch controlling the operon is turned on, transcription is initiated, the genes are expressed, and tryptophan is synthesized.

The five genes that are needed to synthesis tryptophan in *E. coli* are located next to each other in the trp opraon. When tyrpothapn is plentful, two tryptophan molecules bind the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan genes. When tryptophan is absent, the repressor protein does not bind to the operator and the genes are transcribed.

A DNA sequence that codes for proteins is referred to as the coding region. The five coding regions for the tryptophan biosynthesis enzymes are arranged sequentially on the chromosome in the operon. Just before the coding regiono is the **transcriptional start site.** This is the region of DNA to which RNA polyomerase binds to initiate transcription. The promoter sequence is upstream of the transcriptional start site ; each operon has a sequence within or near the promoter to which proteins (activators or repressor) can bind and regulate transcription.

A DNA sequence called the operator sequence is encoded between the promoter region and the first trp coding gene. This **operator** contains the DNA code to which the repressor protein can bind. When tryptophan is present is the cell, two tryptophan molecules bind to the trp repressor, which changes shape to bind to the trp operator. Binding of the tryptophan-repressor complex at the operator physically prevents the RNA polymerase from binding, and transcribing the downstream genes.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator ; therefore, the operator is active and tryptophan is synthesized. Because the repressor protein actively binds to the operator to keep the genes turned off, the *trp* operon is negatively regulated and the proteins that bind to the operator to silence *trp* expression are **negative regulators.**

9.3.4 The *lac* operon

This is an inducer operon and thus the gene regulation in prokaryotic cells occurs through **inducible operons,** which have proteins that bind to activate or repress

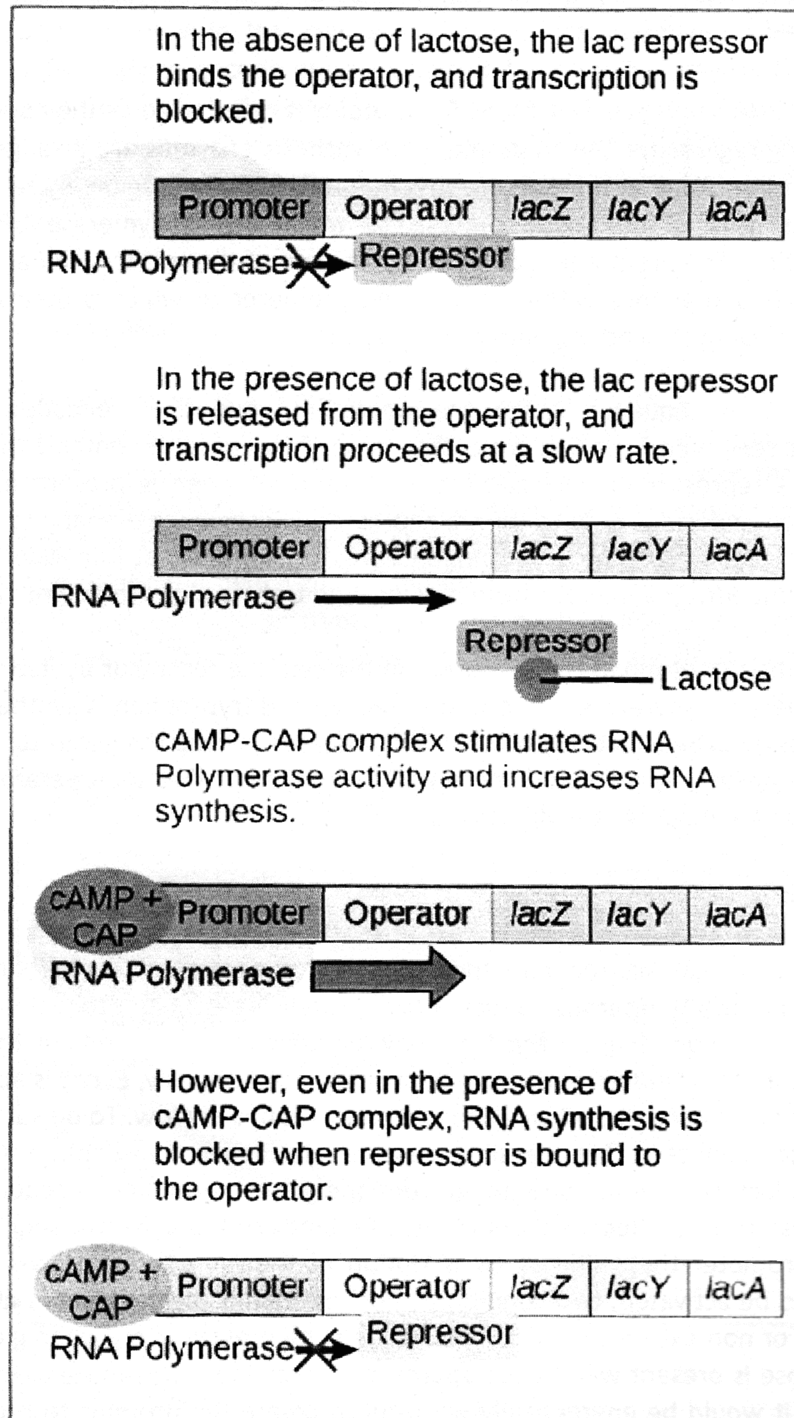

Fig. 9.3 : The *lac* operon function

transcription depending on the local environment and the needs of the cell. The *lac* operon is a typical inducible operon. As mentioned previouly, *E. coli* is able to use other sugars as energy sources when glucose concentrations are low. To do so, the cAMP-CAP protein complex serves as a positive regulator to induce transcription. One such sugar source is lactose. The ***lac* operon** encodes the genes necessary to acquire and process the lactose from the local environment. CAP binds to the operator sequence upstream of the promoter that initiates transcription of the *lac* operon. However, for the *lac* operon to be activated, two conditions must be met. First, the level of glucose must be very low or non-existent. Second, lactose must be present. Only when glucose is absent and lactose is present will the *lac* operon be transcribed. This makes sense for the cell, because it would be energetically wasteful to create the proteins to process lactose if glucose was plentiful or lactose was not avaialable (Fig. 9.3).

Transcription of the *lac* operon is carefully regulated so that its expression only occurs when glucose is limited and lactose is present to serve as an alternative fuel source.

If glucose is absent, then CAP can bind to the operator sequence to activate transcription. If lactose is absent, then the repressor binds to the operator to prevent transcription. If either of these requirements is met, then transcription remains off. Only when both conditions are satisfied is the *lac* operon transcribed (Table 1).a

Table 1 Signal that induce or repress transcription of the lac operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	–	–	+	No
+	–	+	–	Some
–	+	–	+	No
–	+	+	–	Yes

9.4 Transcription regulation in eukaryotes

Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply. The expression of eukaryotic genes is controlled primarily at the level of initiation of transcription, although in some cases transcription may be attenuated and regulated at subsequent steps. As in bacteria

transcription in eukaryoric cells is controlled by proteins that bind to specific reglatory sequences and modulate the activity of RNA polymerase. The intricate task of regulating gene expression in the many differentiated cell types of multicellular organisms is accomplished primarily by the combined actions of multiple different transcriptional regulatory proteins. In addition, the packaging of DNA into chromatin and its modification by methylation impart further levels of complexity to the control of eukaryotic gene expression.

9.4.1 Gene regulation

The human genome encodes over 20,000 genes ; each of the 23 pairs of human chromosomes encodes thousands of genes. The DNA in the nucleus is precisely wound, folded, and compacted into chromosomes so that it will fit into the nucleus. It is also organized so that specific segments can be accessed as needed by a specific cell type.

The first level of organization, or packing, is the winding of DNA strands around histone proteins. Histones package and order DNA into structural units called nucleosome complexes, which can control the access of proteins to the DNA regions (Fig. 9.4). Under the electron microscope, this winding of DNA around histone proteins to form nucleosome looks like small beads on a string. These beads (histone proteins) can move along the string (DNA) and change the structure of the molecule.

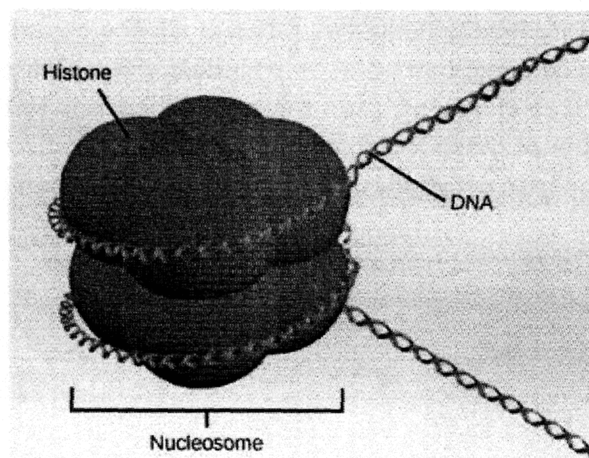

Fig. 9.4 : A nuclesome complex

DNA is folded around histone proteins to create (a) nucleosome complexes. These nucleosomes control the access of proteins to the underlying DNA.

If DNA encoding specific gene is to be transcribed into RNA, the nucleosomes surrounding that region of DNA can slide down the DNA to open that specific chromosomal region and allow for the transcriptional machinery (RNA polymerase) to initiate transcription (Fig. 9.5.). Nucleosomes can move to open the chromosome structure to expose a segment of DNA, but do so in a very controlled manner. How the histone proteins move is dependent on signals found on both the histone proteins and on the DNA. These signals are tags added to histone proteins and DNA that tell the histones if a chromosomal region should be open or closed.

A gene can be turned on or off depending upon the location and modifications to the histone proteins and DNA. If a gene is to be transcribed, the histone proteins and DNA are modified surrounding the chromosomal region encoding that gene. This opens the chromosomal region to allow access for RNA polymerase and other proteins, called transcription factors, to bind to the promoter region, located just upstream of the gene, and initiate transcription. If a gene is to remain turned off, or silenced, the histone proteins and DNA have different modifications that signal a closed chromosomal configuration. In this closed configuration, the RNA polymerase and transcription factors do not have access to the DNA and transcription cannot occur (Fig. 9.5.).

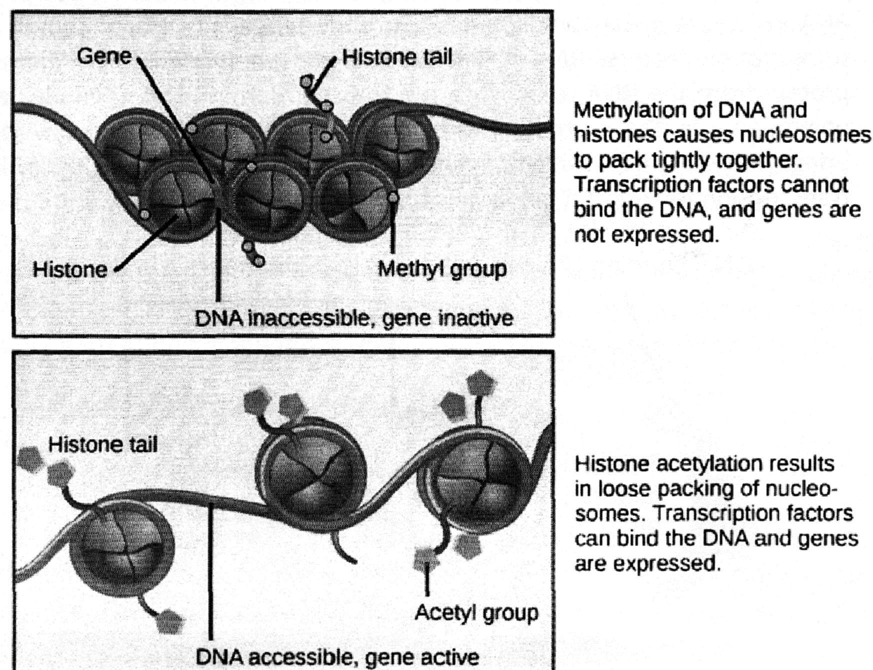

Fig. 9.5 : Initiation of transcription

Like prokaryotic cells, the transcription of genes in eukaryotes requires the actions of an RNA polymerase to bind to a sequence upstream of a gene to initiate transcription. However, unlike prokaryotic cells, the eukaryotic RNA polymerase requires other proteins, or transcription factors, to facilitate transcription initiation. Transcription factors are proteins that bind to the promoter sequence and other regulatory sequences to control the transcription of the target gene. RNA polymerase by itself cannot initiate transcription in eukaryotic cells. Transcription factors must bind to the promoter region first and recruit RNA polymerase to the site for ranscription to be established.

Nucleosome can slide along DNA. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the nucleosomes are spaced far apart (bottom), the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. Modifications to the histones and DNA affect nucleosome spacing.

9.4.2 The promoter and the transcription machinery

Genes are organized to make the control of gene expression easier. The promoter region is immediately upstream of the coding sequence. This region can be short (only a few nucleotides in length) or quite long (hundreds of nucleotides long). The longer the promoter, the more available space for proteins to bind. This also adds more control to the transcription process. The length of the promoter is gene-specific and can differ dramatically between genes. Consequently, the level of control of gene expression can also differ quite dramatically between genes. The purpose of the promoter is to bind transcription factors that control the initiation of transcription.

Within the promoter region, just upstream of the transcriptional start site, resides the TATA box. This box is simply a repeat of thymine and adenine dinucleotides (literally, TATA repeats). RNA polymerase binds to the transcription initiation complex, allowing transcription to occur. To initiate transcription, a transcription factor (TFIID) is the first to bind to the TATA box. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH to the TATA box. Once this complex is assembled. RNA polymerase can bind to its upstream sequence.When bound along with the transcription factors, RNA polymerase is phosphorylated. This releases part of the protein from the DNA to activate the transcription initiation complex and places RNA polymerase in the correct orientation to begin transcription ; DNA-bending protein brings the enhancer, which can be quite a distance from the gene, in contact with transcription factors and mediator proteins (Fig. 9.6).

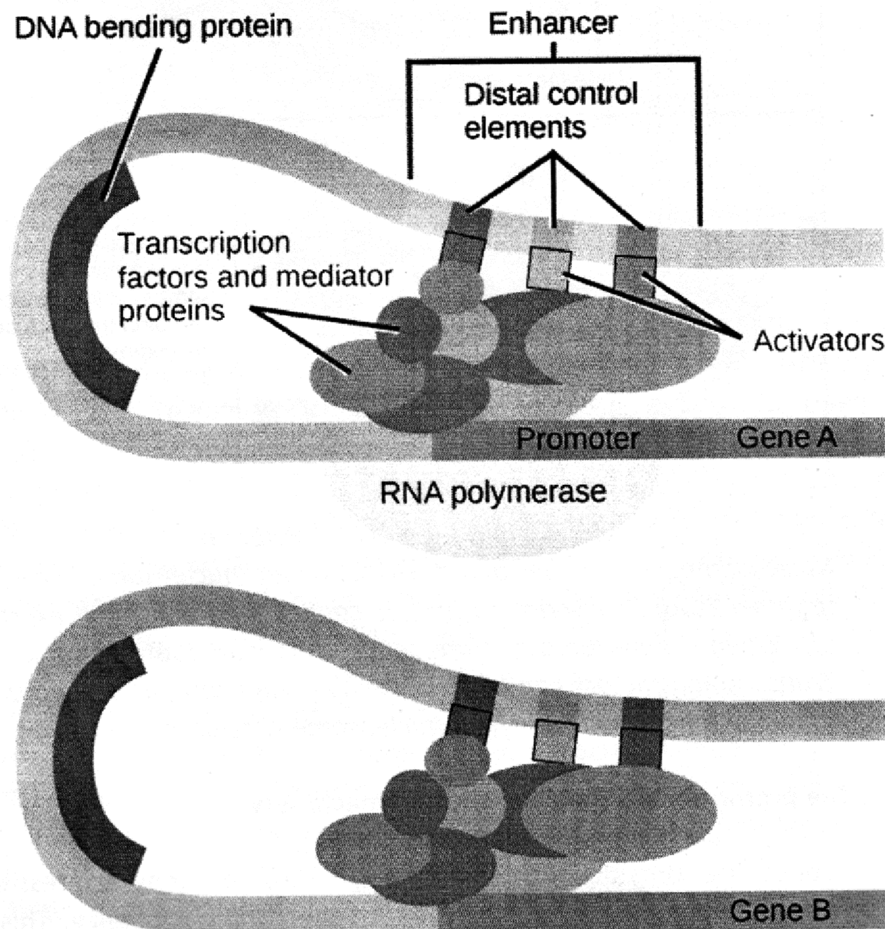

Fig. 9.6 : An Enhancer

An enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genesmay have the same promoter but different distal control elements, enabling differential gene expression.

In addition to the general transcription factors, other transcription factors can bind to the promoter to regulate gene transcription. These transcription factors bind to the promoters of a specific set of genes. They are not general transcription factors that bind to every promoter complex, but are recruited to a specific sequence on the promoter of a specific gene. There are hundreds of transcription factors in a cell that

each bind specifically to a particular DNA sequence motif. When transcription factors bind to the promoter just upstream of the encoded gene, it is referred to as a *cis*-acting element, because it is on the same chromosome just next to the gene.The region that a particular transcription factor binds to is called the transcription factor binding site. Transcription factors respond to enviromental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

9.4.3 Enhancers and transcription

In some eukaryotes genes, there are regions that help increase or enhance transcription. These regions, called enhancers, are not necessarily close to the genes they enhance. They can be located upstream of a gene, within the coding region of the gene, downstream of a gene, or may be thousands of nucleotides away.

Enhancer regions are binding sequences, or sites, for transcription factors. When a DNA-binding protein binds, the shape of the DNA changes (Fig. 9.6). This shape change allows for the interaction of the activators region and the RNA polymerase. Whereas DNA is generally depicted as a straight line in two dimensions, it is actually a three-dimensional object. Therefore, a nucleotide sequence thousands of nucleotides away can fold over and interact with a specific promoter.

9.4.4 Turning genes Off : transcriptional repressors

Like prokaryotic cells, eukaryotic cells also have mechanisms to prevent transcription. Transcriptional repressors can bind to promoter or enhancer regions and block transcription. Like the transcriptional activators, repressors respond to external stimuli to prevent the bindingof activating transcription factors.

9.5. Summary

(i) Gene regulation is the process of controlling which genes in a cell's DNA are expressed (used to make a functional product such as a protein).

(ii) Different cells in a multicellular organism may express very different sets of genes, even though they contain the same DNA.

(iii) The set of genes expressed in a cell determines the set of proteins and functional RNAs it contains, giving it its unique properties.

(iv) In eukaryotes like humans, gene expression involves many steps, and gene regulation can occur at any of these steps. However, many genes are regulated primarily at the level of transcription.

9.6. Model questions

1. Describe gene regulation in prokaryotes.
2. Describe gene regulation in eukaryotes.
3. Explain the operation in *lac* operon.
4. Why lac operon is considered as an inducible operon ?
5. What are activators and repressors ?
6. How enhancers helps in gene regulation in eukaryotes ?

Unit-10 □ Suggested Readings

- Molecular Biology of the Cell, 4th Edition-Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter, New York: Garland Science; 2002

  ISBN-10 : 0-8153-3218-1ISBN-10: 0-8153-4072-9

- The Cell: A Molecular Approach (7th Edition)-Geoffrey M. Cooper, Robert E. Hausman., Sinaur Inc., New York

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